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Evaluation of Dose Distribution Verification of Treatment Plans by Using Electronic Portal Imaging Device at Different Gantry Angles in Intensity Modulated Radiation Therapy

Alper ÖZSEVEN[1]

Introduction

Intensity Modulated Radiation Therapy (IMRT) is a radiation treatment that has become a standard therapy modality, allowing the target volume to be treated with intended doses compared to 3-dimensional conformal radiation therapy, while the normal tissues in the vicinity of the tumor are exposed to less radiation (Van Esch et al., 2004:223). Electronic portal imaging device (EPID) is a retractable component of linear accelerator used in radiation therapy and is one of the most functional equipment in terms of verifying the accuracy of the dose distribution in IMRT treatments as well as validating the position accuracy before the daily treatment of the patients (van Elmpt et al., 2008:289-290). In addition to the fact that IMRT is a complex treatment, the accuracy of treatment depends on many parameters that makes it vital to ensure patient-based quality assurance (QA) (Bailey et al., 2012:83); (van Zijtveld et al., 2006:168); (Sharma et al., 2010:238). One of the most important parameters in LINACs that affecting the treatment dose distribution accuracy is the correct movement of the multi-leaf collimators (MLC), which form the treatment field depending on the shape of the target tumor (Chang et al., 2004:2091); (Richart et al., 2012:263). The studies can be found in the literature that describe in detail how EPID is used in pretreatment verification of IMRT (van Elmpt et al., 2008:290-294); (Jornet et al.,2014:381-382).

Gamma index analysis method has been used as a standard technique for patient-based pretreatment dose verification for many years. Gamma index analysis evaluation is performed by comparing the dose distribution fluence obtained from EPID with the dose distribution fluence calculated and estimated before the irradiation by the treatment planning system for a certain dose difference (DD) at a certain distance to agreement points (DTA) (Low et al., 1998:657); (Spezi et al., 2006:225); (Hussein et al., 2013:370); (Chaikh et al., 2016:32). While it is accepted as standard that to perform gamma analysis with 3% dose difference at 3mm (3%/3mm), it is possible to make comparisons at lower values as an evaluation criterion. Area gamma index <1 value above 95% is considered to be within the acceptance limit. Moreover, the value of area gamma <1 represents the passing rate of the total points for gamma analysis.

In routine practice, the verification of the dose accuracy of the treatment plans of the patients who will receive treatment with the IMRT modality is carried out with the technique of irradiating the EPID with treatment head (gantry) at a fixed angle, which is 0^{o}. This procedure is also accepted as the reference verification position, as it allows faster results. On the other hand, approximately 64% of clinical centers perform the verification of dose distribution with the technique named as non-fixed gantry angles, which are predefined in treatment planning process (Nelms and Simon, 2007:81). Theoretically, it is expected that the treatment devices have the same

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dose output and therefore the same dose distribution at all gantry angles, however the MLC and EPID systems, which are the more sensitive parts of the LINACs to the gravity, are likely to behave differently depending on the angle. This can cause slight variations in the dose distributions (Chin et al.,2004:102); (Monti et al.,2013:205); (Cyriac et al.,2014:2).

The aim of this study is to compare the effects of pretreatment patient-based quality assurance in IMRT patients on the dose distribution of two different techniques, which are the irradiation technique at a fixed gantry angle, 0º, and the irradiation technique using gantry angles, which are predefined in the treatment planning procedure.

METHOD

In this study, 30 randomly selected treatment fields of patients treated with IMRT were compared using two different pretreatment quality assurance techniques (Figure 1). Treatment plans were created using the Eclipse treatment planning system on Varian DHX linear accelerator, with dose calculation by an anisotropic analytical algorithm (AAA). 6 MV x-rays were used. First, the dose distributions on the EPID were recorded by irradiating at a fixed angle at 0º. After that, irradiation was repeated using the non-fixed gantry angles of the fields in the treatment plan and the dose distributions on the EPID were recorded. First of all, by using the Eclipse Portal Dosimetry software, area gamma index <1 values from the resultant dose distributions obtained from EPID for each measurement technique and the predicted dose distributions estimated by the software were compared using the 3%/3mm evaluation criteria (Figure 2). Wilcoxon signed-rank test was used to evaluate the statistical significance. The p-value less than 0.05 was considered statistically significant for all statistical analyses.

Figure 1 – Experiment set-up: pretreatment verification with fixed gantry angle (a), pretreatment verification with non-fixed gantry angle (b)

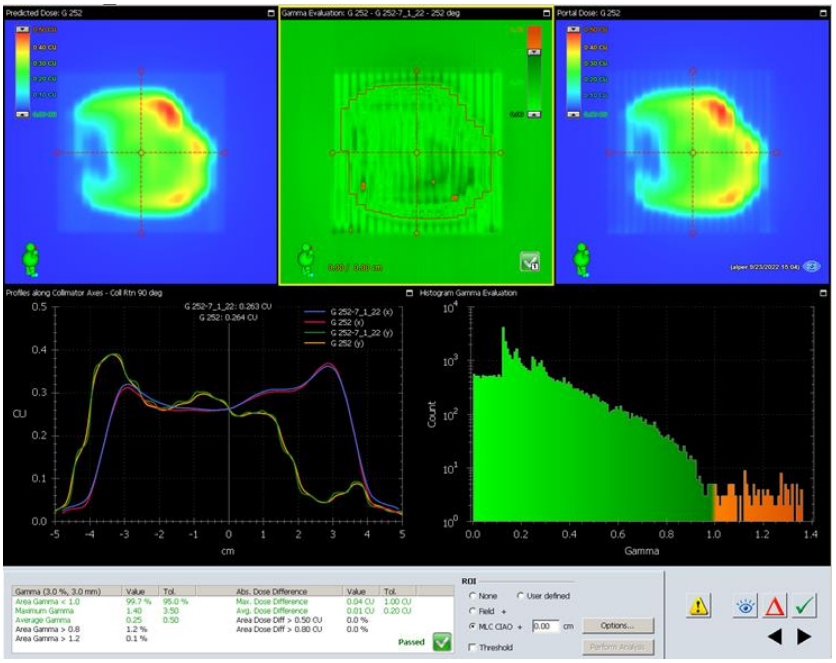

Figure 2 – Gamma analyses in the Eclipse Portal Dosimetry software: dose distributions obtained after irradiation (portal dose, upper left), estimation by the software (predicted dose, upper right), and blended view of comparison (upper middle)

In addition, by using Eclipse Portal Dosimetry software, fixed and non-fixed angle dose distributions, obtained as a result of irradiation were directly compared with each other using area gamma index <1 values in 3%/3mm and 1%/1mm evaluation criteria (Figure 3).

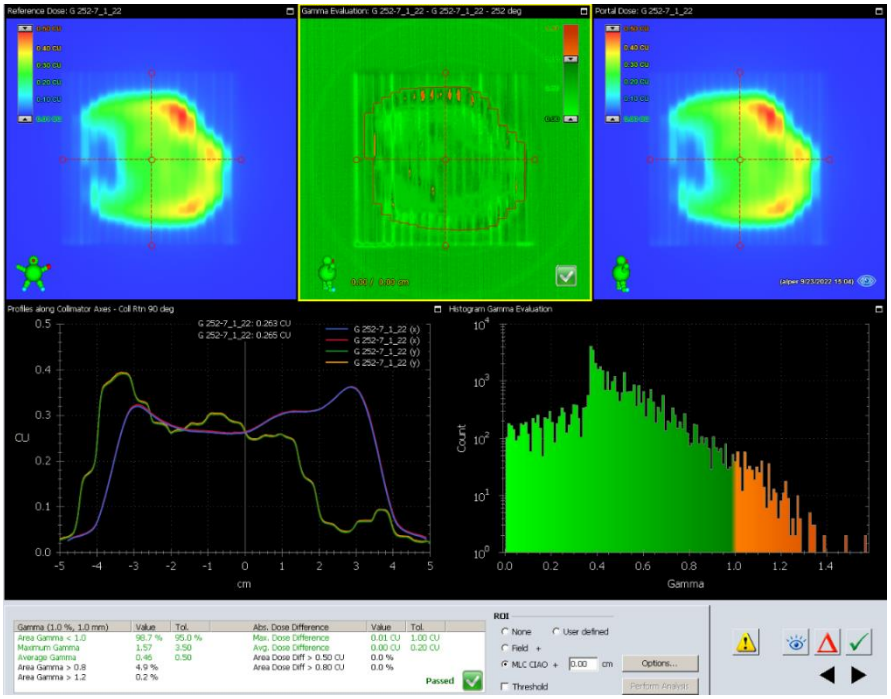

Figure 3 – Gamma analyses of direct comparison in Eclipse Portal Dosimetry software: fixed (upper left) and non-fixed (upper right) angled dose distributions obtained as a result of irradiation of EPID with 1%/1mm evaluation criteria and blended view of comparison (upper middle)

RESULTS and DISCUSSION

The area gamma index <1 median (min-max) values, which are obtained from EPID and the dose distributions estimated by the software with the 3%/3mm dose evaluation criterion for fixed and non-fixed gantry angles are 98.55% (93.70% - 100.00%) and 99.35% (93.90% - 100.00%), respectively (p=0.03) (Table 1).

In measurements where dose distributions were directly compared with each other, all the results were found to be 100% for 30 treatment fields in 3%/3mm evaluation criteria. Since it was a statistically constant value, the result was given as 100%±0% with the notation of mean ± standard deviation. The area gamma index <1 median (min-max) values with the 1%/1 mm evaluation criteria are 98.75% (88% - 100.00%) (Table 1).

Table 1 – Area gamma<1 values as a result of patient-based quality assurance process with two different techniques

	Predicted Dose vs Portal Dose		**Direct Comparison of the Fields**	
Gantry Angle	**0^0**	**Planned**	**(Planned vs Gantry 0^0)**	
Evaluation Criteria	**3%/3mm**		**1%/1mm**	**3%/3mm**
Area Gamma<1 Value*	98.55% (93.70% - 100.00%)	99.35% (93.90% - 100.00%)	98.75% (88% - 100.00%)	100%±0%
p value	0.03**			

** denotes that correlation is significant at the 0.05 level (2-tailed)

* median (min-max) or mean±standard deviation which is applicable

In cases where dose differences in dose distributions need to be evaluated at a certain distance more precisely, this evaluation criterion may decrease to 2%/2mm or even 1%/1mm. In this study, the 3%/3mm criterion was chosen, because it is the reference evaluation criterion. Besides, to analyze the precision of the difference between fixed and non-fixed gantry angle techniques, the 1%/1mm criterion was chosen. The obtained results showed that there was no statistically significant difference for the 3%/3mm evaluation criterion in the comparison of fixed and non-fixed gantry angle techniques with the predicted dose distributions. In addition, the median values found for the two techniques were very close to each other and were higher than 95%, which is the lower limit for area gamma index <1 in clinical routine. On the other hand, no difference was found for the 3%/3mm criterion with the obtained results when these two techniques were directly compared with each other. In measurements where the evaluation criterion was 1%/1mm, the obtained median area gamma index<1 value was 98.75% and this value was higher than the lower limit of 95% and within the acceptance limit. It was remarkable that even with an evaluation criterion such as the 1%/1 mm, the attained result was within the acceptance limit.

In their multicenter study, Jornet et al. stated that pretreatment quality assurance irradiation of patients with head-neck and prostate cancer with fixed and non-fixed gantry angle, did not make a significant difference in the results and yielded values higher than 95%, which is the lower limit for area gamma index <1 (Jornet et al.,2014:386). Similarly, in the study of Cyriac et al., it was reported that the use of these two different techniques in the pretreatment quality assurance processes of patients with prostate cancer did not affect the outcomes. In addition to this, even if no dosimetric difference was found between these techniques; it was also noted that the pretreatment quality assurance process may vary from device to device (Cyriac et al., 2014:4). Monti et al. stated that the pretreatment quality assurance process at different angles caused a position

change in the EPID system due to mechanical conditions Nevertheless, no difference was measured on the dose distributions with this procedure (Monti et al., 2013:206).

CONCLUSIONS

As a consequence, although the difference was statistically significant for gamma analyzes with the predicted dose distributions, all the results were within the acceptance limits. In cases where the techniques were directly compared with each other, the results were within the acceptance limit, and the difference between them varied with regard to the selected evaluation criteria. With a significant time savings for each patient, the pretreatment IMRT QA may also be carried out at fixed (0º) gantry angles.

REFERENCES

Bailey, D. W., Kumaraswamy, L., Bakhtiari, M., Malhotra, H. K., & Podgorsak, M. B. (2012). EPID dosimetry for pretreatment quality assurance with two commercial systems. *Journal of applied clinical medical physics*, *13*(4), 82-99. doi: 10.1120/jacmp.v13i4.3736

Chaikh, A., Desgranges, C., & Balosso, J. (2016). The use of gamma indices with medical imaging as quality assurance tool to validate the dose calculation algorithm in the modern practice of medical physics. *Nucl Med Biomed Imaging*, *1*(2), 31-4. doi: 10.15761/NMBI.1000112

Chang, J., Obcemea, C. H., Sillanpaa, J., Mechalakos, J., & Burman, C. (2004). Use of EPID for leaf position accuracy QA of dynamic multi-leaf collimator (DMLC) treatment. *Medical physics*, *31*(7), 2091-2096. doi: 10.1118/1.1760187

Chin, P. W., Lewis, D. G., & Spezi, E. (2004). Correction for dose-response variations in a scanning liquid ion chamber EPID as a function of linac gantry angle. *Physics in Medicine & Biology*, *49*(8), N93. doi: 10.1088/0031-9155/49/8/N01

Cyriac, S., Musthafa, M. M., Ganapathi Raman, R., Abdul Haneefa, K., & Hridya, V. T. (2014). Pretreatment patient specific quality assurance and gamma index variation study in gantry dependent EPID positions for IMRT prostate treatments. *Journal of Radiotherapy*, *2014*. doi: 10.1155/2014/325057

Hussein, M., Rowshanfarzad, P., Ebert, M. A., Nisbet, A., & Clark, C. H. (2013). A comparison of the gamma index analysis in various commercial IMRT/VMAT QA systems. *Radiotherapy and Oncology*, *109*(3), 370-376. doi: 10.1016/j.radonc.2013.08.048

Jornet, N., Carrasco, P., Beltrán, M., Calvo, J. F., Escudé, L., Hernández, V., et al. (2014). Multicentre validation of IMRT pretreatment verification: comparison of in-house and external audit. *Radiotherapy and Oncology*, *112*(3), 381-388. doi: 10.1016/j.radonc.2014.06.016

Low, D. A., Harms, W. B., Mutic, S., & Purdy, J. A. (1998). A technique for the quantitative evaluation of dose distributions. *Medical physics*, *25*(5), 656-661. doi: 10.1118/1.598248

Monti, A. F., Berlusconi, C., & Gelosa, S. (2013). Gantry angle dependence in IMRT pretreatment patient-specific quality controls. *Physica Medica*, *29*(2), 204-207. doi: 10.1016/j.ejmp.2012.01.002

Nelms, B. E., & Simon, J. A. (2007). A survey on planar IMRT QA analysis. *Journal of applied clinical medical physics*, *8*(3), 76-90. doi: 10.1120/jacmp.v8i3.2448

Richart, J., Pujades, M. C., Perez-Calatayud, J., Granero, D., Ballester, F., Rodriguez, S., & Santos, M. (2012). QA of dynamic MLC based on EPID portal dosimetry. *Physica Medica*, *28*(3), 262-268. doi: 10.1016/j.ejmp.2011.06.046

Sharma, D. S., Mhatre, V., Heigrujam, M., Talapatra, K., & Mallik, S. (2010). Portal dosimetry for pretreatment verification of IMRT plan: a comparison with 2D ion chamber array. *Journal of applied clinical medical physics*, *11*(4), 238-248. doi: 10.1120/jacmp.v11i4.3268

Spezi, E., & Lewis, D. G. (2006). Gamma histograms for radiotherapy plan evaluation. *Radiotherapy and oncology*, *79*(2), 224-230. doi: 10.1016/j.radonc.2006.03.020

Van Elmpt, W., McDermott, L., Nijsten, S., Wendling, M., Lambin, P., & Mijnheer, B. (2008). A literature review of electronic portal imaging for radiotherapy dosimetry. *Radiotherapy and oncology*, *88*(3), 289-309. doi: 10.1016/j.radonc.2008.07.008

Van Esch, A., Depuydt, T., & Huyskens, D. P. (2004). The use of an aSi-based EPID for routine absolute dosimetric pretreatment verification of dynamic IMRT fields. *Radiotherapy and oncology*, *71*(2), 223-234. doi: 10.1016/j.radonc.2004.02.018

van Zijtveld, M., Dirkx, M. L., de Boer, H. C., & Heijmen, B. J. (2006). Dosimetric pretreatment verification of IMRT using an EPID; clinical experience. *Radiotherapy and oncology*, *81*(2), 168-175. doi: 10.1016/j.radonc.2006.09.008

Hyperhidrosis

Aykut ELİÇORA

Introduction

Sweating is a vital physiological process. Hyperhidrosis is defined as sweat secretion that affects the daily life of more people than physiological. It can be considered as a frequently recurring chronic autonomic disease. The most affected organs are the hands, feet and axillary region.

Hyperhidrosis can be primary or secondary. Primary hyperhidrosis is idiopathic and is usually bilaterally symmetrical. Secondary hyperhidrosis can be focal or generalized and is seen due to another underlying cause such as drug use, malignancies, alcohol, chronic pulmonary disease, congestive heart failure, endocrine metabolic disorders, febrile diseases. (Walling, 2011),(Hornberger & et al.,2004)

It has been shown that hyperhidrosis is inherited in an autosomal dominant manner. Positive family history was found in approximately 31.5-65% of them.

Thermoregulatory Sweating

Sweat glands are responsible for thermoregulation to maintain body temperature. It helps regulate body temperature in case of heat, fever or physical exertion. The sweating center is in the hypothalamus and is under the control of the frontal cortex. The sympathetic nervous system regulates thermoregulation by controlling the sweat glands and the circulation of the skin. Regulation is provided by the feedback loop of afferent somatosensory and central temperature sensitive neurons.(Schlereth & Dieterich & Birklein, 2009)

Emotional Sweating

Emotional sweating serves as a "feedback" signal from the emotional to the physical. This type of sweating is regulated by the neocortical and limbic centers. It also occurs with the stimulation of sweat glands in the hands, soles of feet and axilla, which are called “emotional sweating areas” in various emotional situations. Vasodilation accompanies stress-induced thermoregulatory sweating.(Bini & et al.,1980)

But it should be kept in mind that the distinction between thermoregulatory and emotional sweating is not an absolute distinction. It has been shown that they mutually affect each other Surgical treatment.

Focal Hyperhidrosis

Focal hyperhidrosis is most commonly seen as axillary hyperhidrosis. Other forms seen include palmar and plantar hyperhidrosis. (Haider & Solish, 2005) focal hyperhidrosis first begins in puberty or adolescence. Although hyperhidrosis can continue for life, it may end spontaneously with advancing age. (Vlahovic, 2016) The sweating described here is at a level that cannot be explained by external factors. (Sammons & Khachemoun, 2017)

Three types of glands are involved in the realization of sweating. These are the eccrine, apocrine, and apoeccrine sweat glands. Eccrine sweat glands begin to perform their duties from

the moment of birth. Glands that start to function after puberty are apocrine and apoeccrine glands. The increased sweating seen in primary hyperhidrosis is not caused by an increase in the number of these glands, but by an increase in their activity. (Vlahovic, 2016)

Excessive stimulation of the sympathetic nervous system is often cited as the cause of the increase in sweating, and therefore most treatments also show efficacy on the sympathetic nervous system. (Abu & Elhamami, 2021)

Excessive sweating, wet hands, staining on clothes, and odors that can be described as "unpleasant" by people may affect the social life of the person. In conclusion, if hyperhidrosis patients are examined outside of their physical distress, they suffer from higher rates of depressed mood; It is seen that they complain of reduced comfort and self-confidence in daily life conditions such as work, school, and leisure. (Sammons & Khachemoun, 2017)

Axillary Hyperhidrosis

Axillary hyperhidrosis, which is the most common form of primary hyperhidrosis, can be described as excessive sweating in the axilla. Although it manifests itself in puberty or adolescence, if treatment methods are not evaluated, it often continues for life. All three types of sweat glands are found in the axilla. As a result of the formation of conditions such as staining on clothes and odor that can be described as "unpleasant", problems may arise in the patient's relations with other people. (Sammons & Khachemoun, 2017)

Palmar Hyperhidrosis

Palmar hyperhidrosis, a form of focal hyperhidrosis, is a large amount of sweating on the hands that cannot be explained by external factors or emotional state, usually bilaterally symmetrical, and seen throughout the year. During this sweating, which is more severe in the palms, the color of the palm may be normal or close to red. Usually this sweating is cold and wet. (Romero, 2016)This sweating, which affects life socially, like axillary hyperhidrosis, can cause problems in the interpersonal relationships of the person and cause them to develop behaviors such as avoiding shaking hands. (Abu & Elhamami, 2021)

Plantar Hyperhidrosis

Focal hyperhidrosis that can occur on the soles of the feet is then called plantar hyperhidrosis. Affected patients talk about the anxiety felt while performing sports activities. As the reason for this, they show the necessity of changing foot accessories such as socks and shoes more than once during the day. (Vlahovic, 2016) As this sweat is metabolized by standing microfauna, malodorous sweating called bromohidrosis may occur. This condition, which is frequently seen in plantar hyperhidrosis, may cause the social complaints of patients to increase exponentially. (Rieger &Pedevilla&Lausecker, 2015) Plantar hyperhidrosis, which creates a moist environment on the feet, invites fungal infections, which are often referred to as foot, in addition to social complaints. The cutaneous infection that may occur may be of bacterial or viral origin apart from fungal. (Vlahovic, 2016)

Treatment

It can be divided into two as conservative and surgical options. However, full cure cannot be achieved. Treatment choices vary from patient to patient. As the main headings, treatment options can be listed as follows.

Topical treatments

Systemic treatments

Device-based treatments

Surgical treatments

HDSS (hyperhidrosis disease severity scale) score system is used in the selection of treatment. In HDSS, questions are asked to the patient to determine the severity of the disease and scoring is done. Scoring is used to determine treatment.

Topical Treatments

Antiperspirants

It is a frequently beneficial treatment method used in patients with HDSS step 1-2. Aluminum chloride hexahydrate is used in amounts ranging from 6.25-40%. Lower concentrations are suitable for sensitive skin, but efficacy also decreases with intensity.

This solution is used once a day. It is applied on dry skin, especially before going to bed at night. It destroys secretory cells and mechanically obstructs eccrine sweat glands. May cause temporary skin irritation. Aluminum zirconium tetrachlorohydrex (AZT), which has fewer side effects, is another treatment option.

Topical Anticholinergics

HDSS step 1-2 is a more commonly used treatment modality in craniofacial type hyperhidrosis. 1-2% glycopyrrolate is used as spray or solution once or twice a day. Typical anticholinergic effects such as xerostomia, mydriasis and urinary stasis can be seen as side effects. It has been shown to provide 96 percent success.

Cryotherapy

It has been observed that eccrine sweat glands are damaged by the cold application method using the nitrous oxide cryoprobe applicator.

Botulinum Toxin

Botulinum Toxin Type A is administered locally by intradermal injection. It provides temporary success.

Systemic Treatments

Anticholinergics

It is one of the most commonly used systemic treatments. Dose adjustment can be increased depending on the development of tolerance. It is not preferred by patients due to the excess of side effects.

It is contraindicated in cases of Myasthenia Gravis, Paralytic Ileus, pyloric stenosis. The most preferred oral anticholinergics are glycopyrrolate and oxybutynin. It is preferred in patients with HDSS score of 3 and above. Typical anticholinergic side effects (urinary stasis, constipation, dry mouth, tachycardia, etc.) are present.

Alpha adrenergic agonists

Clonidine, an alpha-2 adrenergic agonist, is used in the treatment of hyperhidrosis by reducing sympathetic outflow.

Calcium channel blockers and other oral medications

Helps reduce sweating by reducing the release of acetylcholine

Injectable treatments

Botulinum toxin is a neurotoxin that blocks acetylcholine. It is the most commonly used non-surgical treatment. It is especially preferred in the treatment of axillary hyperhidrosis. Botulinum Toxin A is divided into three. Onabotulinum, Abobotulinum, Incobotulinum.

Rimabotulinum is used in Botulinum Toxin B. In the side effects. hand muscle paralysis is seen especially in those who are injected into the hand.

Device Treatments

Microwave

Microwave thermolysis is used in the treatment of hyperhidrosis by causing local inflammation.

Iontophoresis

It is used in the treatment of palmoplantar hyperhidrosis. The exact mechanism has not been elucidated. An ionized substance is caused to damage the sweat glands through water and using electric current.

Radiofrequency

Microneedle radiofrequency is ablation of eccrine sweat glands by heating the hypodermis.

Photodynamic therapy, Laser and Ultrasound are among the other treatment methods.

Surgical Treatments

Surgical methods are used in the failure of conventional treatment methods. Localized surgery can be performed in axillary hyperhidrosis. Endoscopic thoracic sympathectomy (thoracic 2nd and 3rd sympathetic ganglia are removed) is preferred in more severe cases. Even if the hyperhidrosis in the relevant region decreases, compensatory sweating in different regions (abdomen, back, hips) is common as a late complication. Other complications related to surgery are hemothorax pneumothorax horner syndrome ductus thoracic injury and N. phrenicus palsy, wound infection, skin necrosis, skin discoloration, and scar formation.

References

Walling HW. (2011) Clinical differentiation of primary from secondary hyperhidrosis. *J Am Acad Dermatol.* 64(4):690-5. doi: 10.1016/j.jaad.2010.03.013.

Hornberger J, Grimes K, Naumann M, Glaser DA, Lowe NJ, Naver H, Ahn S, Stolman LP; Multi-Specialty Working Group on the Recognition, (2004) Diagnosis, and Treatment of Primary Focal Hyperhidrosis. Recognition, diagnosis, and treatment of primary focal hyperhidrosis. *J Am Acad Dermatol.* 51(2):274-86. doi: 10.1016/j.jaad.2003.12.029.

Schlereth T, Dieterich M, Birklein F. (2009) Hyperhidrosis--causes and treatment of enhanced sweating. *Dtsch Arztebl Int.* 106(3):32-7. doi: 10.3238/arztebl.2009.0032.

Bini G, Hagbarth KE, Hynninen P, Wallin BG.(1980) Thermoregulatory and rhythm-generating mechanisms governing the sudomotor and vasoconstrictor outflow in human cutaneous nerves. *J Physiol.* 306:537-52. doi: 10.1113/jphysiol.1980.sp013413.

Haider A, Solish N.(2005) Focal hyperhidrosis: diagnosis and management. *Cmaj.* 172(1):69-75. doi: 10.1503/cmaj.1040708.

Vlahovic TC. (2016) Plantar Hyperhidrosis: An Overview. *Clin Podiatr Med Surg.* 33(3):441-51. doi: 10.1016/j.cpm.2016.02.010.

Sammons JE, Khachemoune A. (2017) Axillary hyperhidrosis: a focused review. *J Dermatolog Treat.* 28(7):582-590. doi: 10.1080/09546634.2017.1309347.

Abu Arab WS, Elhamami MM. (2021)Plantar hyperhidrosis associated with primary palmar hyperhidrosis: Outcome following video-assisted thoracoscopic sympathectomy. *Asian Cardiovasc Thorac Ann.* 29(4):310-317. doi: 10.1177/0218492321996508.

Rieger R, Pedevilla S, Lausecker J.(2015) Quality of life after endoscopic lumbar sympathectomy for primary plantar hyperhidrosis. *World J Surg.* 39(4):905-11. doi: 10.1007/s00268-014-2885-4.

Romero FR, Haddad GR, Miot HA, Cataneo DC. (2016)Palmar hyperhidrosis: clinical, pathophysiological, diagnostic and therapeutic aspects. *An Bras Dermatol.* 91(6):716-725. doi: 10.1590/abd1806-4841.20165358.

Chest Wall Deformities

Aykut ELİÇORA

Introduction

Chest wall deformities are structural anomalies that affect the sternum, ribs and chest wall muscles and change the anatomical structure and/or function of the thorax. (Andreescu & et al., 2018) Chest wall deformities are a large group of anomalies that are seen with an incidence of 1/400 - 1/1000 in the general population and have a significant impact on the life of the patients. (Yüksel, 2015) Although the majority of the patients are in the childhood, it can also be encountered in the early adult period in the clinic. The incidence is higher in men. Although the etiology and/or pathogenesis cannot be clearly explained, family history is reported in most of the cases. (Yüksel, 2015)

The psychosocial and cosmetic concerns of the patients have made these deformities more important and remarkable in clinical practice, and have led to the development of different surgical and non-surgical treatment methods.

Pectus excavatum and pectus carinatum are the most common chest wall deformities. Apart from these, sternal cleft, ectopia cordis, Poland syndrome, Jeune syndrome are seen more rarely. Chest wall deformities may be accompanied by other musculoskeletal anomalies, cardiovascular, gastrointestinal and genitourinary anomalies.

Pectus Excavatum

Pectus excavatum or "funnelchest" (funnel chest), popularly known as "shoemaker's chest", is the most common chest wall deformity. It accounts for approximately 90% of all chest wall deformities. It occurs in 1 in 400 live births and is more common in males (male/female ratio approximately 4:1). Although it is mostly noticed during birth or in the first years after birth, the severity of the deformity may increase with the growth of the child.

It is caused by the posterior depression of the sternum as a result of abnormal growth and inward collapse of the costal cartilages. It usually affects the middle and lower 1/3 of the sternum. Although the etiology is not clearly known, it has been reported that 37% of the cases have a family history. Previous studies have shown that inheritance in pectus excavatum is autosomal dominant; however, it is thought that autosomal recessive inheritance, X-linked inheritance and multifactorial inheritance may also play a role in genetic transmission. The coexistence of pectus excavatum with anomalies such as scoliosis, long extremities, high palate, mitral valve prolapse, cardiac arrhythmias shows that there may be a relationship between pectus excavatum and connective tissue disorders. (Andreescu & et al., 2018), (Creswick, et al., 2006) It can be seen in mild, moderate and severe forms in the clinic. Although the deformity can be centralized or asymmetrical, it is often asymmetrical, most commonly depression in the right hemithorax. It can rarely be seen in the axillary or lateral regions. The severity of symptoms may vary according to the degree of deformity. In mild cases, patients may be mostly asymptomatic. In severe cases, cardiac pressure, dyspnea, chest pain, palpitations, decreased exercise tolerance and syncope can be seen. In addition, patients with pectus excavatum often have conditions such as lack of self-confidence, decrease in social relations, anxiety and avoidance of social activities.

Abnormal changes can also be detected in the electrocardiography (ECG) and pulmonary function tests of patients with deformities. ST and T changes, P wave elevation, right axis deviation, right bundle branch block, left atrium and left ventricular hypertrophy can be seen

on ECG due to cardiac compression. Mitral valve prolapse can be detected by echocardiography in 18% of cases. Restrictive changes occur in pulmonary function tests. (Beiser, et al., 1972), (Cahill, Lees & Robertson, 1984) Compared to the general population, it was determined that there was a significant decrease in maximal voluntary ventilation in patients. Frequent recurrent pulmonary infections are also seen in approximately 30% of patients. After the treatment of the deformity, improvement in cardiac and pulmonary findings is observed. (Fonkalsrud, 2009) In addition, psychosocial recovery is also provided.

Pectus Carinatum

Pectus carinatum, popularly known as "pigeon chest", is the second most common chest wall deformity after pectus excavatum. It accounts for approximately 16% of chest wall deformities, more common in men than women (4:1). In contrast to pectus excavatum, it is rare for it to occur soon after birth, often not recognized until puberty. (Demirkaya & Cansever, 2018) As in pectus excavatum, the etiology is not known clearly, but a positive family history has been reported in 25-30% of the cases. Scoliosis, kyphosis, congenital heart diseases and various musculoskeletal anomalies may accompany.

Pectus carinatum is characterized by anterior protrusion of these structures as a result of abnormal growth of the costal cartilages and sternum. It can be classified into three types.

Chondrogladiolar type:

It is the anterior projection of the lower costal cartilages together with the sternum corpus. It is the most common type. It can be symmetrical (most common) or asymmetrical.

Mixed type

It is the type in which pectus excavatum and carinatum deformities are seen together.

Chondromanubrial type

It results from the anterior projection of the manubrium and the second and third rib cartilages and relative depression of the sternum corpus. It is rare.

Although functional disorders due to cardiac and pulmonary compression are less common than pectus excavatum, complaints such as chest pain, dyspnea with exertion, and an increase in respiratory tract infections can also be seen in patients with pectus carinatum. In addition, psychosocial and cosmetic concerns are the most important reasons for seeking treatment.

Treatment Methods In Pectus Deformities

The choice of the right treatment method should be carefully determined according to the shape and severity of the deformity, the age, gender and social status of the patient. Treatment protocols can be grouped under two headings as surgical and non-surgical methods. Traditional Ravitch method for pectus excavatum in surgical treatment protocols and Minimally Invasive Pectus Correction (MIRPE) operation "Nuss procedure" (Molik & et al., 2001); For pectus carinatum, traditional Ravitch method and today's minimally invasive equivalent "Abramson procedure" are applied. Vacuum bell for pectus excavatum and dynamic external compression (orthosis / corset treatment) for pectus carinatum are used in non-surgical protocols.

Surgical treatment

Surgical indications in the treatment of pectus excavatum can be evaluated in two groups as functional and psychological-cosmetic indications. The aim is to prevent the compression of the deformity on the heart and lungs, to allow the normal development of the thorax, to correct the posture and to improve the psychological state. The treatment protocol should be decided according to the shape of the deformity and the clinical condition of the patient, after a detailed

evaluation with the patient and his family. Although there is no clear consensus on the timing of surgery, the age range of 10-16 is considered to be the most appropriate. (Nuss & et al., 1998)

The Haller index (HI), which was presented as a result of Haller's studies in 1987, is the most commonly used index in the typing of pectus excavatum. It is the ratio of the transverse diameter at the deepest point of the deformity to the distance between the posterior surface of the sternum and the anterior aspect of the vertebrae at the same point in thorax computed tomography. In normal people, the HI is 2.5. An HI above 3.25 is an indication for surgery. (Haller, Kramer & Lietman, 1987)

The first surgical treatment for pectus deformities was performed by Ludwig Meyer in 1911 in a 16-year-old patient with pectus excavatum. Secondly, it was performed by Sauerbruch in 1913. Over the years, open surgical procedures with different techniques have continued to be applied and improvements have been made in the technique. The classical operation technique that will be used from the past to the present is the Ravitch Method. The first series of 8 cases, started by Mark M. Ravitch in 1947, was published in the April 1949 issue of the Annals of Surgery journal. (Ravitch, 1949) Complications after open surgery include atelectasis, pneumothorax, hemothorax, wound infections, pneumonia, intrathoracic organ injury, pericarditis, sternocostal joint dislocation, and recurrences in the late period.

Today, the most preferred method for pectus excavatum is the minimally invasive pectus excavatum correction operation (MIRPE), which was defined by Donald Nuss in 1988. In this technique, which is applied under general anesthesia, a nickel-steel alloy bar is placed videothoracoscopically in the retrosternal area. The bar is removed at the end of 2-4 years. In the minimally invasive method, short operation time, minimal blood loss, earlier initiation of daily activities and shorter hospital stay are important advantages compared to other open surgical methods. Despite this, open surgical methods are still used in selected cases. The most serious complication of Nuss operation is cardiac injury. Early complications include pneumothorax, wound infection, hemothorax, nickel allergy, pleural effusion, pericarditis, and pneumonia, while late complications include bar and stabilizer dislocation, pain, infection, allergic reactions, hemothorax, recurrence, and overcorrection. With the developing surgical techniques and increasing surgical experience, the risk of complications and recurrence in pectus excavatum surgery has decreased.

The traditional method in the surgical treatment of pectus carinatum is the Ravitch method. It is an open technique that involves resection of deformed cartilages with or without sternal osteotomy, depending on the condition of the deformity. The Abramson Procedure, a modified version of the Nuss procedure for pectus carinatum, was described by Argentine thoracic surgeon Dr. Horacio Abramson in 2005. In this method, which is also known as minimally invasive pectus carinatum correction surgery (MIRPC), the chest cavity is not entered. Under general anesthesia, it is aimed to place a nickel-steel alloy metal bar presternally through a tunnel created under the skin and to correct the deformity by pressing on the sternum. At the end of 2 years, the bar is removed again under general anesthesia.

Non-Surgical Treatment

Non-surgical treatment of pectus excavatum is vacuum bell application. It consists of a silicone suction cup and a hand pump, which are produced to provide negative pressure that will create a vacuum effect on the chest wall. Successful results are obtained with its regular application in the prepubertal period, when bone development is not yet completed. (Haecker, 2011)

In the non-surgical treatment of pectus carinatum, the dynamic external compression method (orthosis/corset treatment) is frequently used in suitable patients as an alternative to surgical treatments. With a specially developed device, the anterior chest wall is kept under pressure for certain periods. The treatment is applied for 1-1.5 years and the patient is followed up at regular

intervals throughout the treatment. Determining the appropriate pressure level and the patient's compliance with the treatment are important in the success of the treatment. Compression-related pain and vasovagal symptoms, local subcutaneous hematomas and skin ulcerations are rare complications of this treatment.

Poland Syndrome And Treatment

Poland syndrome is characterized by hypoplasia or aplasia of the pectoralis muscles; It may be accompanied by anomalies such as hypoplasia (brachydactyly), finger fusion (syndactyly), breast anomalies, rib defects, absence of pectoral and axillary hair, and weakness of subcutaneous adipose tissue. It occurs in approximately one in 30 000 - 32 000 live births. (Shamberger, Welch &Upton)Generally, familial predisposition is not observed. Its etiology is not clearly known. The syndrome affects only one side of the body, 60-75% is seen on the right side.

Functional complaints are rare in patients, mostly aesthetic concerns. In addition, respiratory complaints can be seen in the presence of costal agenesis and severe chest wall defect. In patients with chest wall defects, surgical treatments should not be delayed as much as possible in order to protect vital organs such as the heart and lungs. Surgical procedures for the hand, such as syndactyly correction surgery, should also be performed as early as possible.

The treatment to be applied is determined according to the components accompanying the syndrome. The main purpose of treatment is to protect vital organs and functions. In addition, it is aimed to ensure the stability of the rib cage and to give the maximum symmetrical appearance.

Sternal Cleft And Ectopia Cordis

It is a rare congenital chest wall deformity. The sternum is formed between the 7th and 10th weeks of pregnancy as a result of the fusion of two mesenchymal leaves, starting from the manubrium and down to the xiphoid. Sternal defects occur due to complete or partial deficiency of this fusion.

In the sternal cleft, the heart, pericardium and diaphragm are in their normal localization, with normal skin cover. Cardiac defects in this group are rare and usually asymptomatic. It occurs in 1 in 50,000 to 100,000 live births. Diagnosis can be made by inspection and palpation in the neonatal period. It is very important to see paradoxical chest movements and heart movements under the skin in the diagnosis. Imaging methods are also important to detect additional malformations that may accompany.

In case of defect in the lower part of the sternum, ectopiacordis can be seen. In cases with thoracic ectopia cordis, the heart is outside the thorax without a pericardial sheath. Thoracoabdominal ectopia cordis is associated with sternal cleft, diaphragmatic defects, omphalocele, perdicardial defect, and intrinsic cardiac anomalies (Cantrell pentalogy). (Gorlin & et al.,1994)

Surgical applications include different techniques from primary closure of the defect to closure with various prostheses and grafts. Surgical treatment should be performed as soon as possible after birth to prevent intrathoracic organ damage, protect it from external factors, and restore normal cardiac and pulmonary functions.

Jeune Syndrome

It was first described in 1955 by Jeune et al. It can be congenital or acquired. Acquired Jeune syndrome is a severe complication that can be seen after open pectus excavatum surgery. Congenital Jeune syndrome, asphyxiating thoracic dystrophy (ATD), is an autosomal recessive polychondrodysplasia disease. It occurs with a frequency of 1 in every 100,000 to 130,000 live births. (Kozlowski & Masel, 1976)

The thorax is narrow in the transverse and sagittal axis; It also lost its flexibility. The lungs are hypoplasic because of the narrow rib cage. Expansion of the thorax is impaired due to short horizontal ribs. As a result, alveolar hypoventilation develops due to insufficient expansion of the lungs. The morbidity and mortality of the disease vary according to the degree of lung involvement. Pneumonia and respiratory failure may occur after birth due to lung hypoplasia. Untreated babies with June syndrome may die early. (Kozlowski & Masel, 1976) In moderately severe cases, recurrent pneumonia, respiratory failure and eventually ventilator dependence may occur. In mild cases, respiratory problems may decrease with advancing age. In some patients, lung functions may be found to be completely normal.

Jeune syndrome is a multisystemic disease and other accompanying skeletal anomalies, kidney failure, hepatic and biliary fibrosis, pelvic anomalies, retinal anomalies can be seen.

The main goal in the treatment of Jeune syndrome is to widen the rib cage and allow adequate lung expansion. Surgical treatment should be performed as soon as possible after delivery.

References

Andreescu N, Sharma A, Mihailescu A, Zimbru CG, David VL, Horhat R, Kundnani NR, Puiu M & Farcas S. (2022) Chest wall deformities and their possible associations with different genetic syndromes. *Eur Rev Med Pharmacol Sci*, 26(14):5107-5114. doi: 10.26355/eurrev_202207_29298.

Yüksel M. (2015) Göğüs Duvarı Deformiteleri ve Cerrahi Tedavisi. Minimal İnvaziv Cerrahi. *Göğüs Cerrahisi Kırmızı Kitap* (s. 571-579). Yüksel M, Balcı AE, (eds). İstanbul: Nobel Tıp Kitapevleri.

Creswick HA, Stacey MW, Kelly RE Jr, Gustin T, Nuss D, Harvey H, Goretsky MJ, Vasser E, Welch JC, Mitchell K & Proud VK.(2006) Family study of the inheritance of pectus excavatum. *J Pediatr Surg.* 41(10):1699-703. doi: 10.1016/j.jpedsurg.2006.05.071.

Beiser GD, Epstein SE, Stampfer M, Goldstein RE, Noland SP & Levitsky S.(1972) Impairment of cardiac function in patients with pectus excavatum, with improvement after operative correction. *N Engl J Med.* 287(6):267-72. doi: 10.1056/NEJM197208102870602.

Cahill JL, Lees GM & Robertson HT. (1984) A summary of preoperative and postoperative cardiorespiratory performance in patients undergoing pectus excavatum and carinatum repair. *J Pediatr Surg.* 19(4):430-3. doi: 10.1016/s0022-3468(84)80268-7.

Fonkalsrud EW. (2009) 912 open pectus excavatum repairs: changing trends, lessons learned: one surgeon's experience. *World J Surg.* 33(2):180-90. doi: 10.1007/s00268-008-9793-4.

Demirkaya A, Cansever L.(2018) Pektus Karinatum Deformitesinde Tanı, Sınıflandırma ve Tarihçe. *Göğüs Duvarı Deformiteleri, Türk Göğüs Cerrahisi Derneği.* Bilgin M, Özpolat B (eds.). Ankara: Nobel Tıp Kitapevleri.

Molik KA, Engum SA, Rescorla FJ, West KW, Scherer LR & Grosfeld JL.(2001) Pectus excavatum repair: experience with standard and minimal invasive techniques. *J Pediatr Surg.* 36(2):324-8. doi: 10.1053/jpsu.2001.20707.

Nuss D, Kelly RE Jr, Croitoru DP & Katz ME. (1998) A 10-year review of a minimally invasive technique for the correction of pectus excavatum. *J Pediatr Surg.* 33(4):545-52. doi: 10.1016/s0022-3468(98)90314-1.

Haller JA Jr, Kramer SS & Lietman SA. (1987) Use of CT scans in selection of patients for pectus excavatum surgery: a preliminary report. *J Pediatr Surg.* 22(10):904-6. doi: 10.1016/s0022-3468(87)80585-7.

Ravitch MM. (1949) The Operative Treatment of Pectus Excavatum. *Ann Surg.* 129(4):429-44. doi: 10.1097/00000658-194904000-00002.

Haecker FM. (2011) The vacuum bell for conservative treatment of pectus excavatum: the Basle experience. *Pediatr Surg Int.* 27(6):623-7. doi: 10.1007/s00383-010-2843-7.

Shamberger RC, Welch KJ & Upton J. (1989) Surgical treatment of thoracic deformity in Poland's syndrome. *J Pediatr Surg.* 198924(8):760-5.766. doi: 10.1016/s0022-3468(89)80532-9.

Gorlin RJ, Kantaputra P, Aughton DJ & Mulliken JB. (1994)Marked female predilection in some syndromes associated with facial hemangiomas. *Am J Med Genet.* 52(2):130-5. doi: 10.1002/ajmg.1320520203.

Kozlowski K, Masel J. (1976) Asphyxiating thoracic dystrophy without respiratory disease: report of two cases of the latent form. *Pediatr Radiol.* 5(1):30-3. doi: 10.1007/BF00988659.

Preventing Liver Toxicity Induced By Methotrexate

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Introduction

Methotrexate (MTX), formerly known as amethopterin, is one of several folic acid antagonists originally utilized in children with acute leukaemia (Conway and Carey, 2017; Fiehn, 2008; Martin et al., 2009; Salim et al., 2008). The data on the effectiveness of MTX derive from studies conducted in the 1960-1970s when there are no current methodological standards, but clinical experience with MTX is more than studies (Chen et al., 2009; Ginnani et al., 1992; Jolivet et al., 1983; Pınarbaşlı et al., 2008). Methotrexate is also an effective drug for the treatment of adult malignancies. Toxic reactions can occur when patients use high doses of MTX, but the relationship between toxicity and concentration in adults is controversial (Yang et al., 2018). Wippel et al., in their study conducted in 2018, they investigated the safety and efficacy of high-dose MTX for osteosarcoma in young adults aged 7-38 years and difference in clearance time between the two study groups (≤18 years vs >18 years) (Wippel et al., 2019). When MTX treatment is planned, pre-treatment complete blood count, erythrocyte sedimentation rate, kidney function tests (urea, creatinine), urine analysis, liver function tests (AST, ALT, GGT, ALP), hepatitis serology (HBV and HCV), HIV and tuberculosis tests must be done. Blood count, liver and kidney function tests should be followed during the treatment. Biochemical tests should be repeated once a month for the first 3 months of treatment, then every 3 months thereafter. Blood count should be applied once a week in the first 3 months of treatment and if there are no side effects, the test interval should be extended (Lindsay et al., 2009). MTX is used orally or intravenously. While it is almost completely absorbed from the gastrointestinal system at low doses, the intravenous route is preferred since the absorption of the gastrointestinal system will decrease in high doses. Oral and intravenous administration of MTX provides good tissue distribution except the central nervous system (Armağan, 2015). It reaches the maximum blood level in 45-120 minutes after oral intake. Serum half-life period is 6-8 hours. MTX is excreted in the urine rate of 65-80% within the first 12 hours by binding to albumin in the circulation. MTX accumulates in erythrocytes and hepatocytes. The clinical effect begins in 2-3 weeks, reaching a maximum in the 8th week (Güven et al., 2017; Kavala et al., 2014; Roenigk et al., 1998). Anemia, thrombocytopenia, leukopenia, tuberculosis, infections, peptic ulcer, ulcerative colitis, alcoholism, immunodeficiency, pregnancy and lactation are contraindications for MTX treatment. Excessive alcohol consumption, liver enzyme elevation, liver disease, obesity, diabetes mellitus, presence of hepatitis B and C, exposure to hepatotoxic drugs are also important risk factors for hepatotoxicity (Nast et al., 2012). The severity of the side effects of MTX is variable. The most common side effects are mild and reversible. Side effects such as nausea, vomiting, increased transaminases and stomatitis are often dose related. Generally,

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these side effects resolve after the end of treatment or dose reduction (Van et al., 2001). MTX causes side effects such as nephrotoxicity and hepatotoxicity. These side effects may occur as a result of oxidative damage caused by reactive oxygen species. There are studies in the literature stating that it should be used together with antioxidants to avoid MTX toxicity. In these studies, the occurrence of hepatotoxicity due to the use of MTX was explained as follows; the use of MTX, which is kept in the form of polyglutamate inside the cell, increases the amount of polyglutamate form in the cell and decreases folic acid levels. This causes hepatocyte necrosis (Akbulut et al., 2014; Cetin et al., 2011; Jahovic et al., 2003; Kahraman et al., 2013; Kolli et al., 2009; Kose et al., 2012; Soliman, 2009; Şentürk, 2016; Vardı et al., 2010. Approximately 30% of the patients receiving MTX treatment are discontinued due to drug toxicity (Van et al., 2001). Long-term and high-dose use of MTX, especially due to hepatotoxicity, may be interrupting the treatment or prolonging the treatment process by reducing the dose to be used. Therefore, with this study, we aimed to collect studies in the literature to prevent hepatotoxicity caused by MTX. This study was produced from my doctoral thesis, which was supported by Cukurova University Research Fund as project number TDK-2018-10773 and was finished on September 1, 2022 (decision no: 24/68-65).

Methotrexate Clinical Applications

MTX is one of the first drugs used for the treatment of rheumatoid arthritis since the 1980s. Friedman and Cronstein examined the mechanism of MTX activity in rheumatoid arthritis in their study in 2019. The reason why MTX is preferred for the treatment of rheumatoid arthritis is the explanation considered as Adenosine signal because MTX increases adenosine levels and causes adenosine to associate with its extracellular receptors, interacting intracellularly with a general anti-inflammatory effect. Studies have shown that MTX is used effective as an antagonist folate pathway by inhibiting dihydrofolate reductase when administered in very high doses (as high as 1 gram in a single dose) for leukemia, and in much lower doses (15-25 mg / week) for rheumatoid arthritis patients (Visentin et al., 2012). Rheumatoid arthritis is a common autoimmune disease that seriously endangers human health (Boone et al., 2019; Burmester et al., 2017; Pang et al., 2018). Another department where MTX treatment is dermatology. MTX is most commonly used in psoriasis in dermatology (Milojevic et al., 2001; Van et al., 2019; Thi et al., 2019). When studies investigating MTX treatment for other dermatological disorders are examined; Systemic retinoids take the first place in the treatment of Pityriasis Rubra Pilaris (PRP), which is a papulosquamous dermatosis. Dicken et al. they reported that when patients with PRP used 10-25 mg MTX a week for an average of 6 months, they had very positive results in all patients (Dicken, 1987; Lower and Baughmann, 1995; Paul et al., 1994). In addition, it has been reported that various immunosuppressives, especially systemic steroids, are used in the treatment of dermatomyositis (DM) with inflammatory myopathy and characteristic skin findings (Kasteler and Callen, 1997). In cutaneous lupus erythematosus, which is one of the cutaneous manifestations of systemic lupus erythematosus, the first choice is hydroxychloroquine, but it has been shown that it can be used in systemic steroids and other immunosuppressives in recalcitrant cases (Wenzel et al., 2005). In recent studies, it has been reported that low-dose MTX has beneficial effects on atopic eczema, which is a chronic and recurrent disease, with dry skin, itching and dyshydrosic eczema with itchy vesicles in hairless areas (Egan et al., 1994; Seyger et al., 1998). In the literature, there is only one study with MTX in Behçet's disease, which is characterized by vascular involvement such as oral and genital aphthae, ocular involvement, erythema nodosum, arthritis and thrombophlebitis

(Jorizzo et al., 1991; Lally et al., 1985; Melton et al., 1991; Torti et al., 2007). In another study by Van et al. in 2019, they evaluated the effectiveness and safety of MTX together methylprednisolone dose in the treatment of severe alopecia areata. As a result, they found that the combination of MTX and methylprednisole was effective and safe in the treatment of alopecia areata (Van et al., 2019) .

Studies to Prevent Toxicity Caused By Methotrexate

MTX inhibits mitosis of the cells by antagonizing folic acid required for deoxyribonucleic acid (DNA) synthesis of cells. MTX toxicity targets vital organs and structures of the body namely skin, GI mucosa, kidney, liver, and bone marrow. Major toxic effects of MTX, such as hepatic, renal, pulmonary, and bone marrow disorders, occur less frequently than the minor effects but may be lifethreatening. Signs and symptoms of acute MTX toxicity are based on extent and severity of organ involvement (Madke et al., 2015).

Table 1 - Studies to prevent liver toxicity caused by MTX

MTX Toxicity	**Experimental animals**	**Human**
Hepatotoxicity	Molsidomine Metformin Alpha lipoic acid Rutin trihydrate (Santa Cruz, USA) Turmeric Timokino Balanites aegyptiaca extract, Melatonin Ursodeoxycholic acid Sitagliptin Metformin Vitamin E Metallothionein Indole-3-Carbinol Inulin Tempol Glycyrrhizin acid Moringa oleifera leaf extract Coenzyme Q10 Ellagic acid Ginkgo biloba extract Acori Graminei Rhizoma	Silymarin Laurencia undulate Folic acid Leflunomide
Testicular toxicity	Carvacrol	
Nephrotoxic effect	Testicular toxicity Infliximab Lycopene Melatonin Corn oil Alpha lipoic acid Nigella sativa (black seeds) Berberine Caffeic acid phenethyl ester	Silymarin
Intestinal mucositis	Arginine, Omega 3 fatty acids nucleotides (oral formula enriched)	
Pulmonary fibrosis	Clarithromycin	
Pancreatic toxicity	Tumor necrosis factor alpha inhibitors	
Inflammation	Coconut oil	
Splen toxicity	Moringa oleifera leaf extract	
Oral mucositis	Alpha lipoic acid	
Fibrosarcoma-bearing	Vitamin c	
Over toxicity	Alpha lipoic acid	

When the literature studies on MTX toxicity in experimental animals are examined (Table 1); Daggulli et al. examined the protective effects of Carvacrol against testicular toxicity caused by MTX in rats. Twenty-four rats equally divided into three groups: group I; control treatment, group II; MTX treatment, group III; received MTX + Carvacrol treatment. On the first day of the experiment, group III received a single dose of Carvacrol, and on the second day of the experiment, groups II and III received a single dose of MTX. They found that the levels of MDA, TOS and OSI in serum and testis sampling were significantly higher in the MTX group only, compared to the control group, and significantly decreased in the MTX group compared to the control group in the TAS group. They noted that with the administration of Carvacrol, levels of MDA, TOS, OSI, and TAS were reduced relative to rats treated with MTX alone (Daggulli et al., 2014). In another study made by Aslaner et al. In 2015, they investigated the effectiveness of ozone therapy on the nephrotoxic effect of MTX. For this purpose, 18 rats is divided into three equal groups as Group 1; control, Group 2; MTX given, Group 3; MTX and ozone therapy given. Ozone therapy (25 mcg / ml) was applied to all three groups every day, and a single dose of MTX (20 mg / kg) was given to Groups 2 and 3 on the fifteenth day, and tissue and blood samples were taken at the end of the third week. Ozone therapy has been found to heal antioxidant enzymes-activated kidney tissue in rats and prevent nephrotoxicity caused by MTX (Aslaner et al., 2015). In a study conducted by Kirbas et al. in 2015, they examined the effect of anti-inflammatory infliximab active ingredient on preventing renal damage caused by MTX on rats. It was studied in three groups with 10 rats in each group. Group 1; receiving a single dose of MTX (20mg/kg), Group 2; receiving a single dose of infliximab (7mg/kg) and Group 3; receiving MTX (20mg/kg) and infliximab (7mg/kg). After five days, blood and tissue samples were taken from the rats. Consequently, at the end of five days, levels of tumor necrosis factor-α ($P=0.008$), interleukin-1β ($P=0.036$), nitric oxide ($P<0.001$) and adenosine deaminase ($P<0.001$) were compared to Group 2 and 3. They found that it was higher, and infliximab was shown to have a strong protective effect against MTX-induced nephrotoxicity by suppressing cytokine release (Kirbas et al., 2015). In addition, in the study by Oğuz et al. examined the effects of lycopene alone or combination with melatonin on nephrotoxicity caused by MTX in rats. They divided 26 rats into four groups; Group 1; control, Group 2; given MTX (20 mg / kg), Group 3; given MTX and corn oil, Group 3 given MTX and lycopene, Group 4; MTX, lycopene and melatonin. In Group 3 and Group 4, a significant decrease was achieved in tumor necrosis factor-alpha, interleukin 1-beta and ceruloplasmin levels compared to Group 2. In addition, a significant decrease was observed in the nitric oxide level in Group 4. In histological examination, while there was damage in Group 2, it was found that there was an obvious improvement in Group 4 (Oguz et al., 2015). Çakır et al. investigated the antioxidant and anti-inflammatory effects of alpha lipoic acid (ALA) on MTX-induced kidney damage on rats. They divided thirty-two rats equally into four groups; Group 1; Control, Group 2; ALA, Group 3; MTX and Group 4; ALA+MTX. They gave a single dose of MTX (20 mg / kg) intraperitoneally to Groups 3 and 4 for kidney injury. On the sixth day, blood samples and kidney tissues received measurement of TNF-α, IL-1β, malondialdehyde, glutathione, myeloperoxidase and sodium potassium-adenosine triphosphatase levels and histological examination results. They stated that the application of MTX caused a significant decrease in GSH and Na +, K + -ATPase activity in the tissue, and a significant increase in MDA and MPO activities. They also found that proinflammatory cytokines (TNF-α, IL-β) were significantly increased in the MTX group. They found that ALA treatment reversed all biochemical indices as well as histopathological changes induced by MTX++++ (Çakır et al., 2015). In the Medical Speciality Thesis made by Akyüz in 2015, investigated the effects of arginine, omega 3 fatty acids and an oral formula enriched with nucleotides in the MTX-induced experimental intestinal mucositis model. The study was conducted

on 32 rats by forming four groups. Group 1; Saline only by gavage was given for 5 days, Group 2; three times a day immunonutritional oral formula (5 mg/kg) was given, Group 3; After intraperitoneal single dose MTX (20mg/kg), saline by gavage was given for 5 days and Group 4; immunonutritional oral administration was given three times a day for 5 days following a single dose of MTX. Tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) levels were studied in serum on the 6th day, and luminol, lucigenin, glutathione, myeloperoxidase, malondialdehyde and Na^+-K^+ ATPase levels were studied in jejunal tissue samples. As a result, serum TNF-α and IL-1β levels were significantly higher in the Group 3 compared to the Group 1 ($p<0.001$). TNF-α and IL-1β levels in the Group 4 were found to be statistically lower than the Group 3 ($p<0.001$). In jejunal tissue, glutathione and Na^+-K^+ ATPase levels were lower in Group 3 than Group 1 ($p<0.05$, $p<0.01$, respectively), while the mean value in Group 4 was higher than in Group 3 ($p<0.05$). In addition, tissue myelopexidase and malondialdehyde levels were statistically higher in Group 3 than Group 1 ($p<0.001$, $p<0.01$, respectively). In Group 4, myelopexidase and malondialdehyde levels were found to be statistically lower than Group 3 ($p<0.001$, $p<0.05$, respectively). Luminol and lucigenin levels in Group 3 were significantly higher than Group 1 ($p<0.01$, $p<0.001$, respectively); Luminol and lucigenin levels in Group 4 were significantly lower than Group 3 ($p<0.01$). This study has shown that chemotherapy has negative effects on the small intestine mucosa, and the immunonutritional oral formula has demonstrated the protective effect of intestinal damage due to MTX (Yilmaz et al., 2018).

In a study conducted by Ahmed et al. in 2017, they examined the effect of Nigella sativa (black seeds) on nephrotoxicity caused by MTX in mice. There are eight mice (Swiss albino) in each group. Group 1; control, Group 2; MTX receiving (10 mg / kg), Group 3; Nigella sativa (black seed) receiving (0.125ml/day), Group 4; They studied with four groups receiving MTX (10 mg/kg) and Nigella sativa (black seed) (0.125ml/day). Malondialdehyde (MDA) and glutathione (GSH) measurements were made on the kidney homogenate on the 23rd day, and they found a increase in MDA value in Group 2 and a lower GSH level in Group 4 compared to Group 1. While in Group 2 had nephrotoxicity, no changes were found in mice in Group 4 (Ahmed et al., 2017).

In the study made by Kalemci et al. in 2018, they examined the effectiveness of clarithromycin as a protective agent in the pulmonary fibrosis model induced by MTX. They examined 30 rats as three groups; Group 1; control, Group 2; MTX (3 mg/kg) and Group 3; MTX (3 mg/kg) and clarithromycin (200 mg/kg). Histopathological changes were evaluated and normal changes were observed in the control group. In group 2, histopathological changes were found to be significantly higher in the pulmonary fibrosis MTX group compared to the other groups. As a result, they have shown that clarithromycin is effective in protecting against pulmonary fibrosis caused by MTX (Kalemci et al., 2018). In addition, Mercantepe et al. investigated the protective effects of tumor necrosis factor alpha inhibitors on pancreatic toxicity caused by MTX. They divided the rats equally and randomly into 3 groups (Group 1, healthy controls, Group 2, MTX group and Group 3, MTX+ADA group) and rats have applied intraperitoneal injection of a single dose of MTX (20 mg/44kg) in Group 2, Group 3. Group 3 received a single dose of 5 mg/kg ADA (INFLECTRA®) ip and all rats were sacrificed under anesthesia 5 days after receiving the MTX injection. Compared to those recorded in Group 1, they recorded higher edema, necrotic cell and inflammatory scores in Groups 2 and 3, edema, number of necrotic cells and inflammatory scores in Group 3, and a more pronounced decrease in Group 2. A decrease in Langerhans cell insulin and somatostatin positive interneurons islets was observed after MTX administration. They found that the islets of Langerhans and the restructuring of the pancreatic structure occurred after ADA treatment (Mercantepe et al., 2018). In the study of Rong et al., they examined that MTX

improved spinal cord injury and found that CASPASE 3, GRP 78 apoptotic factors increased in spinal cord injury, but the values decreased as MTX was administered (Rong et al., 2018). In the study conducted by Famurewa et al. in 2018, 24 rats (Group 1; control, Group 2; MTX 20 mg/kg, Group 3; MTX and 5% coconut oil and Group 4; MTX and 15% coconut oil) for the prevention of oxidative stress caused by the use of MTX with coconut oil. The parameters of SOD, CAT, GPx, GSH, MDA, Interleukin-6, C-reactive protein and nitric oxide were evaluated. They found a significant decrease in SOD, CAT, GPx and GSH values in the groups using coconut oil extract and a significant increase in Interleukin-6, C-reactive protein and nitric oxide parameters. SOD, CAT, GPx (U/mg protein), GSH (mg/g protein), MDA (nmol/mg protein) values were found respectively; in Group 1, 0.22±0.01, 9.83±0.20, 44.3±1.70, 4.50±0.16, 1.25±0.30 in Group 2; 0.18±0.01, 7.46±0.22, 19.6±2.00, 3.36±0.24, 2.18±0.20 in Group 3; 0.22±0.01, 8.67±0.19, 32.2±0.66, 3.91±0.02, 0.93±0.23 and in Group 4; It was 0.23±0.01, 10.2±0.28, 34.5±1.28, 4.29±0.10, 1.02±0.03 (Famurewaa et al., 2018).

In another study examining the protective effect of berberine against MTX-induced nephrotoxicity, the anti-apoptotic properties of berberine were demonstrated by suppressing BAX and CASPASE-3 and increasing BCL2 expression when compared to the MTX-administered group (Hassanein et al., 2019). Similarly, in another study conducted to prevent the damage of Moringa oleifera leaf extract to the spleen caused by MTX in mice, BAX and CASPASE 3 values were found to be higher in the MTX group compared to the Moringa+MTX group (Soliman, 2009).

When the literature studies with humans on MTX toxicity, Taylor et al., examined the evaluation of biomarkers of oxidative stress and apoptosis in patients with severe MTX neurotoxicity. Central nervous system therapy is an important part of ALL treatment and the most common treatment is intrathecal and high dose intravenous MTX. Treatment with MTX can cause neurotoxicity, which often leads to neurological changes, delays in treatment, and prolonged hospital stays. Oxidative stress assessment, oxidized phosphatidylcholine (PC), oxidized phosphatidylinositol (Pi) and F2 isoprostanes; have measured apoptosis with caspase 3/7 activity. They found the most consistent biomarker changes in the cases they examined, an increase in caspase 3/7 and F2 isoprostanes before acute toxicity. They found the most consistent biomarker changes in the cases they examined, an increase in caspase 3/7 and F2 isoprostanes before acute toxicity. They also found that these findings support the role of oxidative stress in MTX-induced neurotoxicity.58 Pulmonary damage occurs as a serious adverse event in 0.3% to 8% of patients, usually during the first year of MTX treatment. Pneumonitis caused by alveolar damage, perivascular inflammation and lung infections. MTX can precipitate in renal tubules, leading to renal failure and nephrotoxic side effects due to acute tubular necrosis (Imokawa et al., 2000; Romão et al., 2014; Widemann et al., 2006). In another study investigating apoptosis in SKOV-3 ovarian cells during treatment with MTX, they noted that MTX caused an increase in BAX, BAX / BCL2 ratio and a decrease in BCL 2 ($P < 0.01$). As a result, they found that MTX caused moderate apoptosis in SKOV-3 ovarian cells (AlBasher et al., 2018).

MTX is a drug used in the treatment of various malignancies. Unfortunately, it leads to life-threatening complications, including hepatorenal toxicity. Hepatotoxicity due to drugs is one of the most common causes of liver damage (Chen et al., 2015; Lucena et al., 2008). This is because the liver is the main organ for the metabolism of many drugs or chemical agents. There are more than 1100 toxic substances that are harmful to the liver (Abboud and Kaplowitz, 2007). The increase in liver enzymes as a result of drug use is an important factor in stopping the production and distribution of the drug (Eren et al., 2004). Although severe drug-induced liver injury is an

extremely rare event, it may require orthotopic liver transplantation or result in the death of the patient (Massart et al., 2017). For example, there are studies demonstrating the protective effects on hepatorenal toxicity in other models in addition to its anticancer effects (Isoda et al., 2006; Nasri and Rafieian-Kopaei, 2014). When the literature studies with experimental animals on hepatotoxicity induced MTX, in the study made by Ascı in 2010, examined the prophylactic effect of Misoprostol in preventing liver and kidney damage caused by MTX. In the study, 24 Wistar albino male rats were used and formed 4 groups, Group 1; Control, Group 2; MTX (20 mg/kg, ip, single dose), Group 3; MTX (20 mg/kg, ip, single dose)+Misoprostol (200µg/kg, oral, 5 days) and Group 4; Misoprostol (200µg/kg, oral, 5 days). As a result of the biochemical analysis in liver tissue, it was found a significant increase was observed in MDA levels in rats using MTX compared to the control group, while MDA decreased with the use of MP. The antioxidant enzyme CAT and SOD levels were found to be significantly decreased in Group 2, while the use of MP increased these enzyme levels. In the kidney tissue, biochemically, it was found that MTX increased the MDA level and decreased the CAT, SOD values, while MP significantly decreased the MDA level and increased the CAT and SOD values. In parallel with biochemical findings, histological examinations of liver and kidney tissues also observed findings that MTX caused oxidative damage. As a result, it was concluded that the toxic effects of MTX on rat liver and kidney tissue can be healed with Misoprostol (Aşcı and Özer, 2011). In a study by Ramachandran et al., they examined the effect of acetaminophen on mitochondrial oxidative stress, DNA and liver damage, they found an increase in AIF and BAX values (Ramachandran et al., 2011). Similarly, Çakır et al. examined the effect of alpha lipoic acid on rats in the prevention of hepatotoxicity caused by MTX. Thirty two rats is divided into four groups; Group 1; Control, Group 2; ALA, Group 3; MTX, Group 4; MTX+ALA. Groups 3 and 4 were given a single dose of MTX (20 mg / kg), while groups 2 and 4 were given intraperitoneal alpha lipoic acid for five days. On the 6th day, blood and liver tissue taken from rats were examined. TNF-α (pg / ml) and IL-1β (pg / ml) values were found respectively in Group 1; 9.7±1.3, 10.5±1.3, in Group 3; 33.1±3.5, 31.6±3.1 in Group 4; 13.9±2.8, 16.3±2.7. It was concluded that MTX caused a significant decrease in GSH and Na +, K + ATPase activity while it caused a significant increase in MDA and MPO activity. In addition, TNF-a, IL-β values also increased significantly in the MTX group. As a result, they found that ALA treatment reversed all these biochemical indices and histopathological changes induced by MTX (Çakır et al., 2015). In another study, Erdogan et al. investigated the possible protective effect of rutin (Rutin trihydrate, Santa Cruz, USA) on MTX-induced hepatotoxicity. Twenty-two rats were divided into three experimental groups for the study; Group 1; Control-saline, Group 2; MTX, Group 3; MTX+Routine. They took hepatic tissue for histological evaluation and biochemical analysis, analyzed the oxidative stress parameters (malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD)) and liver markers in serum (aspartate aminotransferase (AST), alanine aminotransferase (ALT)). They found that there was less damage in histological examinations and increased levels of MDA, ALT, SOD and GSH-Px in the MTX+Routine group compared to the MTX group. They found that MDA and ALT levels were higher, SOD and GSH-Px levels were lower, and AST levels were similar in the MTX group compared to the MTX + Rutin group (Erdogan et al., 2015). In another study made with experimental animals, Moghadam et al. examined the effect of turmeric against liver toxicity and oxidative stress caused by MTX. Wistar albino male rats were fed for 30 days and they were divided into 6 groups; Group 1; control, Group 2; turmeric (100mg/kg), Group 3; turmeric (200mg/kg), Group 4; MTX (20mg/kg), Group 5; turmeric (100mg/kg)+MTX (20mg/kg), Group 6; turmeric (200mg/kg)+MTX (20mg/kg). SOD, CAT and GSH-Px for cellular antioxidant defense activities and MDA for lipid peroxidation were investigated. They obtained significant signs of liver damage in the MTX group. In the

microscopic examination of liver tissue, they found that MTX treatment caused severe centrilobular degeneration, periportal degeneration, portal vein hyperemia, increased arterial inflammatory cells infiltration, necrosis, and all histopathological changes were reduced in the group given turmeric (200 mg / kg) (Moghadam et al., 2015). In addition, El-Sheikh et al. examined the effect of Timokino (TQ) (10mg / kg) on MTX-induced hepatorenal toxicity on rats orally for 10 days. For this study, four groups were designed as Group 1; control, Group 2; TQ given, Group 3; MTX given and Group 4; MTX+TQ given. They observed that caused impairment in kidney and liver functions in urea nitrogen, creatinine, alanine aminotransferase and aspartate aminotransferase in the groups given MTX (20mg/kg i.p.) at the end of the 3rd day. Moreover, they found that MTX caused a decrease in glutathione, catalase, increase in malondialdehyde levels and nitrosative stress associated with increased nitric oxide in liver and kidney. They also found that MTX caused an apoptotic effect as it lowered the caspase 3 level. When the groups given TQ at the end of the 10th day were examined, they found that MTX also reversed the inflammatory and apoptotic findings, oxidative and nitrosative stress (El-Sheikh et al., 2015). In another study by Ramachandran et al., they examined the effect of hepatitis C virus structural proteins on acetaminophen-induced liver damage in mice, and stated that there was an increase in AIF and GADD153 values compared to the control group and a decrease in GRP78 in mice with hepatitis c with liver damage (Ramachandran et al., 2015). Hamed et al. investigated the protective effect of strawberries (fragaria ananassa) against hepatotoxicity due to carbon tetrachloride and found that the value of BAX, CASPASE 3 decreased and BCL2 value increased in the strawberry given group (Hamed et al., 2016). Montasser et al. in 2017 examined the protective effects of balanites aegyptiaca extract, melatonin and ursodeoxycholic acid against hepatotoxicity caused by MTX in male rats. Eighty adult male rats (Sprague Dawely) weighing 190±10 g were randomly divided into eight equal groups: Group 1; Control, Group 2; MTX, Group 3; melatonin, Group 4; balanites aegyptiaca extract, Group 5; ursodeoxycholic acid, Group 6; MTX + melatonin, Group 7; MTX + balanites aegyptiaca extract, Group 8; MTX + ursodeoxycholic acid. They examined liver function biomarker enzymes, liver tissue oxidative stress parameters, total antioxidant capacity, and tumor necrosis factor (TNF-a). They also performed histopathological and immunohistochemistry examinations. In the MTX group, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total and direct bilirubin, TNF-a levels, oxidized glutathione (GSSG), malodialdehyde (MDA), nitric oxide (NO) found a significant increase. They noted that there was a significant decrease in total protein, albumin, total antioxidant capacity, glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) levels in the MTX-treated group. They stated that while there were positive changes in the treatment of melatonin and balanites aegyptiaca extract, no improvement was seen in ursodeoxycholic acid treatment (Montasser et al., 2017). In another experimental study, Abo-Haded et al. investigated the protective effect of Sitagliptin, a selective dipeptidyl peptidase-4 inhibitor used clinically as a novel oral anti-diabetic agent, against MTX-induced liver toxicity. They divided forty mice into four groups (10 mice each); Control, MTX, and two sitagliptin groups (pre-treated with sitagliptin 10 mg/kg/day and 20mg/kg/day) during the five days prior to MTX injection. They found that there was hydropic degeneration, apoptosis and focal necrosis in all liver regions and in biochemical analysis, there was a significant increase in serum transaminases, alkaline phosphatase and lactate dehydrogenase in the MTX group. They observed that MTX induced oxidative stress and depressed antioxidant system of hepatic tissues, activation of inducible nitric oxide synthase and increased nitrite / nitrate level in MTX group. When investigating hepatic cellular apoptosis, they found that there was an increase in Bax and caspase-3 levels and a decrease in Bcl2 levels. They

stated that all biochemical, histopathological, and immunohistochemical markers were significantly improved in the groups in sitagliptin given groups (Abo-Haded et al., 2017). In another study made in mice to prevent acetaminophen-induced hepatotoxicity, the protective effect of Folium Microcos was examined, and an increase in CASPASE3, BAX values and a decrease in BCL2 were observed in the acetaminophen given group compared to the acetaminophen+Folium Microcos group (Wu et al., 2017). In addition, Rizk et al. investigated the effect of MET (Metformin) on hepatotoxicity of MTX induced. They divided the thirty rats into 3 groups: Group 1; control, Group 2; receiving MTX treatment and Group 3; receiving MET/MTX treatment. After 7 days, they observed hepatorenal toxicity in the group receiving MTX and performed histological examinations and biochemical analysis of liver and kidney function. As a result, they observed an increase in hepatic and renal malondialdehyde levels, a significant decrease in hepatic and renal total antioxidant capacity levels and Na + / K + -ATPase in the other groups compared to the control group. They have demonstrated that MET significantly reduces hepatorenal toxicity and can prevent toxic effects on all measured parameters (Rizk et al., 2018). Amirfakhrian et al. in their study in 2018 evaluated the protective effect of vitamin E against hepatotoxicity caused by MTX using 99mTc-phytate as a radiopharmaceutical agent. They divided the rats into five groups; Group 1; control, Group 2; solvent, Group 3; Vit E (100 mg/kg), Group 4; MTX (20 mg/kg) and Group 5; Vit E+MTX. The rats continued intraperitoneal injection of Vit E for 17 days before MTX injection and for 4 days after MTX injection. 99mTc-phytate (% ID/g) in the livers, significantly increased the ID/g % level in MTX-injected rats. This study shows that 99mTc-phytate is an acceptable radiopharmaceutical agent for the evaluation of liver and spleen injuries in an animal model (Amirfakhrian et al., 2018). In another study examining the effect of vitamin E and Metallothionein in fish to prevent the toxicological effect of cadmium on the liver, an increase in CASPASE 3, AIF, BAX values and a decrease in BCL2 value were found in the group given saline compared to the group given vitamin and Metallothionein (Duan et al., 2018). In the same way, Hasan et al. in 2018 examined the therapeutic benefits of indole-3-Carbinol in adjuvant-induced arthritis and its protective effect against MTX-induced hepatic toxicity. Arthritic Sprague Dawley rats injected Complete Freund's Adjuvant (CFA) and administered Indole-3 Carbinol and/or MTX. In order to examine the anti-inflammatory and anti-arthritic effect, they examined the values of body weight, macroscopic scoring of the hind paw, level of pivotal cytokines (TNF-α, IL-6), pathogenesis, spleen index and erythrocyte sedimentation rate. They stated that both I3C and MTX by reducing inflammatory markers and mediators, alleviated inflammation and histopathological changes affecting the tibiotarsal joint. In this study, they found that I3C alleviated arthritis and liver failure induced by MTX therapy by shrinking prooxidants, regulating antioxidant defenses, and reducing pathological changes affecting the liver (Hasan et al., 2018). In another study examining the effect of coenzyme Q10 on lung and liver fibrosis in rats treated with MTX, combined therapy of CoQ10 and methotrexate was used, and blood samples for biochemical analysis, lung and liver tissue were examined for biochemical and histopathological analysis.

They found that combined treatment with CoQ10 and methotrexate resulted in improved histological picture of lung and liver tissues, modulation of liver function (elevations in serum ALT and AST and decrease in albumin), and oxidative stress biomarkers (increase in GSH level and decrease in MDA level). Autophagy genes [rapamycin mammalian target (m-TOR), Microtubule-associated proteins 1A/1B light chain 3 (MAP1LC3B) and Sequestosom 1 ubiquitin binding protein p62 (p62 / SQSTM1)] and Protein B1 (HMGB1) with simultaneous reduction in the High Mobility Group). Based on their results, they found that CoQ10 up had protective properties against methotrexate-induced lung and liver fibrosis in the current study rat model (Mohamed et al., 2018). Ebrahimi et al. examined the hepatoprotective effect of Ellagic acid (EA) against MTX-

mediated oxidative stress (OS). Thirty two Wistar rats divided into 4 groups; Group 1; control, Group 2; 10 days of oral corn oil (0.5%)+a single 20 mg/kg dose of MTX at the end of the third day (ip), Group 3; 5 mg/kg oral EA for 10 consecutive days+a single 20 mg/kg dose of MTX (ip) at the end of the third day and Group 4; 10 mg/kg oral EA for 10 consecutive days+a single 20 mg/kg dose of MTX (ip) at the end of the third day. After biochemical analysis, liver enzymes and malondialdehyde were found to be significantly higher in the MTX group. They stated that oxygen species (ROS) overproduction, decrease in mitochondrial outer membrane potential, mitochondrial swelling, cytochrome c release and increase in caspase-3/9 resulted apoptosis. Also, they noted that overexpression of proinflammatory factors, nuclear factor kappa B (NF-κB) and interleukin 6 (IL-6) showed MTX-induced inflammation in the MTX-treated group. But they found that EA can significantly prevent OS, mitochondrial dysfunction, apoptosis, and the inflammation. Furthermore, the EA-treated rats also found that both nuclear factor erythroid 2-associated factor 2 (Nrf2) showed significant upregulation (Ebrahimi et al., 2019). Moreover, in a study of Nho et al. examined the effect of Acori Graminei Rhizoma on type II collagen-induced arthritis (CIA). They evaluated the water extract of Acori Graminei Rhizoma (WAG) in Tus, CIA mouse models. Male DBA / 1 mice were divided into five groups (NOR; n = 10, CON; n = 10, CIA + methotrexate (MTX); n = 10, CIA + 100 mg / kg WAG; n = 10, CIA + 500 mg / kg. WAG; n = 10). They analyzed hematological parameters, liver and kidney serum toxicity markers. Also, they analyzed serum levels of interleukin (IL) -6, TNF-and type II collagen IgG by enzyme-linked immunosorbent assay (ELISA). As a result, they found that WAG did not alter hematological parameters, kidney and liver toxicity markers, but could be used to treat arthritis by reducing inflammation (Nho et al., 2019). Samdanci et al. investigated the protective effect of molsidomine against hepatotoxicity caused by the use of MTX in rats. Fourty Wistar albino rats were divided into five groups as Group 1 (control), Group 2 (Molsidomine), Group 3 (MTX), Group 4 (Molsidomine-MTX) and Group 5 (MTX-Molsidomine). Average values of SOD (U/mg), CAT (k/g), GSH-Px (U/g), MDA (nmol/g), GSH (micromole/g), MPO (ng/mL) parameters was found respectively in Group 1; 0.32, 0.22, 0.83, 3.27, 2.07, 12.75 in Group 2; 0.026, 0.50, 4.69, 6.98, 2.46, 13.76 in Group 3; 0.02, 0.22, 0.96, 13.97, 1.58, 14.65 in Group 4; 0.031, 0.41, 1.52, 8.86, 2.37, 13.86 and in Group 5; 0.036, 0.32, 1.24, 11.18, 1.97, 13 (Oguz et al., 2015). Significant differences were found between groups in all parameters except MPO parameter. While GSH, GSH-Px, and GSH were significantly higher in the group receiving molsidomine and in given the first molsidomine group, the CAT level was significantly higher in the group treated with molsidomine only (Samdanci et al., 2019). In another experimental study, Ge et al. investigated the protective effect of tempol against acute hepatotoxicity caused by acetaminophen and found that it reduced pro-apoptotic protein expressions (split CASPASE 3 and BAX) and increased anti-apoptotic BCL2 (Ge et al., 2019). Kalantarive et al. examined the protective effect of inulin on MTX-induced liver toxicity in mice. In the study, 48 male mice were divided into 6 equal groups and the mice were treated orally with inulin (100, 200 and 400 mg/kg) for 9 days and given MTX (10 mg/kg, intraperitoneally) on days 7 and 9. Serum samples were taken and they evaluated the expression levels of oxidative stress biomarkers, pathological changes, apoptotic factors such as Bcl-2, caspase -3 and miR-122. The results showed that MTX induced significant liver damage and serum factors in all mice, and found a decrease in Bcl2 and an increase in caspase-3 activity and miR-122 expression compared to the control group (Kalantaria et al., 2019). Similarly, Kury et al. examined the effect of Ginkgo biloba on preventing MTX-induced liver toxicity. Rats were divided into the saline, MTX, and Gb treatment groups (Gb was administered as 60, 120, or 180 mg/kg) and the silymarin group. Overall, a seven-day protocol was adopted, in which animals received either a single daily dose of saline (with 5% DMSO) or a daily oral dose of Gb (60, 120, or 180 mg/kg) or

a daily dose of silymarin (100 mg/kg). Looking at histopathological results, they found that MTX had a significant increase in various hepatic enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and serum alkaline phosphatase (ALP). In addition, MTX contributed to the significant release of inflammatory mediators such as apoptotic (caspase-3 and c-Jun N-terminal kinases (JNK)) and tumor necrosis factor (TNF-a). As a result, they found that treatment with Gb was protective against MTX-mediated apoptosis and inflammation, as well as correcting innate antioxidative mechanisms such as glutathione (GSH) and glutathione S-transferase (GST) (Al Kury et al., 2020). In the study made by Orazizadeh et al., examining the effect of glycyrrhizin acid on BAX and BCL2 expression in hepatotoxicity caused by Titanium dioxide nanoparticles in rats, they found that there was an increase in BAX expression and a decrease in BCL2 expression (Orazizadeh et al., 2020). In the study examining the protective effects of Moringa oleifera leaf extract against oxidative stress and apoptosis in the liver and kidney due to MTX in mice, the BAX value was found to be higher in the MTX group compared to the group receiving Moringa+MTX, while the BCL2 value was found to be lower (Soliman et al., 2020). In addition, Zhang et al. investigated the protective effect of aspirin on acute liver injury due to paraquat in rats, and found that BAX, AIF and CASPASE 3 decreased, while BCL2 value increased after aspirin treatment (Zhang et al., 2020).

When the literature studies with humans on hepatotoxicity induced MTX, in meta-analysis study, over 1000 patients with osteosarcoma and acute lymphoblastic leukemia were given high doses of MTX and investigated whether the 677C> T variant was associated with the risk of liver toxicity associated with MTX therapy. All patients included in the study were evaluated for hepatotoxicity and classified according to the National Cancer Institute criteria. As a result, no relationship was found between the presence of the genetic variant in the MTHFR gene and the development of liver toxicity (Hagleitner et al., 2014). They found no relationship between the two functional MTHFR genetic variants and the occurrence of liver toxicity or other toxicities. In rheumatoid arthritis patients, MTX doses are much lower than used in cancer treatment. Generally, higher doses of MTX are thought to cause liver toxicity. Therefore, it is much more important to determine toxicity in cancer patients receiving high doses of MTX. Yang et al. found a significant correlation between the 677C> T genetic variant and the occurrence of liver toxicity in their study with adult and pediatric patients of different ethnic origins with acute lymphoblastic leukemia (Yang et al., 2012). They also classified the patients according to their ethnic origin and found that the risk of liver toxicity was high, especially in Africans and Caucasians, while Asians were not at high risk. When the age factor was evaluated, they found that the presence of liver toxicity with the 677C> T variable was more dominant in adults compared to children. However, while high doses (3.5 g / m2) of MTX are used in the treatment of childhood acute lymphoblastic leukemia, the use of lower doses (15-30 mg / m2) of MTX in adults also makes this result interesting. Certainly, the number of patients in the studies is insufficient to confirm this result. Lopez-Lopez et al. in another meta-analysis study, they investigated the relationship between the presence of 677C> T and 1298A> C genetic variants of the MTHFR gene and MTX-induced toxicity in children with acute lymphoblastic leukemia. In the pediatric population included in the study, they showed that there was no relationship between these genetic variables and MTX-induced toxicity in children, as in the study by Yang and colleagues (Lopez et al., 2013). In another study in patients with rheumatoid arthritis, Owen et al. examined 309 patients with toxicities caused by MTX treatment (Owen et al., 2013). Feinsilber et al. emphasized the importance of defining and managing toxicity in high-dose MTX administration in their study in 2018. In the literature, Lin et al., selected 1130 records with liver toxicity risk from 5903 records in 2015. All features were included in a 3-month section for classification and analyzed the records with Apache clinical Text Analysis and Knowledge

Extraction System. They emphasized the importance of classifying RA patients with MTX-induced liver damage (Lin et al., 2015). Hagag et al. investigated the role of protective silymarin on hepatic and renal toxicity caused by MTX-based chemotherapy in children with acute lymphoblastic leukemia (Hagag et al., 2016).

Conclusion

These recent studies have shown that the development of liver toxicity of MTX is associated with free oxygen radicals and hydrogen peroxide. For this purpose, melatonin, nicotinamide, methionine, vitamin E, vitamin C, n-acetylcysteine, ALA, coconut, folic acid, antioxidant agents, anti-inflammatories and vasodilator agents have been used to protect tissues from liver toxicity of MTX (Table 1). Thus, discontinuation of MTX treatments due to side effects will be prevented.

References

Abboud G, Kaplowitz N.(2007). Drug-ınduced liver ınjury. *Drug Safety* 30 (4): 277-94.

Abo-Haded HM, Elkablawy MA, Al-johani Z, Al-ahmadi O, El-Agamy DS. (2017). Hepatoprotective effect of sitagliptin against methotrexate induced liver toxicity. *Plos One 12(3)*:1-16.

Ahmed JH, Abdulmajeed IM.(2017). Effect of Nigella sativa (black seeds) against methotrexate-induced nephrotoxicity in mice. *Journal of Intercultural Ethnopharmacology 6(1)*: 9-13.

Akbulut S, Elbe H, Eris C, Doğan Z, Toprak G, Otan E, Erdemli E, Turkoz Y. (2014) Cytoprotective effect of amifostine ascorbic acid and n- acetylcysteine against methotrexate ınduced hepatotoxicity in rats. *World Journal of Gastrpenterology 20(29)*:10158-65.

Al Kury LT, Dayyan F, Ali Shah F, Malik Z, Khalil AAK, Alattar A, Alshaman R, Ali A, Khan Z. (2020 May). Ginkgo biloba Extract Protects against methotrexate-ınduced hepatotoxicity: *A Computational and Pharmacological Approach. Molecules 25(11)*:2540. doi: 10.3390/molecules25112540.

AlBasher G, AlKahtane AA, Alarifi S, Ali D, Alessia MS, Almeer RS, Abdel-Daim MM, Al-Sultan NK, Al-Qahtani AA, Ali H, Alkahtani S. (2018). Methotrexate-induced apoptosis in human ovarian adenocarcinoma SKOV-3 cells via ROS-mediated bax/bcl-2-cyt-c release cascading. *Onco Targets Ther 12*:21-30. doi: 10.2147/OTT.S178510.

Amirfakhrian H, Abedi SM, Sadeghi H, Azizi S, Hosseinimehr SJ. (2018). The use of 99mTc-phytate for assessment the protective effect of vitamin E against hepatotoxicity induced by methotrexat in rat. *Nuclear Medicine Review 21(1)*:8-13.

Armağan İ. (2015). Metotreksat'ın karaciğerde be böbrekte neden olduğu hasarda oksidatif stresin rolü. *Süleyman Demirel Üniversitesi Tıp Fakültesi Dergisi 22(4)*:152-5.

Aşcı H, Özer M. (2011). Protective effect of misoprostol in methotrexate induced liver and kidney damage. *Süleyman Demirel Üniversitesi Sağlık Bilimleri Dergisi 2(2)*:125-126.

Aslaner A, Çakır T, Çelik B, Doğan U, Mayir B, Akyüz C, Polat C, Baştürk A, Soyer V, Koç S, Şehirli AÖ.(2015). Does intraperitoneal medical ozone preconditioning and treatment ameliorate the methotrexate induced nephrotoxicity in rats? *Int J Clin Exp Med 8(8)*: 13811-7.

Boone NW, Sepriano A, Van Der Kuy PH, Janknegt R, Peeters R, Landewé RBM. (2019). Cotreatment with methotrexate in routine care patients with rheumatoid arthritis receiving biological treatment yields better outcomes over time. *RMD Open 5*: 1-6.

Burmester GR, Kaeley GS, Kavanaugh AF, Gabay C, MacCarter DK, Nash P, Takeuchi T, Goss SL, Rodila R, Chen K, Kupper H, Kalabic J.(2017). Treatment efficacy and methotrexaterelated toxicity in patients with rheumatoid arthritis receiving methotrexate in combination with adalimumab. *Rheumatic Musculoskeletal Diseases 3*:1-9.

Çakır T, Baştürk A, Polatı C, Aslaner A, Durgut H, Şehirli AÖ, Gül M, Öğünç AV, Gül S, Sabuncuoglu MZ, Oruç MT.(2015). Does alfa lipoic acid prevent liver from methotrexate induced oxidative injury in rats? *Acta Cirúrgica Brasileira 30(4)*:247-54.

Çakır T, Polat C, Baştürk A, Gül M, Aslaner A, Durgut H, Şehirli AÖ, Aykaç A, Bahar L, Sabuncuoğlu MZ.(2011). The effect of alpha lipoic acid on rat kidneys in methotrexate induced oxidative injury. *European Review for Medical and Pharmacological Sciences 19*:2132-9.

Cetin A, Kaynar L, Eser B, Karada C, Saraymen B, Öztürk A. (2011). Beneficial effects of propolis on methotrexate-induced liver injury in rats. *Acta Oncologica Turcica 44*: 18-23.

Chen M, Suzuki A, Borlak J, Andrade RJ, Lucena MI.(2015). Drug induced liver injury interactions between drug properties and host factors. *Journal of Hepatology 63*: 503-14.

Chen YX, Lv WG, Chen HZ, Ye F, Xie X. (2009). Methotrexate induces apoptozis of human choriocarsinoma cell line JAR via a mitachondrial pathway. *Eur j Obstet Gynecol Reprod Biol 143(2)*:107-11.

Conway R, Carey JJ.(2017). Risk of liver disease in methotrexate treated patients. *World J Hepatol 18*;9(26):1092-1100. doi: 10.4254/wjh.v9.i26.1092.

Daggulli M, Dede O, Utangac MM, Bodakci MN, Hatipoglu NK, Penbegul N, Sancaktutar AA, Bozkurt Y, Türkçü G, Yüksel H.(2014). Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. *Int J Clin Exp Med 7(12)*:5511-6.

Duan Y, Duan J, Feng Y, Huang X, Fan W, Wang K, Ouyang P, Deng Y, Du Z, Chen D, Geng Y, Yang S.(2018). Hepatoprotective activity of vitamin e and metallothionein in cadmium-ınduced liver ınjury in ctenopharyngodon idellus. *Oxid Med Cell Longev 1-12*:9506543. doi: 10.1155/2018/9506543.

Ebrahimi R, Sepand MR, Seyednejad SA, Omidi A, Akbariani M, Gholami M, Sabzevari O. (2019). Ellagic acid reduces methotrexate-induced apoptosis and mitochondrial dysfunction via up-regulating Nrf2 expression and inhibiting the IκBα/NFκB in rats. *Daru 27(2)*:721-733. doi: 10.1007/s40199-019-00309-9.

Egan CA, Rallis TM, Meadows KP et al.(1994). Low dose methotrexate treatment for recalcitrant palmoplantar pompholyx. *J Am Acad Dermatol 40*:612-4.

El-Sheikh AAK, Morsy MA, Abdalla AM, Hamouda AH, Alhaider IA. (2015). Mechanisms of thymoquinone hepatorenal protection in methotrexate-ınduced toxicity in rats. *Hindawi Publishing Corporation Mediators of Inflammation* 1-12.

Erdogan E, Ilgaz Y, Gurgor PN, Oztas Y, Topal T, Oztas E. (2015). Rutin ameliorates methotrexate induced hepatic injury in rats. *Acta Cirúrgica Brasileira 30(11)*:778-84.

Eren M, Temizel İ, Koçak N. (2004). İlaca bağlı hepatotoksisite. *Çocuk Sağlığı ve Hastalıkları Dergisi 47*: 222–27.

Famurewaa AC, Folawiyob AM, Enohnyaketb EB, Azubuike-Osuc SO, Abid I, Obajee SG, Famurewaf OA.(2018). Beneficial role of virgin coconut oil supplementation against acute methotrexate chemotherapy-induced oxidative toxicity and inflammation in rats. *Integrative Medicine Research 7*: 257-63.

Fiehn C. (2009). Methotrexate in rheumatology. *Z Rheumatol 68(9)*:747-56.

Ge Z, Wang C, Zhang J, Li X, Hu J.(2019). Tempol protects against acetaminophen induced acute hepatotoxicity by inhibiting oxidative stress and apoptosis. *Front Physiol 10*:660. doi: 10.3389/fphys.2019.00660.

Ginnani EH, Brewer EJ, Kuzima N, Shakiov A, Maximov A, Vorontsov I, Fink CW, Newman AJ, Cassidy JT, Zemel LS. (1992). Methotrexate in resistant juvenile rheumatoid arthritis. Results of the USAUSSR double blind placebo controlled trial. *N Engl J Med 326*:1043-9.

Dicken CH. (1987). Isotretinoin treatment of pityriasis rubra pilaris. *Journal of the American Academy of Dermatology 16(2)*:297-301.

Güven E, Erişgin Z, Tekelioğlu Y.(2017). Sıçanlarda metotreksat kaynaklı testis hasarı üzerine shilajitin'in histopatolojik etkisi. *Kocatepe Tıp Dergis 18*:1-6.

Hagag AA, Elgamsy MA, El-Asy HM, Mabrouk MM.(2016). Protective role of silymarin on hepatic and renal toxicity ınduced by mtx based chemotherapy in children with acute lymphoblastic leukemia. *Mediterr J Hematol Infect Dis 8*:1-9.

Hagleitner MM, Coenen MJ, Aplenc R, Patiño-Garcia A, Chiusolo P, Gemmati D, De Mattei M, Ongaro A, Krajinovic M, Hoogerbrugge PM, Vermeulen SHHM, Te Loo DMWM. (2014).The role of the MTHFR 677C>T polymorphism in methotrexate-induced liver toxicity: a meta-analysis in patients with cancer. *Pharmacogenomics J 14(2)*:115-9.

Hamed SS, Al-Yhya NA, El-Khadragy MF, Al-Olayan EM, Alajmi RA, Hassan ZK, Hassan SB, Abdel Moneim AE. (2016). The protective properties of the strawberry (fragaria ananassa) against carbon tetrachloride-ınduced hepatotoxicity in rats mediated by anti-apoptotic and upregulation of antioxidant genes expression effects. *Front Physiol 7*:325. doi: 10.3389/fphys.2016.00325.

Hasan H, Ismail H, El-Orfali Y, Khawaja G. (2018). Therapeutic benefits of Indole-3-Carbinol in adjuvant-induced arthritis and its protective effect against methotrexate inducedhepatic toxicity. *BMC Complementary and Alternative Medicine 18(337)*:1-12.

Hassanein EHM, Shalkami AS, Khalaf MM, Mohamed WR, Hemeida RAM.(2019). The impact of Keap1/Nrf2, P38MAPK/NF-ϰB and Bax/Bcl2/caspase-3 signaling pathways in the protective effects of berberine against methotrexate-induced nephrotoxicity. *Biomed Pharmacother 109*:47-56. doi: 10.1016/j.biopha.2018.10.088.

Imokawa S, Colby T, Leslie K, Helmers R. (2000). Methotrexate pneumonitis: review of the literature and histopathological findings in nine patients. *Eur Respir J 15(2)*:373-381. doi:10.1034/j.1399-3003.2000.15b25.x

Isoda K, Young JL, Zirlik A, MacFarlane LA, Tsuboi N, Gerdes N, Scho¨nbeck U, Libby P. (2006). Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. *Arterioscler Thromb Vasc Biol 26(3)*:611-7.

Jahovic N, Cevik H, Sehirli Ao, Yeğen BC, Sener G. (2003). Melatonin prevents Methotrexate induced hepatorenal oxidative injury in rats. *J Pineal Res 34(4)*: 282-7.

Jolivet JJ, Cowan KH, Clendennin NJ, Chabner BA.(1983). The pharmacokinetics and clinical use of methotrexate. *N Engl J Med 309*: 1094-104.

Jorizzo L, White WL, Wice CM et al. (1991). Low dose methotrexate for unusual neutrophilic vascular reactions: cutaneous polyarteritis nodosa and Behcet's disease. *J Am Acad Dermatol 24*:973-8.

Kahraman H, Kurutas E, Tokur M, Bozkurt S, Cıralık H, Kabakcı B.(2013). Protective effectsof erythropoietin and n-acetylcysteine on methotrexate-induced lung injury in rats. *Balkan Med J 30*: 99-104.

Kalantaria H, Asadmasjedib N, Abyazb MR, Mahdaviniac M, Mohammadtaghvaeid N.(2019). Protective effect of inulin on methotrexate- induced liver toxicity in mice. *Biomedicine & Pharmacotherapy 110*:943-50.

Kalemci S, Akpınar O, Dere Y, Sarıhan A, Zeybek A, Tanrıverdi Ö. (2018). Efficacy of clarithromycin as a protective agent in the methotrexate-induced pulmonary fibrosis model. *Kardiochirurgia Torakochirurgia Polska 15 (4)*: 209-12.

Kasteler JS, Callen JP. (1997). Low dose methotrexate administered weekly is an effective corticosteroid repairnig agent for the treatment of the cutaneous manifestations of dermatomyositis. *J Am Acad Dermatol 36*:67-71.

Kavala M, Türkoğlu Z, Özlü E. (2014). Metotreksat ve dermatolojide klinik kullanımları. *Göztepe Tıp Dergisi 29(2)*:104-10.

Kirbas A, Cure MC, Kalkan Y, Cure E, Tumkaya L, Sahin OZ, Yuce S, Kizilkaya B, Pergel A.(2015).Effect of ınfliximab on renal ınjury due to methotrexate in rat. *Iranian Journal of Kidney Diseases 9(3)*: 221-9.

Kolli VK, Abraham P, Isaac B, Selvakumar D. (2009).Neutrophil infiltration and oxidative stress may play a critical role in methotrexate-induced renal damage. *Chemotherapy 55(2)*:83-90.

Kose E, Sapmaz HI, Sarihan E, Vardi N, Turkoz Y, Ekinci N. (2012). Beneficial Effects of Montelukast against methotrexate-ınduced liver toxicity: a biochemical and histological study. *Scientific World Journal* 1-6.

Lally EV, HG Jr. (1985). A review of methotrexate therapy in Reiter syndrome. *Semin Arthritis Rheum 15*:139-45.

Lin C, Karlson EW, Dligach D, Ramirez MP, Timothy A Miller TA, Mo H, Braggs NS, Cagan A, Gainer V, Denny JC, Savova GK.(2015). Automatic identification of methotrexate-induced liver toxicity in patients with rheumatoid arthritis from the electronic medical record. *J Am Med Inform Assoc 22*: 151-61.

Lindsay K, Fraser AD, Layton A et al. (2009). Liver fibrosis in patients with psoriasis and psoriatic arthritis on long term, high cumulative dose methotrexate therapy. *Rheumatology 48(5)*:569-72.

Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, Garcia-Orad A.(2013). A systematic review and meta-analysis of MTHFR polymorphisms in methotrexate toxicity prediction in pediatric acute lymphoblastic leukemia. *Pharmacogenomics J 13(6)*:498-506.

Lower EE, Baughmann RP.(1995). Prolonged use of methotrexate for sarcoidosis. *Arch Intern Med 55*:846-51.

Lucena MI, Cortes MG, Cueto R, Duran L, Andrade RJ.(2008). Assessment of drug induced liver injury in clinical practice. *Fundam Clin Pharmacol 22*: 141-58.

Madke B, Singh A. (2015). Acute methotrexate toxicity. *Indian Journal of Drugs in Dermatology* 46-9. DOI: 10.4103/WKMP-0110.170764).

Martin SA, McCarthy A, Barber LJ, Burgess DJ, Parry S, Lord CJ, Ashworth A.(2009). Methotrexate induces oxidative DNA damage and is selectively lethal to tumour cells with defects in the DNA mismatch repair gene MSH2. *EMBO Mol Med 1(6-7)*:323-37.

Massart J, Begriche K, Moreau C, Fromenty B. (2017). Role of nonalcoholic fatty liver disease as risk factor for drug-induced hepatotoxicity. *Journal of Clinical and Translational Research 3(S1)*: 212-232.

Melton JL, Nelson BR, Stogh DB et al. (1991). Treatment of keratoacanthoma with intralesional methotrexate. *J Am Acad Dermatol 23*:17-23.

Mercantepe T, Kalkan Y, Tumkaya L, Sehitoglu İ, Mercantepe F, Yıldırmıs S. (2018). Protective effects of tumor necrosis factor alpha inhibitors on methotrexate-induced pancreatic toxicity. *Adv Clin Exp Med 27(6)*:715-20.

Milojevic M, Citotoksiena V, Kardaglı C. (2000). Dermatologia Beograd: *Vojnoizdavacki Zavod* 2251-6.

Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, Yazdi M, Bonyadi F, Mohajeri D, Mazani M, Marzban H, Łos MJ, Ghavami S. (2015). Pre-administration of turmeric prevents

methotrexate-induced liver toxicity and oxidative stress. *BMC Complementary and Alternative Medicine 15(246)*:1-13.

Mohamed DI, Khairy E, Tawfek SS, Habib EK, Fetouh MA.(2019). Coenzyme Q10 attenuates lung and liver fibrosis via modulation of autophagy in methotrexate treated rat. *Biomed Pharmacother 109*:892-901. doi: 10.1016/j.biopha.2018.10.133.

Montasser AOS, Saleh H, Ahmed-Farid OA, Saad A, Marie MAS. (2017). Protective effects of balanites aegyptiaca extract, melatonin and ursodeoxycholic acid against hepatotoxicity induced by methotrexate in male rats. *Asian Pacific Journal of Tropical Medicine 10(6)*:557-65.

Nasri H, Rafieian-Kopaei M. (2012). Metformin: Current knowledge. J Res Med Sci. 2014;19(7):658.

Nast A, Boehncke WH, Mrowietz U et al. Guidelines on the teratment of psoriasis vulgaris (English version) update. *J Dtsch Dermatol Ges 10*:51-99.

Nho JH, Kim AH, Jung HK, Lee MJ, Jang JH, Yang BD, Lee HJ, Lee KH, Woo KW, Cho HW. (2019). Water extract of acori graminei rhizoma attenuates features of rheumatoid arthritis in dba/1 mice. *Evid Based Complement Alternat Med* 3637453. doi: 10.1155/2019/3637453.

Oguz E, Kocarslan S, Tabur S, Sezen H, Yilmaz Z, Aksoy N.(2015). Effects of lycopene alone or combined with melatonin on methotrexate-ınduced nephrotoxicity in rats. *Asian Pac J Cancer Prev 16*: 6061-6.

Orazizadeh M, Khorsandi L, Mansouri E, Fakhredini F. (2020). The effect of glycyrrhizin acid on Bax and Bcl2 expression in hepatotoxicity induced by Titanium dioxide nanoparticles in rats. *Gastroenterol Hepatol Bed Bench 13(2)*:168-176.

Owen SA, Lunt M, Bowes J, Hider SL, Bruce IN, Thomson W, Barton A. (2013). MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms. *Pharmacogenomics J 13(2)*:137-47.

Pang Z, Wang G, Ran N, Lin H, Wang Z, Guan X, Yuan Y, Fang K, Liu J, Wang F. (2018). Inhibitory effect of methotrexate on rheumatoid arthritis ınflammation and comprehensive metabolomics analysis using ultra-performance liquid chromatography-quadrupole time of flight-mass spectrometry (uplc-q/tof-ms). *Int J Mol Sci 19*:2894;1-23.

Paul MA, Hyg MS, Jorizzo JL et al. (1994). Low dose methotrexate treatment in elderly patients with bullous pemphigoid. *J Am Acad Dermatol 31*:620-5.

Pınarbaşlı A, Akman A, Yılmaz E, Basaran E.(2008). Metotreksat verilen psöriazis vulgaris hastalarına folik asit desteği gerekli mi? *Türk Dermatoloji Dergisi 2*: 39- 42.

Ramachandran A, Lebofsky M, Weinman SA, Jaeschke H.(2011). The impact of partial manganese superoxide dismutase (SOD2)-deficiency on mitochondrial oxidant stress, DNA fragmentation and liver injury during acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol 251(3)*:226-33. doi: 10.1016/j.taap.2011.01.004.

Ramachandran A, Lebofsky M, Yan HM, Weinman SA, Jaeschke H. (2015). Hepatitis C virus structural proteins can exacerbate or ameliorate acetaminophen-induced liver injury in mice. *Arch Toxicol 89(5)*:773-83. doi: 10.1007/s00204-015-1498-5.

Rizk F, Saadeny AA, Dawood L, Elkaliny HH. (2018). Metformin ameliorated methotrexate-induced hepatorenal toxicity in rats in addition to its antitumor activity: Two birds with one stone. *Journal of Inflammation Research 11*:421-9.

Roenigk HHJr, Auerbach R, Malbach H et al. Methotrexate in psoriasis: consensus conference. J Am Acad Dermatol.1998;38:478-85.

Romão VC, Lima A, Bernardes M, Canhão H, Fonseca JE. (2014). Three decades of low-dose methotrexate in rheumatoid arthritis: Can we predict toxicity? *Immunol Res* 289-310. doi:10.1007/s12026-014-8564-6.

Rong F, Gao X, Liu K, Wu J. (2018). Methotrexate remediates spinal cord injury in vivo and in vitro via suppression of endoplasmic reticulum stress-induced apoptosis. *Exp Ther Med 15(5)*:4191-4198. doi: 10.3892/etm.2018.5973.

Salim A, Tan E, Ilchyshyn A, Berth-Jones J. (2006). Folic acid supplementation during treatment of psoriasis with methotrexate: a randomized, double-blind, placebo-controlled trial. *Br J Dermatol 154*:1169-74.

Samdanci ET, Huz M, Ozhan O, Tanbek K, Pamukcu E, Akatli AN, Parlakpinar H.(2019). Cytoprotective effects of molsidomine against methotrexate-induced hepatotoxicity: an experimental rat study. *Drug Design Development and Therapy 13*: 13-21.

Şentürk N. (2016). Metotreksat. *Arch Turk Dermatol Venerology 50(1)*:18-21.

Seyger MMB, Hoogen FHJ, Boo T. (1998). Low dose methotrexate in the treatment of widespread morphea. *J Am Acad Dermatol 39*:220-5.

Soliman ME. (2009). Evaluation of the possible protective role of folic acid on the liver toxicity ınduced experimentally by methotrexate in adult male albino rats. *Egypt J Histol 32*: 118-128.

Soliman MM, Aldhahrani A, Alkhedaide A, Nassan MA, Althobaiti F, Mohamed WA.(2020). The ameliorative impacts of Moringa oleifera leaf extract against oxidative stress and methotrexate-induced hepato-renal dysfunction. *Biomed Pharmacother* 128:110259. doi: 10.1016/j.biopha.2020.110259.

Taylor OA, Hockenberry MJ, McCarthy K, Gundy P, Montgomery D, Ross A, Scheurer ME, Moore IM. (2015). Evaluation of biomarkers of oxidative stress and apoptosis in patients with severe methotrexate neurotoxicity: a case series. *J Pediatr Oncol Nurs 32(5)*:320-5.

Thi VB, Minh VN, Ngoc AT, Dang QT, Ngoc DL, Van ED, Van TN, Minh PPT, Thu HDT, Huu ND, Huyen ML, Hau KT, Gandolfi M, Satolli F, Feliciani C, Tirant M, Vojvodic A, Lotti T. (2019). Effectiveness, safety and tolerance of methotrexate in vietnamese psoriatic arthritis patients. *Open Access Maced J Med Sci 7(2)*: 250-2.

Torti DC, Jorizzo JL, Mc Carty MA.(2007). Oral lichen planus: a case series with emphasis on therapy. *Arch Dermatol 143*:511-15.

Van ED, Diem TP, Thi VB, Xuan THT, Tuan KL, Quynh TN, Thu TV, Van TN, Huu SN, Minh PPT, Huu ND, Van TH, Hau KT, Gandolfi M, Satolli F, Feliciani C, Tirant M, Vojvodic A, Lotti T. (2019). Successful psoriasis treatment using nb-uvb with methotrexate: the vietnamese experience. *Open Access Maced J Med Sci 7(2)*:253-5.

Van Ede AE, Laan RFJM, Rood MJ. (2001). Effects of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis. *Arthritis Rheumatism 44*:1515-24.

Vardı N, Parlakpınar H, Çetin A, Erdoğan A, Öztürk IÇ. (2010). Protective Effect of Beta Carotene on Methotrexate Induced Oxidative Liver Damage. *Toxicologic Pathology 38*:592-7.

Visentin M, Zhao R, Goldman ID. (2012). The antifolates. *Hematol Oncol Clin North Am 26*:629-48.

Wenzel J, Braher S, Bauer R et al.(2005). Efficacy and safety of methotreaxate in recalcitrant coutaneous lupus erythematosus: results of a retrospective study in 43 patients. *Br J Dermatol 15*:157-62.

Widemann BC. (2006). Understanding and Managing Methotrexate Nephrotoxicity. *The Oncologist* 694-703. doi:10.1634/theoncologist.11-6-694.

Wippel B, Gundle KR, Dang T, Paxton J, Bubalo J, Stork L, Fu R, Ryan CW, Davis LE.(2019). Safety and efficacy of high-dose methotrexate for osteosarcoma in adolescents compared with young adults. *Cancer Medicine 8*:111-6.

Wu H, Zhang G, Huang L, Pang H, Zhang N, Chen Y, Wang G. (2017). Hepatoprotective effect of polyphenol-enriched fraction from folium microcos on oxidative stress and apoptosis in acetaminophen-ınduced liver ınjury in mice. *Oxid Med Cell Longev 1-14*:3631565. doi: 10.1155/2017/3631565.

Yang L, Hu X, Xu L. (2012). Impact of methylenetetrahydrofolate reductase (MTHFR) polymorphisms on methotrexateinduced toxicities in acute lymphoblastic leukemia:a meta-analysis. *Tumour Biol 33(5)*:1445-54.

Yang Y, Wang X, Tian J, Wang Z.(2018). Renal function and plasma methotrexate concentrations predict toxicities in adults receiving high-dose methotrexate. *Med Sci Monit 24*:7719-26.

Yilmaz Akyuz E, Akyuz C, Kiziltan G, Ozer Sehirli A, Cetinel S.(2018). The effects of oral nutritional formula enriched with arginine, omega-3 fatty acids and nucleotides on methotrexate-induced experimental intestinal mucositis. *Progress in Nutrition 20(2)*: 248-256. DOI: 10.23751/pn.v20i2.6396.

Zhang ZD, Yang YJ, Liu XW, Qin Z, Li SH, Li JY. (2020). The protective effect of aspirin eugenol ester on paraquat-induced acute liver ınjury rats. *Front Med (Lausanne) 7*:589011. doi: 10.3389/fmed.2020.589011.

Nutrition in the Climacteric Period

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Introduction

A woman's life consists of five stages: childhood, adolescence, sexual maturity, menopause and old age. Each of these periods shows unique physical, psychic and hormonal differences. Although each period has its own characteristics, puberty and menopause are the most important periods because of their effects on women's life (Çelik Sis & Pasinlioglu, 2013). The climacteric period, on the other hand, is the transition period between the productive and old age period of women's life, characterized by symptoms that occur as a result of changes in hormonal balance due to morphological and functional changes in the ovaries. Climacterium and menopause are often confused with each other. Menopause is a stage seen in the climacteric period. The climacteric period begins around the age of 45, which is considered the premenopausal stage, and continues until the age of 65, which is considered the old age, including the postmenopausal period. In this respect, it can be defined as the transition from the sexual maturity period to the old age period. The climacteric period; includes premenopausal, menopausal and postmenopausal periods (Görgel & Çakıroğlu, 2007).

It is stated that the average age of menopause worldwide is 51 and varies between 45-55 years. In Turkey, the median age of menopause was found to be 47 years (Özdemir & Çöl, 2004). In the 2019 data of the Turkish Statistical Institute (TUIK), it is stated that 1/7 of the female population in our country is in the menopause period (TUIK, 2019), while the life expectancy in women is 81.3 years in the TUIK 2020 data (TUIK, 2020). Based on these data, it can be said that women spend most of their lives in the menopause and postmenopausal period. The problems to be encountered for the menopausal and post-menopausal female population, which constitute a large part of the society, should be emphasized (Akdağ, Kaner, & Ayer, 2022). Age at menopause is affected by many factors such as genetic, environmental, socioeconomic and nutrition. It is emphasized that many factors such as not giving birth, smoking, high dietary fat intake and caffeine consumption accelerate the onset of menopause (Sapre & Thakur, 2014).

Menopause is not a disease but a natural and inevitable process in the life of a healthy woman (Kurt & Arslan, 2020). A healthy diet, protection and maintenance of health during and after menopause are of great importance in reducing the risks of diseases that may be caused by menopause. Chronic diseases such as obesity, cardiovascular diseases, diabetes and osteoporosis, which increase during and after menopause, can be prevented to a large extent by the combination of medical nutrition therapy and physical activity. The nutritional needs of women in the menopausal and postmenopausal period are quite different from women of childbearing age. For this reason, the individual nutrition programs of women in this period should be arranged by a dietitian, taking into account their chronic diseases and their needs specific to the menopause period (Pekcan, Şanlıer, Baş, Başoğlu, & Acar Tek, 2016).

Nutrition During Menopause

Nutrition; It is defined as the consumption of nutrients that provide the nutrients and bioactive components necessary for the continuation of life, growth and development, protection, improvement and development of health, increasing the quality of life, ensuring productivity.

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Nutrition in menopausal and postmenopausal periods is important, energy, carbohydrate, protein and fat intake, fluid consumption, vitamin and mineral intake, especially calcium and vitamin D, salt and caffeine intake are important in this period (Akdağ et al., 2022).

Energy Intake

With the menopausal period, even if the body weight remains the same, a decrease in muscle mass, an increase in fat mass and a decrease in total body water level occur. Both the absence of the menstrual cycle and the decrease in muscle mass cause the basal metabolic rate to slow down, resulting in a decrease in energy expenditure (Directorate, 2017). In addition, with the advancing age, there is a decrease in metabolic activities and physical activity levels in the body. With the decrease in physical activity levels, total energy expenditure decreases, so the prevalence of obesity in menopausal women increases (Pekcan et al., 2016). It was determined that there was an average increase of 2.1±5.1 kg in body weight in the menopausal period (Guthrie, Dennerstein, & Dudley, 1999). In the American Heart Association 2013 / American College of Cardiology Obesity Guidelines, it is recommended for women to consume 1200-1500 kcal/day energy intake or 500-750 kcal/day energy deficit for body weight loss and to reduce the consumption of foods with high carbohydrate and fat content (Members et al. al., 2014). However, the recommended energy intake level according to Turkey Dietary Guidelines 2015 is 1934 kcal for a moderately active woman aged 40-49, and 1917 kcal for a moderately active woman aged 50-59 (Toplar, Kaner, & Ayer, 2022).

Carbohydrate Intake

Carbohydrates, which are the main energy source of the body, should constitute 45-60% of the total energy taken daily and at least 130 g/day of carbohydrates should be consumed. Bread, which is the most consumed grain product, should be prepared from whole grain flours or mixed whole grain flours. A diet rich in whole grains provides more dietary fiber, vitamins and minerals to the human body than refined grains. In this period, it is recommended that the bread and cereal group consume 3-3.5 portions and the pulp consumption should be 25 g/day. Therefore, at least half of the total daily grain consumption should be whole grain (Akdağ et al., 2022; TÜBER, 2015). It is emphasized that the consumption of vegetables and fruits, which are a source of complex carbohydrates, is rich in vitamins, minerals and phytochemicals, and is effective in preventing cardiovascular diseases, some types of cancer, type 2 diabetes and some eye diseases. Consuming fresh vegetables mostly raw or cooked in their own juices provides more bioavailability. In the Turkish Dietary Guidelines, it is recommended to consume 2.5 portions of vegetables and 2 portions of fruit for this period (Yabancı, Akdevelioğlu, & Rakıcıoğlu, 2012). In a study conducted in England, it was determined that women with a high habit of consuming refined pasta and rice entered menopause on average one and a half years earlier, and women with high consumption of legumes about one year later (Dunneram, Greenwood, Burley, & Cade, 2018). In another study conducted with menopausal women, it was determined that dietary lactose and galactose were not associated with the age of menopause (Rostami Dovom, Moslehi, Mirmiran, Azizi, & Ramezani Tehrani, 2019). In addition, it has been determined that simple carbohydrates increase insulin resistance, impair sex hormone binding globulin production and trigger early menopause (Dunneram et al., 2018).

Protein Intake

Consumption of quality protein in recommended amounts for age and gender is important for tissue formation, repair, and musculoskeletal health in the menopausal period, as it is in every period of life. According to Turkey Dietary Guidelines 2015, adult individuals are recommended to consume 0.8-1.0 grams of protein per body weight per day (TÜBER, 2015). It is stated that the minimum protein consumption level that should be taken to prevent lean mass loss in postmenopausal women is 0.8 g/kg per day. It is recommended that 10-20% of the daily needed energy should be met from protein sources and this ratio should be regulated according to the quality of the consumed protein source (Akdağ et al., 2022). In addition, it is recommended to consume 1.5 servings/day (120 g) of meat, chicken, fish, eggs, legumes and oilseeds group (Akdağ et al., 2022).

Osteoporosis is one of the biggest health problems that occur with the menopause period. In the Turkish Dietary Guidelines 2015, it is emphasized that high protein consumption should be avoided. In a randomized controlled study with obese women in the postmenopausal period, it was determined that medical nutrition therapy containing 1.2 g/kg/day protein was effective in reducing the loss of lean body mass. However, it has been shown that 1.2 g/kg/day protein intake causes changes in muscle cell structure and organization, and body weight loss prevents oxidative stress caused by low protein intake by regulating muscle-insulin signaling (Smith et al., 2016). Unlike this study, in a randomized controlled study with postmenopausal women, it was concluded that 1.2 g/kg/day protein intake did not support the increase in lean tissue. It is stated that more studies with a longer duration and involving more participants are needed to evaluate the effect of low or high protein intake recommendation on lean tissue gain in postmenopausal women (Rossato et al., 2017).

Oil Intake

The menopausal period is a process in which many chronic diseases occur (32). In the Turkish Dietary Guidelines, it is stated that the energy from fat should be 20-35% of the total energy need for healthy individuals. However, it is recommended that cholesterol intake be below 300 mg/day and omega-3 intake should be 250 g/day (TÜBER, 2015).

In the literature, it is thought that the type and amount of dietary fat may have effects on bone health. It has been reported that excessive dietary intake of saturated fatty acids adversely affects bone density (Wang et al., 2016). In a study on the subject, it was stated that omega-3 fatty acids affect the age of menopause. It has been determined that as oily fish consumption increases, women enter menopause later (3.3 years) (Dunneram et al., 2018). In a randomized controlled study with postmenopausal women, it was reported that 900 mg of omega-3 consumption per day significantly reduced body mass index, waist circumference, blood pressure, serum triglyceride, interleukin-6 and insulin resistance (Tardivo et al., 2015).

It is emphasized that adequate adherence to the energy-restricted Mediterranean diet is important in achieving improvements in body weight loss and metabolic health in postmenopausal women (Bajerska et al., 2018).

Vitamins and Minerals

It is emphasized that the consumption of vegetables and fruits, which are good sources of potassium and magnesium, should be increased due to the increase in the risk of chronic diseases during the menopause period. Green leafy vegetables, especially rich in folic acid, reduce the risk of coronary heart disease by preventing the increase in homocysteine levels in the blood (TÜBER, 2015).

A diet rich in antioxidant vitamins A, C and E significantly reduces serum lipid peroxide levels in postmenopausal women. For this reason, it is recommended that women in the postmenopausal period increase their intake of antioxidants such as vitamin A, beta carotene, vitamin C, vitamin E, plant flavonoids and soy isoflavones. These nutrients are generally found in fruits, vegetables, soybean, cocoa and tea leaf extracts (Ko & Kim, 2020).

Adequate intake of calcium in all age groups reduces bone mineral loss and maintains bone health. For this reason, with increasing age in women, consumption of foods with high calcium content such as milk, yogurt, cheese, green leafy vegetables should be increased for bone health. The daily calcium requirement in women is determined as 950-1000 mg/day for 19-50 years old and 950 mg/day for 50 years old and later according to Turkish Nutrition Guidelines. It is recommended to consume 3 portions of milk and products, which are sources of calcium, in the menopausal and postmenopausal periods (TÜBER, 2015).

According to the Nurses' Health Study evaluation, women with the highest dietary intake of vitamin D (528 IU/day) had a 17% lower rate of early menopause than those with the lowest (128 IU/day), and women with the highest dietary calcium intake (1246 IU/day). mg/day) compared to those with the lowest consumption (556 mg/day), it was determined that the rate of entering early menopause was 13% lower (Özcan & Oskay, 2013). In a study conducted with postmenopausal

women in Korea, it was stated that vitamin D concentration should be measured regularly to maintain the optimum vitamin D level, and if the level is insufficient, individuals should be made aware of the issue and additional supplementation should be given (Lee et al., 2019).

Water and Salt Consumption

Adequate fluid intake is important for all age groups to maintain normal kidney functions, prevent urinary tract infections, kidney stones and constipation. It is very important to ensure adequate fluid consumption in the menopausal and postmenopausal periods, since the sense of thirst decreases with age. With menopause, there is a decrease in total body water as a result of frequent urination and less fluid retention in the body. For daily fluid requirement, at least two liters of water (8-10 glasses of water) should be consumed per day (TÜBER, 2015).

Since the risk of osteoporosis increases with menopause, hard water with high mineral content should be preferred. In terms of bone and dental health, it is recommended that the fluoride content of drinking and potable water should be at the level of 0.7-1.2 milligrams per liter (Akdağ et al., 2022).

In a randomized controlled study to investigate the effectiveness of increasing daily water consumption in premenopausal women on the frequency of recurrent cystitis, 140 participants were divided into a control group consuming a normal amount of water and an intervention group consuming an extra 1.5 liters of water per day. As a result of the research, it was stated that increasing water intake is beneficial in reducing the risk of recurrent cystitis in women who consume little water and have a history of frequently recurring cystitis (Hooton et al., 2018).

Salt is an essential mineral source that plays a role in the body's fluid-electrolyte balance with its sodium content. It is stated that excessive salt consumption increases urinary sodium and calcium excretion, decreases bone mineral density, and therefore increases the risk of osteoporosis. It is stated that adequate dietary calcium intake is effective in preventing osteoporosis, and the recommended amount of salt consumption affects this situation positively (Yılmaz Taşkın, Demirel, & Kumsar Karakoç, 2015). The World Health Organization recommends that adults consume 5 grams of salt per day (WHO, 2012). Excessive salt consumption during menopause is associated with many diseases, especially hypertension and osteoporosis (Öztürk & Garipağaoğlu, 2018). However, in the results of the Salt Consumption and Hypertension Community Surveys SALTURK 1 and SALTURK 2, it was stated that the average salt consumption in Turkey is 18 g/day and 15 g/day. With these studies, it has been determined that the Turkish population consumes much more salt than recommended, and it has been emphasized that applications to reduce salt consumption should be given priority (Erdem et al., 2017; Erdem et al., 2010).

Conclusion and Recommendations

As in every period of life, a healthy diet, protection and maintenance of health in the menopause and postmenopausal period are of great importance in reducing the risks of diseases that may be caused by menopause. During the climacteric period, the nutritional needs of women differ from previous years. In this context, the energy, macro and micro nutrient needs of the individual should be provided with individually planned nutrition programs. Individual nutritional recommendations should be made, taking into account the symptoms experienced by women in this period, hormonal changes and their needs specific to the menopause period.

REFERENCES

Akdağ, S., Kaner, G., & Ayer, Ç. (2022). Menopoz Döneminde Beslenmenin Yönetimi. İzmir Katip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi, 7(1), 191-197.

Bajerska, J., Chmurzynska, A., Muzsik, A., Krzyżanowska, P., Mądry, E., Malinowska, A. M., & Walkowiak, J. (2018). Weight loss and metabolic health effects from energy-restricted Mediterranean and Central-European diets in postmenopausal women: A randomized controlled trial. Scientific reports, 8(1), 1-11.

Çelik Sis, A., & Pasinlioglu, T. (2013). Klimakterik dönemde yaşanan semptomlar ve hemşirenin rolü. ERÜ Sağlık Bilimleri Fakültesi Dergisi, 1(1), 50-56.

Dunneram, Y., Greenwood, D. C., Burley, V. J., & Cade, J. E. (2018). Dietary intake and age at natural menopause: results from the UK Women's Cohort Study. J Epidemiol Community Health, 72(8), 733-740.

Erdem, Y., Akpolat, T., Derici, Ü., Şengül, Ş., Ertürk, Ş., Ulusoy, Ş., . . . Arıcı, M. (2017). Dietary sources of high sodium intake in Turkey: SALTURK II. Nutrients, 9(9), 933.

Erdem, Y., Arici, M., Altun, B., Turgan, C., Sindel, S., Erbay, B., . . . Caglar, S. (2010). The relationship between hypertension and salt intake in Turkish population: SALTURK study. Blood pressure, 19(5), 313-318.

Görgel, E. B., & Çakıroğlu, F. P. (2007). Menopoz Döneminde Kadin. In: Ankara.

Guthrie, J., Dennerstein, L., & Dudley, E. (1999). Weight gain and the menopause: a 5-year prospective study. Climacteric, 2(3), 205-211.

Hooton, T. M., Vecchio, M., Iroz, A., Tack, I., Dornic, Q., Seksek, I., & Lotan, Y. (2018). Effect of increased daily water intake in premenopausal women with recurrent urinary tract infections: a randomized clinical trial. JAMA internal medicine, 178(11), 1509-1515.

Ko, S.-H., & Kim, H.-S. (2020). Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women. Nutrients, 12(1), 202.

Kurt, G., & Arslan, H. (2020). Kadınların menopoz döneminde yaşadıkları sağlık sorunları ve baş etme yöntemleri. Cukurova Medical Journal, 45(3), 910-920.

Lee, C. J., Kim, S. S., Suh, W. Y., Kim, J. S., Jung, J. G., Yoon, S. J., . . . Yang, H. J. (2019). The Effect of Education and Vitamin D Supplementation on the Achievement of Optimal Vitamin D Level in Korean Postmenopausal Women. Journal of bone metabolism, 26(3), 193-199.

Members, E. P., Jensen, M. D., Ryan, D. H., Donato, K. A., Apovian, C. M., Ard, J. D., . . . Jakicic, J. M. (2014). Executive summary: guidelines (2013) for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Obesity Society published by the Obesity Society and American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Based on a systematic review from the The Obesity Expert Panel, 2013. Obesity, 22(S2), S5-S39.

T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü Sağlıklı Beslenme ve Hareketli Hayat Dairesi Başkanlığı. Menopoz Döneminde Beslenme, 2017 [cited 2021 Apr 2].Available from:https://hsgm.saglik.gov.tr/tr/beslenme/menapoz-doneminde-beslenme.html

Özcan, H., & Oskay, Ü. (2013). Menopoz döneminde semptom yönetiminde kanıta dayalı uygulamalar. Göztepe Tıp Dergisi, 28(4), 157-163.

Özdemir, O., & Çöl, M. (2004). The age at menopause and associated factors at the health center area in Ankara, Turkey. Maturitas, 49(3), 211-219.

Öztürk, R. İ., & Garipağaoğlu, M. (2018). Tuz tüketimi ve sağlık. Türkiye Klinikleri Sağlık Bilimleri Dergisi.

Pekcan, A., Şanlıer, N., Baş, M., Başoğlu, S., & Acar Tek, N. (2016). TC Sağlık Bakanlığı Türkiye Halk Sağlığı Kurumu, Obezite, Diyabet ve Metabolik Hastalıklar Daire Başkanlığı.

Rossato, L. T., Nahas, P. C., de Branco, F. M., Martins, F. M., Souza, A. P., Carneiro, M. A., . . . De Oliveira, E. P. (2017). Higher protein intake does not improve lean mass gain when compared with RDA recommendation in postmenopausal women following resistance exercise protocol: a randomized clinical trial. Nutrients, 9(9), 1007.

Rostami Dovom, M., Moslehi, N., Mirmiran, P., Azizi, F., & Ramezani Tehrani, F. (2019). Habitual dietary lactose and galactose intakes in association with age at menopause in non-galactosemic women. PloS one, 14(3), e0214067.

Sapre, S., & Thakur, R. (2014). Lifestyle and dietary factors determine age at natural menopause. Journal of mid-life health, 5(1), 3.

Smith, G. I., Yoshino, J., Kelly, S. C., Reeds, D. N., Okunade, A., Patterson, B. W., . . . Mittendorfer, B. (2016). High-protein intake during weight loss therapy eliminates the weight-loss-induced improvement in insulin action in obese postmenopausal women. Cell reports, 17(3), 849-861.

Tardivo, A. P., Nahas-Neto, J., Orsatti, C. L., Dias, F., Poloni, P., Schmitt, E., & Nahas, E. A. (2015). Effects of omega-3 on metabolic markers in postmenopausal women with metabolic syndrome. Climacteric, 18(2), 290-298.

Toplar, K. B., Kaner, G., & Ayer, Ç. (2022). Sarkopenide Beslenmenin Rolü. İzmir Katip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi, 7(2), 441-445.

TUIK. (2019). İstatistiklerle kadın. Retrieved from https://data.tuik.gov.tr/Bulten/Index?p=IstatistiklerleKadin-2019-33732. Retrieved 26.08.2022 https://data.tuik.gov.tr/Bulten/Index?p=IstatistiklerleKadin-2019-33732

TUIK. (2020). İstatistiklerle Kadın. Retrieved from https://data.tuik.gov.tr/Bulten/Index?p=IstatistiklerleKadin-2020-37221. Retrieved 26.08.2022 https://data.tuik.gov.tr/Bulten/Index?p=IstatistiklerleKadin-2020-37221

TÜBER. (2015). Türkiye Beslenme Rehberi. Ankara: T.C. Sağlık Bakanlığı Yayın No: 1031 Retrieved from https://dosyasb.saglik.gov.tr/

Wang, Y., Dellatore, P., Douard, V., Qin, L., Watford, M., Ferraris, R. P., . . . Shapses, S. A. (2016). High fat diet enriched with saturated, but not monounsaturated fatty acids adversely affects femur, and both diets increase calcium absorption in older female mice. Nutrition research, 36(7), 742-750.

WHO, I. (2012). Guideline: sodium intake for adults and children. World Health Organization, Geneva, Switzerland.

Yabancı, N., Akdevelioğlu, Y., & Rakıcıoğlu, N. (2012). Yaşlı bireylerin sağlık ve beslenme durumlarının değerlendirilmesi. Beslenme ve Diyet Dergisi, 40(2), 128-135.

Yılmaz Taşkın, F., Demirel, G., & Kumsar Karakoç, A. (2015). Tuz Tüketimi, Kemik Sağlığı Ve Osteoporoz. ERÜ Sağlık Bilimleri Fakültesi Dergisi, 3(1), 67-76.

A Current Practice in Dentistry; Endocrowns

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Introduction

Post-supported basic restorations and crowns are most commonly used in the restoration of teeth with root canal treatment and excessive tissue loss (Dietschi & et al., 2008; Zarow, Devoto & Saracinelli, 2009; Ma & et al., 2009). Despite the fact that some clinical success was achieved with the use of intracanal posts, the disadvantages of this are: it causes loss of healthy tissue in the tooth, requires additional processing and time, and causes fractures in the tooth root (Lazari & et al., 2013). Pissis proposed a new technique in which a pulp chamber was used for the retention of crowns without the need for posts. Bindl and Mormann defined "endocrowns" as adhesive crowns for the purpose of restoring root canal-treated posterior teeth that have complete coronal hard tissue loss (Bindl & Mormann, 1999).

Thanks to developments in advehise dentistry, restoration of teeth that have undergone treatment (such as root canal treatment) can be done more conservatively by using endocrowns. Endocrowns combine the crown, core, and intraradicular post in a single component; therefore, they represent monoblock restorations. Endocrown restorations, unlike traditional approaches such as using intraradicular posts, are fixed to the interior of the pulp chamber and to the cavity margins. This results in a micro and macro-mechanical retention, as provided by the adhesive cementation as well as pulp walls (Cunha & et al., 2017).

Developments in computer-aided design and manufacturing (CAD/CAM) have provided more accurate, aesthetically pleasing, and less time-intensive restorative procedures for both material processing and tooth restoration. The different materials that are made available with this technology give clinicians the opportunity to choose the most suitable materials for them.6 In the literature, for glass ceramics that are reinforced with lithium disilicate or leucite, it has been found that feldspathic glass ceramics and resin composites are the best choices for endocrown production, as they show higher flexural strength and can withstand occlusal forces during chewing (Otto, 2004; Biacchi & Basting, 2012; Bindl, Richter & Mörmann, 2005; Zhu & et al., 2017). Resin-based CAD/CAM blocks developed in recent years constitute an important alternative to traditional ceramic blocks. The mechanical properties of resin-based materials and their abrasive behavior on enamel provide significant advantages over glass ceramics, which cause severe abrasion on the enamel of fragile and opposing teeth (Lin & et al., 2010). This review's purpose is to examine the pros and cons of endocrown restorations and to evaluate current materials and studies.

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Endocrowns

Alıntılar metin içinde ve parantez kullanılarak verilmelidir. Metin içi atıflar tek yazarlı eserlerde (Anaz, 2014), iki yazarlı eserlerde (Bekir & Ulaş, 2003), üç yazarlı eserlerde (Kul, Faruk & Deniz, 2016), üçten fazla yazarlı eserlerde ise (Gerekvar & ark., 2018) şeklinde olmalıdır. Bir yazarın aynı yıl içerisinde yayınlanan eserlerine atıf yapılırken (Yılmaz, 2014a, 2014b) şeklinde verilmelidir.

Deciding on the indications for root canal-treated teeth with excessive dental tissue loss is difficult, as there are many restorative materials and restoration options. It has been stated that the main reason for the extraction of these teeth is prosthetic, periodontal, and endodontic failures. With advances in dentistry, a more conservative approach is recommended to restore root canal-treated teeth using endocrowns. (Figure 1A) (Olcay, Ataoglu & Belli, 2018).

Endocrown restorations can be applied to all teeth on the arch, and they are generally a better recommendation for molars. Premolars and anterior teeth, meanwhile, have a small pulp chamber and are subject to lateral forces of larger magnitudes (Sedrez-Porto & et al., 2016; Rayyan & et al., 2019; Al-Dabbagh, 2020). Endocrowns are also indicated in cases of excessive coronal tissue loss and limited interocclusal space when teeth have short, obliterated, dilated, or curved roots (Biacchi, Mello & Basting, 2013). The performance of endocrowns is affected by many factors, including material types, loading axis, and preparation patterns. (El Ghoul & et al., 2019; Altier & et al., 2018; Taha & et al., 2018).

Advantages of endocrowns

- Endocrowns are a conservative technique.
- They require a much shorter session time.
- The tooth/restoration interface of the restored teeth is better at evenly distributing chewing forces across the tooth structure.
- It can be applied to teeth with curved roots and calcified canals.
- This can be applied in cases where the interocclusal distance is insufficient.
- Healthy tooth tissue is preserved.

Disadvantages of endocrowns

- Endocrowns are unsuccessful in the following cases; if adhesion is not achieved or the depth of the pulp chamber is measured below 3 mm, the thickness of the peripheral walls must be thicker than 2 mm.
- Endocrowns can be contraindicated if there is improper occlusion (parafunction).
- In tooth preparation, a 1.5-2 mm ferrule should be made to prevent fractures in the tooth (Singh & et al; 2019).

Classification of endocrown restorations

The classification of endocrown restorations is based on the amount of tooth tissue that remains after preparation.

1. A tooth preparation with a minimum of two solid walls is described.

2. A tooth preparation is described in which a maximum wall has a height of

>1/2 of its original measured height.

3. A tooth preparation is described in which all of the walls are <1/2 of their original height (Belleflamme & et al., 2017).

Construction Stages of Endocrown

Cavity Preparation

The purpose of this preparation is to obtain a large, stable surface that resists the compressive stresses known to occur in molars (Zogheib & et al., 2011). The surface was prepared parallel to the occlusal plane. This provides resistance to stress along the major axis of the tooth (Fages & Bennasar, 2013). As a result, lower stress levels are achieved compared to teeth with prosthetic crowns (Dejak & Miotkowski, 2013).

Thanks to developments in adhesive cementation systems, the need for macroretentive preparation has decreased for cowns (Lander & Dietschi, 2008). The pulp chamber space also provides stability and retention. The trapezoidal shape of the mandibular molars, as well as the triangular shape of the maxillary molars, help increase the stability of the restoration. In addition, additional preparation isn't required. The saddle form of the pulp base helps increase stability. Due to the adhesive properties of bonding materials, this anatomy makes the use of root canals redundant (Figure 1B).

The quality of the endodontic treatment was evaluated from radiography before starting the endocrown preparation. Before dehydration of the teeth, the color of the material to be used was selected. The existing restorations were removed. Attention is paid to the width of the coronary chamber and the amount of enamel remaining on the edges. It is used with diamond burs to provide sufficient space (about 1.5 mm) for restoration. A diamond bur was used to obtain the proper surface on the mesial and distal walls of the tooth and to remove unsupported enamel. The cavity was finished with the same diamond burs used during the low-speed rotating preparation. All interior walls are rounded to facilitate impression-taking, restoration placement, and removal (Cunha & et al., 2017).

As deep subgingival carious lesions and restorations deepen, the gingival marginal enamel keeps thinning down until it reaches the cement-enamel junction (CEJ), and at this point, bonding to the enamel is impossible. Margins that are placed apically to the cement-enamel junction on dentin are known to be more prone to microleakage caused by differences in thermal expansion between the tooth structure and restorative material and their respective thermal expansion coefficients. Incomplete hybridization between the adhesive system and collagen fibrils is also a cause of microleakage (Stockton & Tsang, 2007).

In cavities containing subgingival margin, a technique known as deep margin elevation, proximal box elevation, or relocating cervical margin, also known as opensandwich technique is used. This technique utilizes a direct restoration approach, placed solely in the deep apical portion, during preparation to raise the cavity margin to a more favorable and coronal position for the final part of restoration fabrication and cementation. The oral environment is exposed to direct restoration by deep margin elevation. This extra interface of direct restoration can potentially leak, and there are some concerns that it may be associated with the increased failure rate with this technique (Frankenberger & et al., 2013).

Vertolli et al. showed that deep margin elevation results in a reduction in the fracture of ceramic restorations when the cavity margins extend below the cement–enamel junction. Marginal integrity has been preserved in restorations, both direct and indirect, by using the deep margin elevation technique. This suggests that deep margin elevation may be a viable treatment option, as restorations are made difficult by subgingival margins. No difference was found between the materials (resin-based composite, glass ionomer, resin-modified glass ionomer, and bulk-fill composite) used for proximal box elevation (PBE) in terms of margin quality or fracture resistance.

It is stated in the literature that any of the resin-modified glass ionomer, composite resin, or glass ionomer materials may be suitable for PBE procedures (Vertolli & et al., 2020).

Material Selection

In studies on endocrowns, nanoceramic resin, reinforced composite resin, lithium disilicate glass-ceramic, zirconium reinforced lithium silicate, feldspathic ceramic, and hybrid ceramic blocks were used. For the purposes of endocrown restorations, the most widely used material is lithium disilicate glass-ceramic (LDS), as its biomechanical properties fit. (Kuijper & et al., 2020; Zhu & et al., 2020; Einhorn & et al., 2019).

CAD-CAM materials used in endocrown restorations: Vita Suprinity (VS; VITA Zahnfabrik, Germany), IPS e.max CAD (EMX; Ivoclar Vivadent AG), Vita Enamic (VE; VITA Zahnfabrik), Grandio blocks (GR; VOCO), and Lava Ultimate (LU; 3M ESPE) were investigated using Finite Element Analysis (FEA) and Weibull Analysis (Hidaka & et al., 1999; Gonzaga & et al., 2011). As a result of these analyses, the LU and GR models showed a more balanced distribution of stress. Weibull analysis has also revealed that the 5 models performed similarly while under normal occlusal forces. During clenching, the VE and LE models achieved the highest chances. The fracture strength of GR is significantly higher than that of other materials (Zheng & et al., 2021).

In recent years, continued research on biocompatible materials that have mechanical and physical properties that are similar to those of natural dental tissues has produced a new generation of resin-based ceramic restorative materials. The hybrid nature of resin-based ceramic blocks provides a measure of elastic modulus (12.8 Gpa) that is similar to dentin. The advantages of resin-based ceramic restorations are that they show better fracture resistance and less crack propagation than some CAD/CAM ceramics (El-Damanhoury, Haj-Ali & Platt, 2014; Chen & et al., 2014; Magne & et al., 2010). Today, CAD/CAM blocks that are resin-based with different contents (Vita Enamic, Lava Ultimate, Brilliant Crios, Cerasmart, Shofu Block, and Grandio Block) are available to physicians.

Cementation

Restoration prepared using different dental design programs was produced with the help of milling devices (Figure 1C). The rod area of the restoration produced in the digital system is cut with the help of a diamond bur and removed. The endocrown is then placed in the patient's mouth to assess interproximal contact and marginal integrity. Occlusion control of the restoration was performed. Corrects sections that prevent occlusion restoration are repositioned and marked. When evenly distributed occlusal contacts are observed, polishing is performed with a diamond-containing tire rotating at low pressure and low speed. Ceramic blocks reinforced with glass ceramic, lithium silicate, and zirconia are sintered according to the instructions of the manufacturer (except resin-based CAD/CAM block). Pigments and glaze materials are used to characterize and polish the restoration. Resin-based blocks are only polished with a rubber diamond polisher.

Before cementation of the endocrown, the cavity and adjacent teeth were isolated. Restoration surfaces produced from ceramic-containing blocks are roughened for 20 seconds with hydrofluoric acid and then rinsed for 20 seconds before leaving them to dry. The inner surface of the block, which is produced from resin-based blocks, is roughened by sandblasting. Adjacent teeth are protected by polyester tape; enamel is roughened with phosphoric acid to increase retention and washed.

Dual-cure resin cement was prepared according to the instructions of the manufacturer and applied to the tooth surface. The restoration was then placed on the prepared tooth surface. Excess cement on the proximal surfaces was removed with dental floss and with an explorer on lingual as well as buccal surfaces and polymerized (Cunha & et al., 2017) (Figure 1D).

In vitro studies on endocrown restorations

Many studies have compared the mechanical and physical properties of endocrowns. When Chang et al. compared the failure modes and fracture resistance of endocrowns to conventional post-core supported crowns in maxillary premolars, they found that the endocrowns showed higher fracture resistance compared to conventional crowns; however, between the two groups, there was no significant difference in terms of failure modes (Chang & et al., 2009).

El-Damanhoury et al., in an in vitro study in which they examined the fracture resistance and marginal leakage concerning ceramic endocrowns produced using 3 different CAD/CAM made of lithium disilicate, resin nano-ceramic, and feldspathic porcelain on maxillary molars. It has been shown that resin nanoceramic endocrowns have noticeably greater fracture resistance (El-Damanhoury, Haj-Ali & Platt, 2014).

An in vitro study concerning mandibular molars found that when the fracture strength with respect to ceramic endocrowns strengthened with lithium disilicate and traditional crowns supported by glass fiber posts was examined compared to conventional crowns, endocrowns showed higher fracture strength. Furthermore, the failure pattern for both groups was similar. In addition, it is characterized by tooth fracture related to restoration displacement (Biacchi & Basting, 2012).

A study conducted on adhesive restorations of endodontically treated anterior teeth found that failure modes and fracture resistance concerning endodontically treated upper incisors that had been restored with endocrowns made of ceramic or composite blocks were examined. No significant difference was observed (Ramirez-Sebastia & et al., 2014).

Taha et al. Conducted an in vitro study to evaluate the effect that occurs when changing the margin designs concerning endodontically treated teeth that have been restored using polymer-infiltrated ceramic by the method of endocrown restorations on fracture resistance; it has been observed that endocrowns that have shoulder finish and axial reduction lines have greater fracture resistance (Taha & et al., 2018).

In the literature, studies evaluating the biomechanical behavior of teeth that have been endodontically treated and restored by utilizing different endocrown extensions in the pulp chamber have concluded that greater elongation of the endocrowns results in greater mechanical performance (Dartora & et al., 2018; Silva-Sousa & et al., 2017).

Altier et al. reviewed the fracture resistance of three endocrowns to compare them. They were made of two different indirect resin composites and lithium disilicate ceramics. Finally, they determined that, compared to the indirect composite groups, lithium disilicate ceramic endocrowns showed a higher fracture strength. (Altier & et al., 2018).

A study by Darwish et al. exhibited that endodontically treated maxillary premolars that were restored using resin nanoceramic endocrowns exhibited superior internal adaptation when compared with endocrowns that were restored using lithium disilicat. Finally, the endocrown preparation with smaller axial wall divergence ("6" grade) provided a better internal fit (Darwish, Morsi & El Dimeery, 2017).

In the study by Anton et al. fifty extracted mandibular molars were used; it has been reported that the fatigue resistance concerning endodontically treated molars restored using fiber-reinforced endocrown restorations does not increase, and the potential effect of fiber supports may be reduced due to the high thickness of the restorations. On the other hand, it has been observed that fiber reinforcement positively affects the failure mode and crack propagation after fatigue failure, thus increasing the chance of repairability (Anton & et al., 2021).

In vivo studies on endocrown restorations

Sedrez-Porto et al. evaluated clinical trials. They compared endocrown restorations to conventional treatments while utilizing direct composite resin, intraradicular posts, or onlay and inlay restorations. Endocrowns have been reported to be an improvement over conventional methods, and in the worst cases performed similarly (Sedrez-Porto & et al., 2016).

Bindl and Mörmann have also reported that 19 adhesive-bonded CEREC endocrowns (15 molars and 4 premolars) operated clinically successfully for 28 months, while only one of the endocrowns failed due to recurrent caries (Bindl & Mormann, 1999).

Belleflamme et al. reported that despite the presence of occlusal risk factors, such as extensive coronal tissue loss, adverse occlusal relationships, or bruxism, endocrowns can still be reliable as an approach to restoring severe cases of damaged premolars and molars (Belleflamme & et al., 2017).

Otto et al. concluded that the success rates of feldspathic endocrowns in premolars and molars were 75 and 90.5, respectively 75%, and 90.5% over a 12-year follow-up. In another clinical study, endocrowns showed a clinical success rate ranging from 94% to 100% in 3 years, while the 10-year success rate was 98.8% (Otto & Mörmann, 2015).

Conclusion

Endocrowns have superior fracture strength compared to teeth restored with post-core retaining crowns. Therefore, it represents a promising treatment alternative for root canal-treated molars, preserves the structure of the tooth, and is suitable for minimally invasive dentistry.

Four essential factors explain the long-lasting and successful effects of endocrowns: correct selection of restorative materials as well as correct preparations of the tooth. In addition, the location of the restoration margin and the correct case selection are important factors.

Although feldspathic and lithium disilicate ceramic blocks were initially used in the treatment of endocrown restorations, resin-containing blocks have recently been used for endocrown restorations.

Despite the gains in popularity of endocrowns, it is doubtful whether this restoration method could replace conventional restorative treatments that utilize the post-core in teeth treated with the anterior root canal. To the extent of our knowledge, laboratory and clinical evidence proving the safety of endocrowns for use in anterior teeth is lacking. Therefore, more evidence is needed, supported by long-term clinical trials.

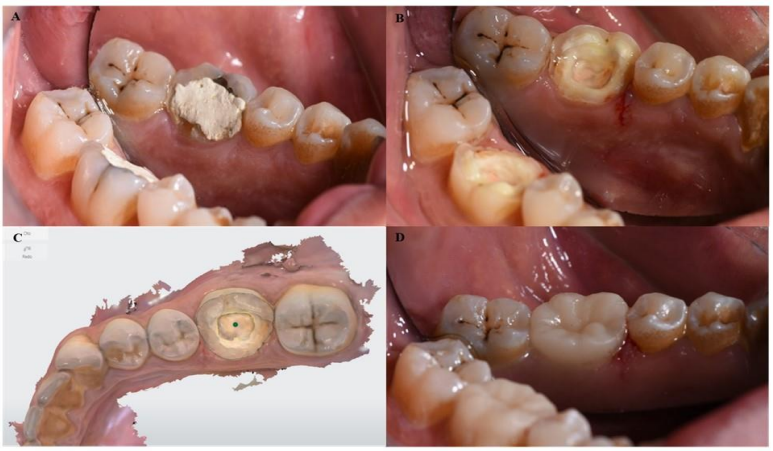

Figure 1A. Intraoral view of tooth before treatment, **B.** Cavity preparation of tooth **C.** Intraoral scanning of the cavity, **D.** Intraoral view of the completed restoration

REFERENCES

1. Dietschi, D., Duc, O., Krejci, I., Sadan, A. (2008). Biomechanical considerations for the restoration of endodontically treated teeth: a systematic review of the literature, Part II (Evaluation of fatigue behavior, interfaces, and in vivo studies). *Quintessence Int.*; 39:117-129.

2. Zarow, M., Devoto, W., Saracinelli, M. (2009). Reconstruction of endodontically treated posterior teeth- with or without post? Guidelines for the dental practitioner. *Eur J Esthet Dent.*, 4:312-127.

3. Ma, O.S., Nicholls, J.I., Junge, T., Phillips, K.M. (2009). Load fatigue of teeth with different ferrule lengths, restored with fiber posts, composite resin cores, and allceramic crowns. *J Prosthet Dent.* 102:229-234.

4. Lazari, P.C., Oliveira, R.C., Anchieta, R.B., Almeida, E.O., Junior A.C.F., Kina, S., Rocha, E.P. (2013). Stress distribution on dentin-cement-post interface varying root canal and glass fiber post diameters: a three-dimensional finite element analysis based on micro-CT data. *J Appl Oral Sci.* 21:511–517.

5. Bindl, A., Mormann, W.H. (1999). Clinical evaluation of adhesively placed Cerec endo-crowns after 2 years-preliminary results. *J Adhes Dent.* 1:255-265.

6. da Cunha, L.F., Gonzaga, C.C., Pissaia, J.F., Correr, G.M. (2017). Lithium silicate endocrown fabricated with a CAD–CAM system: a functional and esthetic protocol. *J Prosthet Dent.* 118:131-134.

7. Otto, T. (2004). Computer-aided direct all-ceramic crowns: preliminary 1-year results of a prospective clinical study. *Int J Periodontics Restorative Dent.* 24:446-455.

8. Biacchi, G.R., Basting, R.T. (2012). Comparison of fracture strength of endocrowns and glass fiber post-retained conventional crowns. *Oper Dent.* 37:130-136.

9. Bindl, A., Richter, B., Mörmann, W.H. (2005). Survival of ceramic computer-aided design/manufacturing crowns bonded to preparations with reduced macroretention geometry. *Int J Prosthodont.* 18:219-224.

10. Zhu, J., Rong, Q., Wang, X., Gao, X. (2017). Influence of remaining tooth structure and restorative material type on stress distribution in endodontically treated maxillary premolars: a finite element analysis. *J Prosthet Dent.* 117:646-655.

11. Lin, C.L., Chang, Y.H., Chang, C.Y., Pai, C.A., Huang, S.F. (2010). Finite element and Weibull analyses to estimate failure risks in the ceramic endocrown and classical crown for endodontically treated maxillary premolar. *Eur J Oral Sci.* 118:87–93.

12. Olcay, K., Ataoglu, H., Belli, S. (2018). Evaluation of related factors in the failure of endodontically treated teeth: a crosssectional study. *J Endod.* 44: 38–45.

13. Sedrez-Porto, J.A., Rosa, W.L., da Silva, A.F., Münchow E.A., Pereira-Cenci, T. (2016). Endocrown restorations: a systematic review and meta-analysis. *J Dent.* 52:8–14.

14. Rayyan, M.R., Alauti, R.Y., Abanmy, M.A., AlReshaid R.M., Ahmad, H.A.B. (2019). Endocrowns versus post-core retained crowns for restoration of compromised mandibular molars: an in vitro study. *Int J Comput Dent.* 22(1):39–44.

15. Al-Dabbagh, R.A. (2020). Survival and success of endocrowns: a systematic review and meta-analysis. *J Prosthet Dent.* 125(3):415-424.

16. Biacchi, G.R., Mello, B., Basting, R.T. (2013). The endocrown: an alternative approach for restoring extensively damaged molars. *J Esthet Restor Dent.* 25:383-390.

17. El Ghoul, W., Ozcan, M., Silwadi, M., Salameh, Z. (2019). Fracture resistance and failure modes of endocrowns manufactured with different CAD/CAM materials under axial and lateral loading. *J Esthet Restor Dent.* 31:378–387.

18. Altier, M., Erol, F., Yildirim, G., Dalkilic, E.E. (2018). Fracture resistance and failure modes of lithium disilicate or composite endocrowns. *Niger J Clin Pract.* 21(7): 821–826.

19. Taha, D., Spintzyk, S., Sabet, A., Wahsh, M., Salah, T. (2018). Assessment of marginal adaptation and fracture resistance of endocrown restorations utilizing different machinable blocks subjected to thermomechanical aging. *J Esthet Restor Dent.* 30(4):319–328.

20. Singh, A., Abrol, K., Agarwal, S., Madan, R. (2019). Endocrown Restorations: A Review. *Chronicles of Dental Research.* 8(2).

21. Belleflamme, M.M., Geerts, S.O., Louwette, M.M., Grenade, C.F., Vanheusden, A.J., Mainjot, A.K. (2017). No post-no core approach to restore severely damaged posterior teeth: an up to 10-year retrospective study of documented endocrown cases. J Dent. 63:1-7.

22. Zogheib, L.V., Siqueira Ferreira Anzaloni Saavedra G., Cardoso, P.E, Valera, M.C, Araújo, M.A.M. (2011). Resistance to compression of weakened roots subjected to different root reconstruction protocols. J Appl Oral Sci. 19(6):648–654.

23. Fages, M., Bennasar, B. (2013). The endocrown: a different type of all-ceramic reconstruction for molars. *J Can Dent Assoc.* 79:d140.

24. Dejak, B., Młotkowski, A. (2013). "3D-finite element analysis of molars restored with endocrowns and posts during masticatory simulation." *Dent Mater.* 29(12): 309–317.

25. Lander, E., Dietschi, D. (2008). Endocrowns: a clinical report. *Quintessence Int.* 39(2):99–106.

26. Stockton, L.W., Tsang, S.T. (2007). Microleakage of class II posterior composite restorations with gingival margins placed entirely within dentin. *J Can Dent Assoc.* 73(3):255.

27. Frankenberger, R., Hehn, J., Hajto, J., Kramer, N., Naumann, M., Koch, A., & Roggendorf, M.J. (2013). Effect of proximal box elevation with resin composite on marginal quality of ceramic inlays in vitro. *Clin Oral Investig.* 17(1): 177-183.

28. Vertolli, T.J., Martinsen, B.D., Hanson, C.M., Howard, R.S., Kooistra, S., Ye, L. (2020). Effect of Deep Margin Elevation on CAD/CAM-Fabricated Ceramic Inlays. *Oper Dent.* 45 (6): 608–617.

29. de Kuijper, M.C.F.M, Cune, M.S., Tromp, Y., Gresnigt M.M.M. (2020). Cyclic loading and load to failure of lithium disilicate endocrowns: Influence of the restoration extension in the pulp chamber and the enamel outline. *J Mech Behav Biomed Mater.* 105.

30. Zhu, J., Wang, D., Rong, Q., Qian, J., Wang, X. (2020). Effect of central retainer shape and abduction angle during preparation of teeth on dentin and cement layer stress distributions in endocrown-restored mandibular molars. *Dent Mater J.* 21.

31. Einhorn, M., DuVall, N., Wajdowicz, M., Brewster, J., Roberts, H. (2019). Preparation ferrule design effect on endocrown failure resistance. *J Prosthodont.* 28(1):237–242.

32. Hidaka, O., Iwasaki, M., Saito, M., Morimoto, T. (1999). Influence of clenching intensity on bite force balance, occlusal contact area, and average bite pressure. *J Dent Res.* 78:1336-44.

33. Gonzaga, C.C., Cesar, P.F., Miranda Jr, W.G., Yoshimura, H.N. (2011). Slow crack growth and reliability of dental ceramics. Dent Mater. 27:394-406.

34. Zheng, Z., He, Y., Ruan, W., Ling, Z., Zheng, C., Gai, Y., Yan, W. (2021). Biomechanical Behavior of Endocrown Restorations with Different CAD-CAM Materials: A 3D Finite Element and in Vitro Analysis. *J Prosthet Dent*. 125(6): 890–899.

35. El-Damanhoury, H., Haj-Ali, R., Platt, J. (2014). Fracture resistance and microleakage of endocrowns utilizing three CAD-CAM blocks. *Oper Dent.* 40:201-210.

36. Chen, C., Trindade, F.Z., de Jager, N., Kleverlaan, C.J., Feilzer, A.J. (2014). The fracture resistance of a CAD/CAM resin nano ceramic (RNC) and a CAD ceramic at different thicknesses. *Dent Mater.* 30: 954-962.

37. Magne, P., Schlichting, L.H., Maia, H.P., Baratieri, L.N. (2010). In vitro fatigue resistance of CAD/CAM composite resin and ceramic posterior occlusal veneers. *J Prosthet Dent.* 104:149-157.

38. Chang, C.Y., Kuo, J.S., Lin, Y.S., Chang, Y.H. (2009). Fracture resistance and failure modes of CEREC endocrowns and conventional post and core-supported CEREC crowns. *J Dent Sci.* 4(3):110-117.

39. Ramirez-Sebastia, A., Bortolotto, T., CattaniLorente, M., Giner, L., Roig, M., Krejci, I. (2014). Adhesive restoration of anterior endodontically treated teeth: Influence of post length on fracture strength. *Clin Oral Investig.* 18(2):545-554.

40. Taha, D., Spintzyk, S., Schille, C., Sabet, A., Wahsh, M., Salah, T., Geis-Gerstorfer, J. (2018). Fracture resistance and failure modes of polymer in filtrated ceramic endocrown restorations with variations in margin design and occlusal thickness. J Prosthodont Res. 62(3):293 –297.

41. Dartora, N.R., de Conto Ferreira, M.B., Moris, I.C.M., Brazao, E.H., Spazin, A.O., Sousa-Neto, M.D., Silva-Sousa, Y.T., Gomes, E.A. (2018). Effect of intracoronal depth of teeth restored with endocrowns on fracture resistance: in vitro and 3-dimensional finite element analysis. *J Endod.* 44(7):1179 –1185.

42. Silva-Sousa, Y., Gomes, E.A., Dartora, N.R., Ferreira, M.C., Moris, I.C., Spazin, A.O., Sousa-neto, M. (2017). Mechanical behavior of endodontically treated teeth with different endocrowns extensions. *Dent Mater.* 33(1):73–74.

43. Darwish, H.A., Morsi, T.S. & El Dimeery, A.G. (2017). Internal fit of lithium disilicate and resin nanoceramic endocrowns with different preparation designs. *Future Dental Journal.* 3(2):67–72.

44. Anton Y Otero, C., Bijelic-Donova, J., Saratti, C.M., Vallittu, P.K., di Bella, E., Rocca, G.T. (2021). The influence of FRC base and bonded CAD/CAM resin composite endocrowns on fatigue behavior of cracked endodontically-treated molars. *J Mech Behav Biomed Mater.* 121:104647.

45. Otto, T. & Mörmann, W.H. (2015). Clinical performance of chairside CAD/CAM feldspathic ceramic posterior shoulder crowns and endocrowns up to 12 years. *Int J Comput Dent.* 18:147-161.

Ocular Delivery Systems and Applications Developed by Qbd Perspective: From the Past to the Future

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1. INTRODUCTION

Eye is one of the most complex organs that converts the light reflected from objects into electrical impulses and transmits them to the brain via the optic nerve. The eye might be investigated in two different perspectives which are anatomical and functional. In context with anatomical. The eye composes two different parts; anterior and posterior segments. While the anterior segment consists of the cornea, anterior chamber, iris, posterior chamber, ciliary body and lens, posterior segment consists of the vitreous, retina, retinal pigment epithelium, and choroid **(Figure 1).**

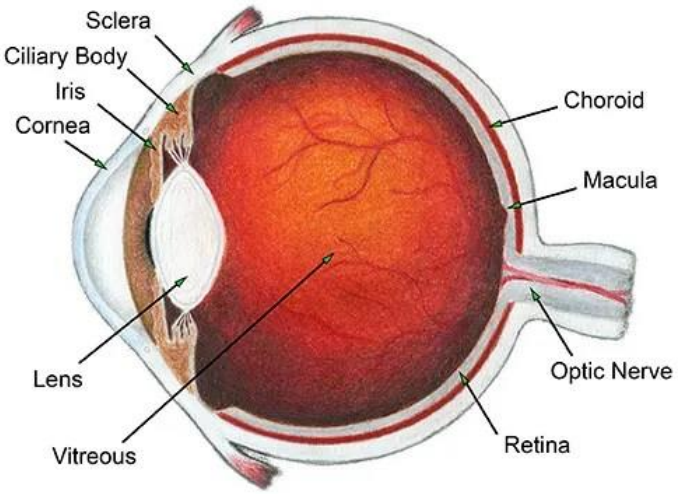

Figure 1. Anatomical investigation of eye division (Pautler, 2021)

The major duty of the components in the anterior segment is permeating the light stimuli to the posterior segment as well as that focus on the macula. The posterior segment, which forms the posterior 2/3 of the eye, includes the vitreous humor, retina, macula, and optic nerves (Gaudana, Jwala, Boddu, & Mitra, 2009; Wadhwa, Paliwal, Paliwal, & Vyas, 2009). In addition, the

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eyeball can be functionally examined in three layers. The outermost protective layer consists of the sclera and cornea. The cornea is a transparent structure with a diameter of 11.7 mm and a thickness of 0.5 - 0.7 mm, which protects the eye against infections, physical damage, and transmits light to the lens and retina by refracting it (Yasukawa et al., 2004). The sclera protects the eye from internal and external forces and ensures that the shape of the eye remains intact is the structure of the uttermost layer of the eye. The middle layer includes the ciliary body, choroid, and iris. The choroid is a highly vascularized structure located among the retina and the sclera. The iris is the structure that plays a role in regulating the amount of light reaching the retina by controlling the pupil opening (DelMonte & Kim, 2011). The retina is the layer that forms the inner layer and contains the basic nerve cells and structures for the sense of sight. And last structure is the ciliary body controls the shape of the lens (Müller, Pels, & Vrensen, 2001).

1.1 Ocular Barriers

The ocular drug delivery process is difficult due to the anatomical and physiological barriers in the eye. In the treatment of superficial diseases of the eye, systemic drug administration is not a preferable option as so the obstacle components such as the blood-aqueous barrier and the blood-retina barrier block desired level of the drug (Figure 2). Hence, for overcoming of this problem, high systemic drug dosage is being applied; unfortunately, this approach causes side effects in patients. For this reason, topical drug administration is frequently preferred in the treatment of superficial diseases of the eye, as well as eye drops constitute more than half of ophthalmic preparations (Singh, Ahmad, & Heming, 2011).

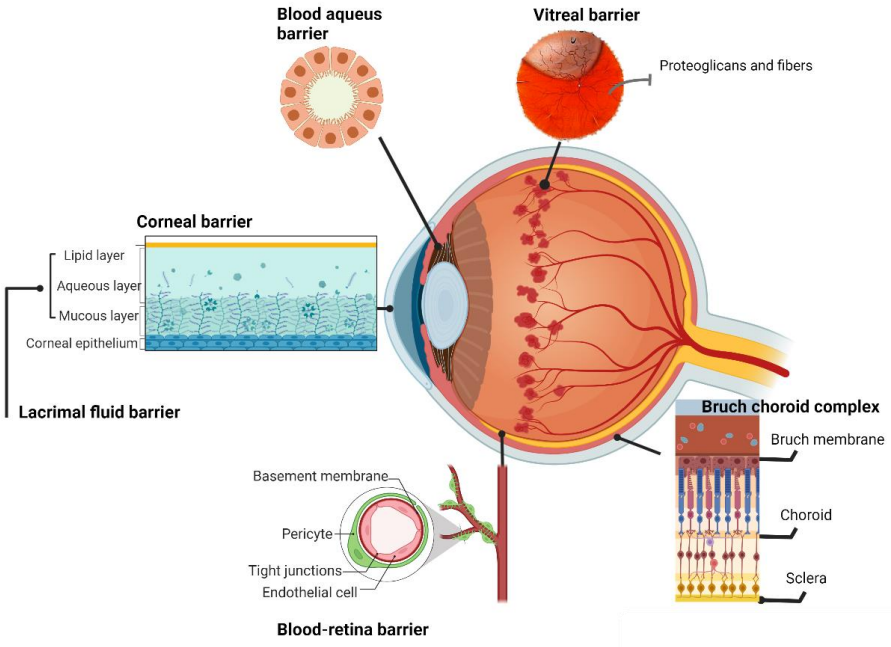

Figure 2: Encountering the barriers on ophthalmic administration

However, low ocular bioavailability (< 5%) is observed with topical administration of drugs to the eye. The reasons for this can be summarized as follows (Kompella, Kadam, & Lee, 2010):

-After the drug is applied to the eye, the drug solution is diluted with lacrimal fluid.

- The volume of lacrimal fluid in the eye is approximately 7 to 10 μL, as well as the volume of each drop applied to the eye is approximately 50 μL. Therefore, approximately 80% of the

amount of drug administered immediately protrudes from the surface of the eye or is removed from the conjunctival tract.

- Topically applied drug continues to dilute with increasing tear, normal tear cycle as well.

- Consequently, regarding other barriers in the eye, the penetration of the drug into the back of the eye is restricted.

Solutions are one of the traditional ophthalmic drug forms that are used ubiquitously forms for the topical application of drugs to the eye. The crucial points regarding the solution's formulation are solubility, ocular toxicity, pKa, the effect of pH, tonicity, buffer capacity, viscosity, compatibility with other ingredients in the formulation, preservatives to be used, comfort in eye application and can be listed as convenience (Gericke et al., 2019; Winter, Anderson, & Braun, 2010).

In order to boost the ocular bioavailability by decreasing the loss of the drug, dozens of strategies (such as increasing viscosity, developing into the ointment, adding a cationic agent, prodrug development, and penetration enhancer addition) had been utilized by the researchers (Gaudana, Ananthula, Parenky, & Mitra, 2010). Unfortunately, these strategies were not very effective at improving bioavailability.

1.2 Novel ocular drug delivery systems

New drug delivery systems are being developed since traditional strategies are not adequately efficient in terms of increasing the accommodation in the precorneal area, preventing it from moving away from this area, and increasing drug penetration through the cornea. These systems can be listed as polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, nanosuspensions, liposomes, micelles, dendrimers, hydrophilic gels, inserts, and ocular minitablets (Hornof, Toropainen, & Urtti, 2005; Irimia et al., 2018; Urtti, 2006)

The pharmaceutical industry is encountered many problems for instance insufficient bioavailability and low water solubility of newly developed drug molecules. Hereby, the requirement is available to occur the action taken promptly for improving the novel drug delivery system that overcomes these disadvantages(Urtti, 2006). These delivery systems should not have acute and chronic toxicity, as well as they should have adequate drug loading capacity, drug targeting, and controlled release properties. Meanwhile, the chemical and physical stability of the drug must be ensured. Size reduction is not one of the best methods only, it is also increasing the solubility of poorly water-soluble agents of biopharmaceutical classification system (BCS) classes II and IV(C. Freitas & Müller, 1999; Fresta, Guccione, Beccari, Furneri, & Puglisi, 2002).

Colloidal systems are drug delivery systems in micron and nanometer sizes, in solid or semi-solid form and show all the advantages of controlled release systems (Yalpani, 1997). In addition, they increase solubility and bioavailability of inferior drugs by acting as preservatives for sensitive drug compounds (Fialho & da Silva-Cunha, 2004). Parameters that need to be controlled to select the most suitable colloidal system are indicated in the **Table 1** (Chrysantha Freitas & Müller, 1998; Müller et al., 2001; Winter et al., 2010).

Table 1: Specifications that affecting on the colloidal systems (Chrysantha Freitas & Müller, 1998; Müller et al., 2001; Winter et al., 2010)

Parameters of colloidal particles				
Electrostatic	**Morphological**	**Solution**	**Optical**	**Quantitative**
Surface charge	Size	Turbidity	Refractive index	Loading capacity
Zeta Potential	Molecular weight	viscosity	Coefficient absorption	Encapsulation efficiency
		stability		

Moreover, regarding the carrier systems: *in-vivo* degradation time, toxicity, biocompatibility with ocular tissues, and excretion time from the body are also important except for the specified parameters in the Table 1.

2. QUALITY BY DESIGN (QBD) – OCULAR APPLICATIONS

Quality by Design (QbD) is a design concept that aims to cover the entire lifecycle of a product, from the design stage to the marketed stage, as well as an initiative initiated by the United States Food and Drug Administration (FDA). QbD is a new paradigm that emphasizes understanding the product and process within the product development process and throughout the product lifecycle. As can be seen from the studies in the literature, the basis of QbD studies is the scientific understanding of the product and the process, hereby statistical experimental designs (Design of Experiments, DoE).

In the literature, reducing the number of experiments and obtaining more useful data. Numerous experimental designs have been described that can provide. If the purpose of an experimental design is to classify many variables as "dependent" and "independent", first-order designs such as Plackett-Burman or Taguchi may be preferred.

However, if the aim is to predict a response variable mathematically (estimation) or to optimize a process, quadratic models such as Box-Behnken, Central Composite, or Optimal designs should be preferred. As seen in the literature examples, the use of appropriate software in the creation is a crucial part of the process as much as selecting the suitable type of the DoE model, and the quality of the data to be obtained from the study as well. By looking at the characteristics given in **Table 1** (dependent), the roadmap used in the selection of the appropriate formulation can be determined. Determining of the levels of independent variables in two-level experimental designs is a critical point. If the gap between the low and high levels is too narrow, a significant factor may be considered meaningless, or on the contrary, if the gap between the two levels is too wide, a nonsignificant factor may be considered significant under normal conditions and may mask the effects of other factors (Ahuja, Ferreira, & Moreira, 2004; Tekade & Chougule, 2013).

Khan et al., investigated the main effects of various adjuvants and process parameters using a seven-factor, twelve-experiment Plackett-Burman design and used X-Stat® software. For this purpose, cumulative drug release was determined as the independent variable, and the lag time and the time when 50% of the total drug was released were considered by constraints. In the quantitative analysis, the most significant factors were determined, and the significance of these factors was tested by analysis of variance (Khan et al., 2000).

Kiss et al. determined the changing active substance (w/w, %), lipid (w/w, %), and surfactant (w/w, %) ratios; investigated their effects on particle size (nm), zeta potential (mV),

loading capacity (%EE) and PDI using two-factor, eight-experimental full factorial design. Consequently, it was observed that the surfactant ratio had a significant effect on both size and EE% ($p<0.05$), it was determined that the lipid ratio did not have a significant effect ($p>0.05$) (Kiss et al., 2020)

The effect of formulation components on the release of besifloxacin- loaded nanostructured lipid carrier was studied using Design-Expert® software (Stat-Ease Inc., USA) with the aid of a Box-Behnken Design by using amount of drug (X_1), lipid:polymer ratio (w/w, %) (X_2) and surfactant concentration (X_3) are independent variants, as well as the particle size (Y_1) and %EE (Y_2) are dependent variants. ANOVA was used to validate the optimization design and correlation of response of 3D plots was generated. As a result of the analysis of variance, it was seen that the factors had significant effects on the response variables ($p<0.05$) (Ferreira et al., 2007).

Fangueiro et al. used Softisan® 100 (w/w, %), Lipoid S75 (soybean phosphatidylcholine) (w/w, %), and Poloxamer 188 (w/w, %) to had a lipid nanoparticle with a water/oil/water structure containing positive potential. In the Taguchi factorial design model performed with CTAB, it has been shown that the lecithin concentration in the formulation (Lipoid S75) is the key factor affecting the nanoparticle size and PDI ($p < 0.05$). The optimized formulation containing CTAB (0.5% of the lipid phase) appeared to be biocompatible in the human retinoblastoma cell line (Fangueiro et al., 2014).

Recently, formulations can be created by using 2 different design models at the same time in order to obtain precise results. Paclitaxel nanoparticles were prepared by Yerlikaya et al. using both Plackett-Burman and Box-Behnken designs. After determining paclitaxel amount (w/w,%), PLGA amount (w/w,%) , PLGA molecular weight, PLGA terminal group, surfactant concentration (w/w,%), surfactant type, homogenization rate (rpm) and homogenization time parameters (min) as potential risk factors, these risk factors effects were specified on the critical quality properties of nanoparticles, which were determined using the Plackett-Burman experimental design. As a result, it was seen that the amount of PLGA (w/w,%), surfactant concentration (w/w,%), and homogenization rate (rpm) of the emulsion affected the particle size, zeta potential and encapsulation efficiency properties of paclitaxel nanoparticles, therefore these factors were critical formulation and process parameters. The formulation and process parameters were investigated in Box-Behnken experimental design after determining critical factors as a result of the Plackett-Burman experimental design.

Fifteen formulations in the Box-Behnken test matrix were prepared and analyzed for particle size, zeta potential, and %EE. When the results obtained were evaluated by regression and analysis of variance, it was seen that the mathematical models established could explain the effect of factors on particle size and encapsulation efficiency ($p<0.05$), but the model established for zeta potential was not statistically significant ($p>0.05$) (Yerlikaya et al., 2013).

4. FUTURE SIGHT

Process validation approaches consisting of different stages adopted in drug production for the last 10 years have brought significant advantages in the production of a targeted quality drug product. Although QbD-based product development, design, and continuous process verification phases require detailed planning and investment, it has been the most beneficial approach in terms of stakeholders (patient, industry, official authority) that concern patient health in general.

For the design and development of desired robust ocular products in terms of quality target product profile (QTTP) is mandatory given the biopharmaceutical properties of the active substance. These properties are called critical quality properties (CQAs) and include physical (particle size distribution/morphology, polymorphism, water solubility), chemical (pKa, chemical,

photolytic, and oxidative stability), biological/microbiological property (partition coefficient, membrane permeability, bioavailability, microbial limits) or the property of an output material, including the finished drug product, that must be within an appropriate limit to achieve the desired product quality.

The criticality of an attribute is decided by the degree of damage done to it. The ocular product design defines whether the product meets the patient's requirements and maintains its performance throughout the shelf life specified both by clinical and stability studies. It is obvious that it provides benefits both on the basis of the patient and company while reducing the possible errors in production to the lowest levels and ensuring the delivery of quality products to the patient. However, since the application of current approaches in process validation requires a significant culture change; regulators, industry, and academics, all together with a multidisciplinary approach needs to work.

5. CONCLUSION

DoE approach provides the most appropriate result by changing many variables at once, instead of changing one variable at a time, which is the traditional method. In this way, many factors are evaluated both simultaneously and independently of each other. Thus, the interactions between the variables are more detailed, interpretable, and clear in a short time. Based on these studies, it is clear that the designs of nanoparticles have been prepared successfully with the QbD perspective. In this way, in the future, it will be possible to use drugs that are clinically critical and costly more effectively and to improve new drug carrier systems in a less complicated and time-consuming manner.

REFERENCES

Ahuja, S. K., Ferreira, G. M., & Moreira, A. R. (2004). Application of Plackett-Burman design and response surface methodology to achieve exponential growth for aggregated shipworm bacterium. *Biotechnology and Bioengineering, 85*(6), 666-675. doi:https://doi.org/10.1002/bit.10880

DelMonte, D. W., & Kim, T. (2011). Anatomy and physiology of the cornea. *J Cataract Refract Surg, 37*(3), 588-598. doi:10.1016/j.jcrs.2010.12.037

Fangueiro, J. F., Andreani, T., Egea, M. A., Garcia, M. L., Souto, S. B., Silva, A. M., & Souto, E. B. (2014). Design of cationic lipid nanoparticles for ocular delivery: Development, characterization and cytotoxicity. *International journal of pharmaceutics, 461*(1-2), 64-73.

Ferreira, S. C., Bruns, R., Ferreira, H. S., Matos, G. D., David, J., Brandão, G., . . . Souza, A. (2007). Box-Behnken design: an alternative for the optimization of analytical methods. *Analytica chimica acta, 597*(2), 179-186.

Fialho, S. L., & da Silva-Cunha, A. (2004). New vehicle based on a microemulsion for topical ocular administration of dexamethasone. *Clin Exp Ophthalmol, 32*(6), 626-632. doi:10.1111/j.1442-9071.2004.00914.x

Freitas, C., & Müller, R. H. (1998). Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. *International journal of pharmaceutics, 168*(2), 221-229.

Freitas, C., & Müller, R. H. (1999). Stability determination of solid lipid nanoparticles (SLN) in aqueous dispersion after addition of electrolyte. *J Microencapsul, 16*(1), 59-71. doi:10.1080/026520499289310

Fresta, M., Guccione, S., Beccari, A. R., Furneri, P. M., & Puglisi, G. (2002). Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. *Bioorg Med Chem, 10*(12), 3871-3889. doi:10.1016/s0968-0896(02)00350-4

Gaudana, R., Ananthula, H. K., Parenky, A., & Mitra, A. K. (2010). Ocular drug delivery. *Aaps j, 12*(3), 348-360. doi:10.1208/s12248-010-9183-3

Gaudana, R., Jwala, J., Boddu, S. H., & Mitra, A. K. (2009). Recent perspectives in ocular drug delivery. *Pharm Res, 26*(5), 1197-1216. doi:10.1007/s11095-008-9694-0

Gericke, A., Wang, X., Ackermann, M., Neufurth, M., Wiens, M., Schröder, H. C., . . . Müller, W. E. G. (2019). Utilization of metabolic energy in treatment of ocular surface disorders: polyphosphate as an energy source for corneal epithelial cell proliferation. *RSC Adv, 9*(39), 22531-22539. doi:10.1039/c9ra04409d

Hornof, M., Toropainen, E., & Urtti, A. (2005). Cell culture models of the ocular barriers. *Eur J Pharm Biopharm, 60*(2), 207-225. doi:10.1016/j.ejpb.2005.01.009

Irimia, T., Ghica, M. V., Popa, L., Anuţa, V., Arsene, A. L., & Dinu-Pîrvu, C. E. (2018). Strategies for Improving Ocular Drug Bioavailability and Corneal Wound Healing with Chitosan-Based Delivery Systems. *Polymers (Basel), 10*(11). doi:10.3390/polym10111221

Khan, M. A., Sastry, S. V., Vaithiyalingam, S. R., Agarwal, V., Nazzal, S., & Reddy, I. K. (2000). Captopril gastrointestinal therapeutic system coated with cellulose acetate pseudolatex: evaluation of main effects of several formulation variables. *Int J Pharm, 193*(2), 147-156. doi:10.1016/s0378-5173(99)00324-5

Kiss, E. L., Berkó, S., Gácsi, A., Kovács, A., Katona, G., Soós, J., . . . Budai-Szűcs, M. (2020). Development and Characterization of Potential Ocular Mucoadhesive Nano Lipid Carriers Using Full Factorial Design. *Pharmaceutics, 12*(7). doi:10.3390/pharmaceutics12070682

Kompella, U. B., Kadam, R. S., & Lee, V. H. (2010). Recent advances in ophthalmic drug delivery. *Therapeutic delivery, 1*(3), 435-456.

Müller, L. J., Pels, E., & Vrensen, G. F. (2001). The specific architecture of the anterior stroma accounts for maintenance of corneal curvature. *Br J Ophthalmol, 85*(4), 437-443. doi:10.1136/bjo.85.4.437

Pautler, S. (2021). Anatomy of the Eye. Retrieved from https://www.scottpautlermd.com/anatomy-of-the-eye/

Singh, V. P., Ahmad, R., & Heming, T. A. (2011). *THE CHALLENGES OF OPHTHALMIC DRUG DELIVERY: A REVIEW*.

Tekade, R. K., & Chougule, M. B. (2013). Formulation Development and Evaluation of Hybrid Nanocarrier for Cancer Therapy: Taguchi Orthogonal Array Based Design. *BioMed Research International, 2013*, 712678. doi:10.1155/2013/712678

Urtti, A. (2006). Challenges and obstacles of ocular pharmacokinetics and drug delivery. *Adv Drug Deliv Rev, 58*(11), 1131-1135. doi:10.1016/j.addr.2006.07.027

Wadhwa, S., Paliwal, R., Paliwal, S. R., & Vyas, S. P. (2009). Nanocarriers in ocular drug delivery: an update review. *Curr Pharm Des, 15*(23), 2724-2750. doi:10.2174/138161209788923886

Winter, K. N., Anderson, D. M., & Braun, R. J. (2010). A model for wetting and evaporation of a post-blink precorneal tear film. *Math Med Biol, 27*(3), 211-225. doi:10.1093/imammb/dqp019

Yalpani, M. (1997). Polymeric Biomaterials, Edited by Severian Dumitriu, Marcel Dekker, New York, ISBN 0-8247-8969-5, x + 845 pp, 1994, $195.00. *Journal of Carbohydrate Chemistry, 16*(2), 249-250. doi:10.1080/07328309708006526

Yasukawa, T., Ogura, Y., Tabata, Y., Kimura, H., Wiedemann, P., & Honda, Y. (2004). Drug delivery systems for vitreoretinal diseases. *Prog Retin Eye Res, 23*(3), 253-281. doi:10.1016/j.preteyeres.2004.02.003

Yerlikaya, F., Ozgen, A., Vural, I., Guven, O., Karaagaoglu, E., Khan, M. A., & Capan, Y. (2013). Development and evaluation of paclitaxel nanoparticles using a quality-by-design approach. *Journal of pharmaceutical sciences, 102*(10), 3748-3761.

Vaginal Infections

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Vaginitis term has a wide definition that is including infectious, inflammatory disorders and substitutions in the normal vaginal flora. Vaginal infections topic will be discussed in this chapter particularly.

General Epidemiology

Epidemiologic studies usually fail to define the real prevalence of vaginal infections because some microorganisms can be found as a normal flora member and cause symptomatic disorder only in specific conditions. In addition, prevalence for vaginal infections is affected by method of diagnosis that can vary widely, from symptomatic questioning to cultures or molecular tests. In general, it is estimated that most of females will be involved with vaginal infection whilst their lifetime that is characterized by malodorous vaginal discharge, itching and burning (1).

Normal Vaginal Discharge

Vaginal discharge is the cardinal symptom for vaginal infections but may be difficult to differenciate the normal discharges from abnormal ones due to hormonal status of women. In reproductive ages, 1 to 4 mL of white or transparent, mostly odorless and not accompanied by itching, burning, irritation, erythema, local erosion and cervical or vaginal fraibility vaginal discharge is accepted as normal.

Vaginal nonkeratinized stratified squamous epithelium becomes rich in glycogen by effect of estrogen. Lactobacillus microbiom of vagina uses glycogen as a source that is shed from epithelium in order to provide acidic environment in the vagina that protects women from sexually transmitted diseases and opportunistic infections (2). In the premenarche and postmenopause, sparse amount of estrogen results in elevated vaginal pH (3). Also, vaginal normal discharge may become more apperent at pregnancy, ovulation and usage of estrogen-progestin contraceptives. Diet, sexual activity, medications and stress may impact on the volume and character of vaginal discharge.

Etiology

The most common causes of vaginal infections are vulvovaginal candidiasis, bacterial vaginosis and trichomoniasis (4,5). These infections are responsible for over 90 percent of infectious vaginitis (6). Also, cervicitis originating from sexually transmitted infections such as gonorrhea, chlamydia and mycoplasma may be found as nonspecific agents.

Common causes of vaginal infections will be discussed seperately below.

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Candida Vulvovaginitis

Vulvovaginal candidiasis is one of the most common infections of vagina causing pruritus and leucorrhoea. It is described as vulvovaginal inflammation in the presence of *Candida* species. Since *Candida sp.* are part of normal vaginal flora of 25 percent of women (7), identifying the presence of *Candida* is not adequate to diagnose candida vulvovaginitis without symptoms.

Epidemiology:

Vulvovaginal candidiasis is the second most common cause of vaginitis symptoms and constitutes one-third of vaginitis cases (8). The exact prevalence is difficult to determine because *Candida sp.* not accompanied by inflammation can be identified in vaginal lumen and endocervical canal in 10 to 20 percent of healthy individuals in the reproductive age, 6 to 7 percent of menopausal women and 3 to 6 percent of prepubertal girls (9,10). The wide use of unprescribed antifungal drugs, diagnostic dependence on symptoms but not microscobic examination and cultures without clinical correlation can be listed as other causes for difficulty of epidemiologic studies. In a survey study, 55 percent of female university students reported at least one health care provider-diagnosed vulvovaginal candidiasis by age 25 (11) and 9 percent of women reported having four or more infections within 12 months, described as reccurent vulvovaginal candidiasis (12).

Microbiology and Pathogenesis

In 80 to 92 percent of vulvovaginal candidiasis cases in the United States, *Candida albicans* is identified and *Candida glabrata* is reported as the second most common cause for candidiasis (13). All *Candida* species cause similar symptoms but severity progresses milder in *C.glabrata* and *C.parapsilosis.* Access to vagina via migration from rectum across the perianal area is suspected since gastrointestinal tract and vagina cultures showing similar *Candida* species (14). Unlike bacterial vaginosis, vulvovaginal candidiasis is not associated with reduction of vaginal lactobacilli (15). Overgrowth of pathogen and penetration of epithelial cells is described as pathogenesis and severity of disease is associated with host inflammatory response and yeast virulance factors (16).

Risk Factors

Diabetes mellitus itself is a known risk factor for vulvovaginal candidiasis (17). Particularly nonalbicans *Candida* species have been identified pathogen among patients with diabetes mellitus type 2 (18). In the other hand, treatment of diabetes mellitus with sodium glucose cotransporter 2 inhibitors (such as canagliflozin, dapagliflozin, and empagliflozin) may increase the risk of colonization and inflammation of *Candida* species (19).

Usage of broad-spectrum antibiotics increases the risk of candida vulvovaginitis caused by decrease of normal vaginal microbiom (20). Besides, administration of oral or vaginal *Lactobacillus* during and after for four days after antibiotic use doesn't decrease the risk for development of postantibiotic vulvovaginitis (21).

Candida vulvovaginitis seems to be more frequent in circumstances progressing with elevated estrogen levels (For example: pregnancy and postmenopausal estrogen replacemet therapy).

Immunosuppressed patients such as undergoing glucocorticoid or other immunosuppressive therapies or human with HIV infections are involved with increased prevalence of candidal infections (22).

Genetic studies have compared women suffering recurrent vulvovaginal candidiasis with control groups and identified polymorphisms in the *SIGLEC15* gene (23), *TLR2* gene and

mannose-binding lectin genes (24), which are not modifiable risk factors but future gene therapy researches may decrease recurrence of vulvovaginal candidiasis.

Diet, sexual behavior, intrauterine or vaginal contraceptive devices and combined oral contraceptives are other known risk factors but studies have not reported consistent result about these risk factors, yet.

Symptoms and Physical Examination

Vaginal pruritus is the major symptom of vulvovaginal candidiasis (13). Vulvar burning and soreness are also common. Vulvar irritation may be present due to candidiasis itself or itching. Symptoms often worsen during the week before menses (25).The severity of symptoms varies from mild to severe except *C.glabrata* and *C.parapsilosis* infections, which is featured with minimal clinical findings or mild symptoms.

Erythema of vulva and vagina can be seen in physical examination. Vulvovaginal edema may be present. There can be minimal or no discharge. If present, vaginal discharge is classically white, thick and adherent to vaginal walls. It's been described as curd-like or cottage cheese-like, clumpy discharge. The malodorus discharge may be absent or minimal. The cervix usually appears to be normal.

Diagnosis

Diagnosis can be assessed via clinical findings, office evalution, culture and nucleic acid tests. The office evaluation comprises of swabbing vaginal wall and discharge, assessing the vaginal pH and performing microscopy. The vaginal pH usually is normal but may be elevated with presence of concomitant bacterial vaginosis. In wet mount of discharge with addition of 10 percent potassium hydroxide, cellular components will be destroyed and microscopic evalution will identify budding yeast, pseudohyphae and hyphae (Figure 1) (26). A surfactant, potassium hydroxyde and blue dye mixture known as Swartz-Lamkins fungal stain may make *Candida* structures easier to identify (27). Nevertheless, microscopy may be negative in 50 percent of cases with culture confirmed candidiasis (16).

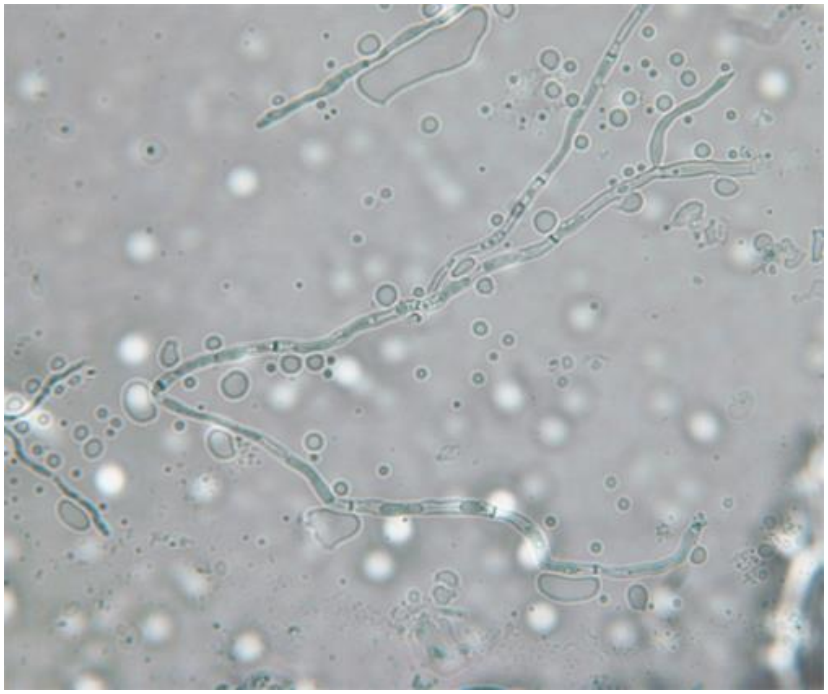

Figure 1. Pseudohyphae of *Candida albicans* on wet mount vaginal discharge miscroscopy

The vaginal culture may be indicated in patients who are demonstrating clinical features of vulvovaginal candidiasis but no pathogens are present on microscopy, in order to avoiding empiric and unindicated therapies. The culture swab should be rubbed against vaginal sidewalls and inoculated onto Sabouraud agar or Microstix-Candida medium which performs equally well against first choice (13).

Polymerase chain reaction and other molecular tests may be considered in resistant and recurrent cases or in studies but clinicians should have regard to high costs and unnecessarity of molecular tests in cases with highly specific clinical features. Also Pap smear may be positive in 25 percent of cases but it's an insensitive testing due to sample origin is cervix, which is not affected by *Candida* vaginitis.

Self-diagnosis is a common situation in vulvovaginal candidiasis and should be discouraged by health care providers. Women with prior infection usually make correct self-diagnosis but this will increase the unprescribed medication uses and may result with unnoticed and more serious gynecologic disorders.

Treatment

Vulvovaginal candidiasis treatment strategy should be set for relief of symptoms since 10 to 20 percent of reproductive aged females have *Candida sp.* as a part of vaginal flora, those do not reqiure any therapy.

Treatment's severity is determined according to candidiasis, whether it's uncomplicated or complicated. Uncomplicated infection criterias are sporadic episodes (≤3 episodes/ year), mild to moderate symptoms, probable infection with *C.albicans,* healthy and non-pregnant patient, non-immunsuppressed individual. To tell that the infection is uncomplicated, all criterias must be present.

The initial treatment may be administered as fluconazole, 150 mg single dose. Therapeutic concentrations in vaginal secretions maintains for 72 hours after ingestion of single 150 mg tablet (28). When compared to topical antifungals, single-dose oral fluconazole regimen is less expensive treatment for candida vulvovaginitis.

Single dose topical regimens are also available in many countries for treatment of candidiasis. Topical agents seem to have less side effects, may lead to possible local burning and irritation, while oral treatment may cause gastrointestinal intolerance, headache, rash and transient liver function disorders. Also, oral treatment may take longer duration to relieve the symptoms. Oral or topical choice for therapy should be considered by cost, patient preference and contraindications. If the topical treatment has been chosen, not only vulvar but vaginal treatment would be crucial to eradicate candidal reservoir (29).

Ibrexafungerp is a single-day oral triterpenoid antifungal drug for use in uncomplicated vulvovaginal candidiasis. Ibrexafungerp shows its effect by inhibiting formation of fungal cell wall and resulting in death of *Candida* species. It's first in its class and may be indicated in patients who have allergic reactions or resistant infections to fluconazole or can not tolarate fluconazole therapy. Clinical trials have shown that ibrexafungerp results in high clinical cure rates compared to placebo, but comparison to azole treatment is yet to be recruited (30,31). It's been administered as two 150 mg tablets twice a day (i.e. two in the morning and two at night). CYP3A inhibitors may interact with ibrexafungerp, so patient who undergone CYP3A inhibitor treatment should reduce the dose to 150 mg twice in a day. Pregnancy and lactation data for humans are yet lacking.

In patients with severe symptoms or immunocompromise, fluconazole is suggested two or three (the number of doses depends on symptom severity) sequential 150 mg doses 72 hours apart.

C.glabrata has low vaginal virulence and infrequently causes vaginal symptoms. When suspected, adequate effort should be made to exclude other causes of symptoms. When *C.glabrata* is identified, vaginal 600 mg boric acid capsules once daily at night for two or three weeks may be administered. The success rates differ between 65 and 70 percent. For better results flucytosine cream or 4% amphotericin B cream may be beneficial with combination of boric acid capsules (32). Neither boric acid capsules nor flucytosine or amphotericin B cream is available commercially and is made by compounding pharmacy. Boric acid capsules may be fatal if swallowed.

In pregnant women, clotrimazole topical agents should be used for one week because oral azole treatments may lead to miscarriage or birth defects, in first trimestr, particularly.

Oteseconazole is another choice of azole antifungal drugs which is more potent than fluconazole in recurrent vulvovaginal candidiasis but has a long half-life (138 days) and contraindicated in women who have potential to get pregnant or lactating (33).

Trichomoniasis

Trichomoniasis is a genitourinary infection caused by protozan *Trichomonas vaginalis*. It's been known that trichomoniasis is the the most common nonviral sexually transmitted infection worldwide. It affects women more than men and is one of three common infectious agent that causing vaginal complaints. It's been estimated that trichomoniasis is affecting 3,7 million women and men in the United States annually and African-Americans are at risk greater four times compared to other races (34). World Helath Organization has estimated 156 million new trichomoniasis cases occured in 2020 (35).

T.vaginalis is the flagellated protozan that is infecting squamous epithelium in the urogenital tract, especillay vagina, urethra and paraurethral glands (34). Cervix, bladder, Bartholin glands, prostate and even oral and rectal cavities are the other sites reported as involved.

Trichomoniasis is almost always sexually transmitted. Females acquire disease from both males and females, while males acquire disease from females but usually don't transmit disease to males. The incubation period is unknown but in-vitro studies have shown that incubation lasts for 4 to 28 days in approximately 50 percent of patients. Coexistance of trichomoniasis and bacterial vaginosis ise common, ranging from 20 to 80 percent. Screening for trichomoniasis is not recommended unless HIV infection is present.

Symptoms in female include a purulent, malodorous and thin vaginal discharge with burning, itching, dysuria, lower abdominal pain and dyspareunia. In physical examination, erythema of the vulva and vaginal mucosa may present. The classically describe yellow-green malodorous vaginal discharge occurs in 10 to 30 percent of women (36). Punctate hemorrhages may be shown on vaginal mucosa and cervix, named as strawberry cervix or colpitis macularis (Figure 2).

Untreated trichomoniasis may result in urethritis or cystitis, posthysterectomy cuff cellulitis and abscess, pelvic inflammatory disease, infertility, increased risk of acquiring HIV and HPV in non-pregnant women. In pregnant women, increased risks for premature rupture of membranes, preterm delivery and low birth weight infants have been reported.

Diagnostic evalution include wet mount microscopy and nucleic acid amplification test (NAAT). Clinicians should consider NAAT's longer turn-around time and higher cost and should prefer this test only in microscopic negative cases. In microscopy, jerky and spinning movement of flagellated *Trichomonas* may be seen in wet mount vaginal discharge preparates. Culture and cervical cytology is less accurate diagnostic tests and not recommended while trichomoniasis is highly suspected on clinical evalution (37).

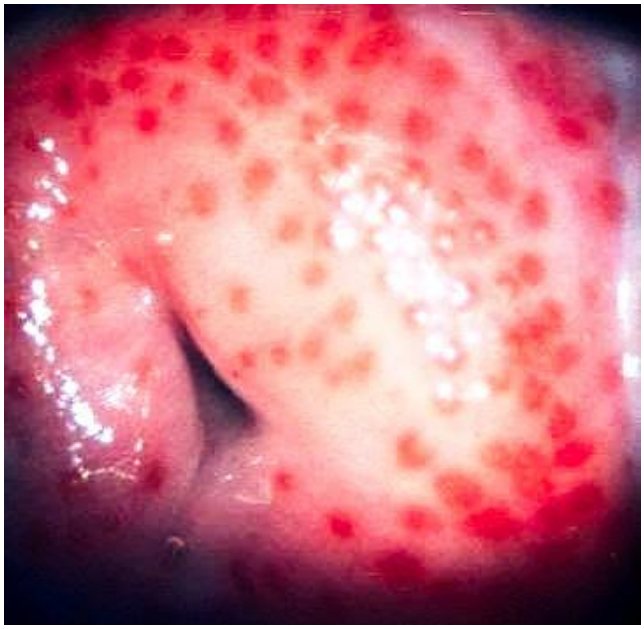

Figure 2. Strawberry cervix or colpitis macularis seen in trichomoniasis

The treatment should be started with avoiding sexual activity until both male and female of couple are treated. Preferred therapy is metronidazole 500 mg orally twice daily for seven days. When it's not available, single dose of 2 g metronidazole is recommended but also associated with lower cure rates. Metronidazole vaginal gel is not recommended alone because doesn't treat all possible anatomic reservoirs.

Gonorrhea

Gonorrhea, the infection with the grma-negative coccus *Neisseria gonorrhoeae*, is a major cause of morbidities among sexually active women worldwide. Over 600,000 cases are reported in the United States annually (38). It's the major cause for urethritis in males and females. Thereafter the infection can result in pelvic inflammatory disease, infertility, ectopic pregnancy, chronic pelvic pain and disseminated gonococcal infection resulting with endocarditis and meningitis with severe morbidities. Gonococcal resistance for antibiotics is common.

The cervix is the most common site for mucosal infection in females. Up to 70 percent of cases may remain asymptomatic, thus the incubation period in females is less characterized than males. When symptoms are present, they manifest as vaginal itching and mucupurulent discharge. Some females may suffer intermenstrual bleeding and menorrhagia. Abdominal pain and dyspareunia may be present if the infection raises to upper genital tract. Urethritis may be presented with dysuria and urgency in posthysterectomy women, in particular. In association with pelvic inflammatory disease, perihepatitis (Fitz-Hugh-Curtis Syndrome) may exist and causes sharp pleuritic pain in the upper right quadrant of abdomen. Fitz-Hugh-Curtis syndrome is diagnosed usually incidental whilst surgery for any other reason. In pregnant women, gonococcal infections may result in chorioamnionitis, premature rupture of membranes, preterm birth, low birth weight and spontaneous abortions. Also, untreated *N.gonorrhoeae* infections may cause neonatal conjunctivitis in infants after delivery.

Treatment for uncomplicated cases of gonococcal infection is 500 mg ceftriaxone, administered intramuscular for individual weighed under 150 kg. Above 150 kg, the dose should revised as 1 g. If the presence of *Chlamydia trachomatis* can't be excluded, additive treatment with doxycycline (100 mg twice daily for seven days) is recommended due to high rates of coinfection.

References

1. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, vd. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep. 23 Temmuz 2021;70(4):1-187.

2. Miller EA, Beasley DE, Dunn RR, Archie EA. Lactobacilli Dominance and Vaginal pH: Why Is the Human Vaginal Microbiome Unique? Front Microbiol. 08 Aralık 2016;7:1936.

3. Lin YP, Chen WC, Cheng CM, Shen CJ. Vaginal pH Value for Clinical Diagnosis and Treatment of Common Vaginitis. Diagnostics (Basel). 27 Ekim 2021;11(11):1996.

4. Danby CS, Althouse AD, Hillier SL, Wiesenfeld HC. Nucleic Acid Amplification Testing Compared With Cultures, Gram Stain, and Microscopy in the Diagnosis of Vaginitis. J Low Genit Tract Dis. 01 Ocak 2021;25(1):76-80.

5. Broache M, Cammarata CL, Stonebraker E, Eckert K, Van Der Pol B, Taylor SN. Performance of a Vaginal Panel Assay Compared With the Clinical Diagnosis of Vaginitis. Obstet Gynecol. 01 Aralık 2021;138(6):853-9.

6. Sobel JD. Vulvovaginitis in healthy women. Compr Ther. 1999;25(6-7):335-46.

7. Gardella C EL, Lentz GM. Genital tract infections. İçinde: Comprehensive Gynecology, 7th Ed. Philadelphia: Elsevier; 2017. s. 542.

8. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 05 Haziran 2015;64(RR-03):1-137.

9. Mj G, B W, N L, Lj M, Jd L, Mp V. Vaginal microbial flora in normal young women. British medical journal [Internet]. 06 Şubat 1979 [a.yer 18 Aralık 2022];1(6176). Web adress: https://pubmed.ncbi.nlm.nih.gov/380743/

10. C T, N C, Ma L, G M, S M, C B. Vaginal and endocervical microorganisms in symptomatic and asymptomatic non-pregnant females: risk factors and rates of occurrence. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases [Internet]. Temmuz 2009 [a.yer 18 Aralık 2022];15(7). Web adress: https://pubmed.ncbi.nlm.nih.gov/19558525/

11. Am G, B F, Bw G. The epidemiology of vulvovaginal candidiasis among university students. American journal of public health [Internet]. Ağustos 1995 [a.yer 18 Aralık 2022];85(8 Pt 1). Web adress: https://pubmed.ncbi.nlm.nih.gov/7625516/

12. B F, R M, Jp D, Jd S, J W. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. Journal of lower genital tract disease [Internet]. Temmuz 2013 [a.yer 18 Aralık 2022];17(3). Web adress: https://pubmed.ncbi.nlm.nih.gov/23486072/

13. Jd S. Vulvovaginal candidosis. Lancet (London, England) [Internet]. 06 Eylül 2007 [a.yer 18 Aralık 2022];369(9577). Web adress: https://pubmed.ncbi.nlm.nih.gov/17560449/

14. Me B, Mj S. Colonization of Candida albicans in vagina, rectum, and mouth. The Journal of family practice [Internet]. Mayıs 1983 [a.yer 18 Aralık 2022];16(5). Web adress: https://pubmed.ncbi.nlm.nih.gov/6341500/

15. B V, C P, E B, M C, S T, G B, vd. Dynamics of vaginal bacterial communities in women developing bacterial vaginosis, candidiasis, or no infection, analyzed by PCR-denaturing gradient gel electrophoresis and real-time PCR. Applied and environmental microbiology

[Internet]. Eylül 2007 [a.yer 18 Aralık 2022];73(18). Web adress: https://pubmed.ncbi.nlm.nih.gov/17644631/

16. Jd S. Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. American journal of obstetrics and gynecology [Internet]. 08 Ocak 1985 [a.yer 18 Aralık 2022];152(7 Pt 2). Web adress: https://pubmed.ncbi.nlm.nih.gov/3895958/

17. Gg D. Lower Genital Tract Infections in Diabetic Women. Current infectious disease reports [Internet]. Aralık 2002 [a.yer 18 Aralık 2022];4(6). Web adress: https://pubmed.ncbi.nlm.nih.gov/12433331/

18. Em de L, Sj J, Jd S, B F. Prevalence and risk factors for vaginal Candida colonization in women with type 1 and type 2 diabetes. BMC infectious diseases [Internet]. 2002 [a.yer 18 Aralık 2022];2. Web adress: https://pubmed.ncbi.nlm.nih.gov/11835694/

19. P N, Y Z, K W, K U. Evaluation of vulvovaginal symptoms and Candida colonization in women with type 2 diabetes mellitus treated with canagliflozin, a sodium glucose co-transporter 2 inhibitor. Current medical research and opinion [Internet]. Temmuz 2012 [a.yer 18 Aralık 2022];28(7). Web adress: https://pubmed.ncbi.nlm.nih.gov/22632452/

20. L W, M K, E H, S S. Relative risk of vaginal candidiasis after use of antibiotics compared with antidepressants in women: postmarketing surveillance data in England. Drug safety [Internet]. 2003 [a.yer 18 Aralık 2022];26(8). Web adress: https://pubmed.ncbi.nlm.nih.gov/12825971/

21. M P, J G, P C, S G, P O, S H, vd. Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. BMJ (Clinical research ed) [Internet]. 09 Nisan 2004 [a.yer 18 Aralık 2022];329(7465). Web adress: https://pubmed.ncbi.nlm.nih.gov/15333452/

22. A D, Cm H, Sf M, S CU, Rs K, A R, vd. Incident and persistent vulvovaginal candidiasis among human immunodeficiency virus-infected women: Risk factors and severity. Obstetrics and gynecology [Internet]. Mart 2003 [a.yer 18 Aralık 2022];101(3). Web adress: https://pubmed.ncbi.nlm.nih.gov/12636961/

23. M J, M P, M B, C C, M D, L van E, vd. A systems genomics approach identifies SIGLEC15 as a susceptibility factor in recurrent vulvovaginal candidiasis. Science translational medicine [Internet]. 06 Aralık 2019 [a.yer 18 Aralık 2022];11(496). Web adress: https://pubmed.ncbi.nlm.nih.gov/31189718/

24. Dc R, Ce D, M J, Ts P, M O, I C, vd. Gene polymorphisms in pattern recognition receptors and susceptibility to idiopathic recurrent vulvovaginal candidiasis. Frontiers in microbiology [Internet]. 23 Eylül 2014 [a.yer 18 Aralık 2022];5. Web adress: https://pubmed.ncbi.nlm.nih.gov/25295030/

25. Lo E, Se H, Ce S, La K, Da E, Kk H. Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. Obstetrics and gynecology [Internet]. Kasım 1998 [a.yer 19 Aralık 2022];92(5). Web adress: https://pubmed.ncbi.nlm.nih.gov/9794664/

26. National guideline for the management of vulvovaginal candidiasis. Clinical Effectiveness Group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases). Sexually transmitted infections [Internet]. Ağustos 1999 [a.yer 19 Aralık 2022];75 Suppl 1. Web adress: https://pubmed.ncbi.nlm.nih.gov/10616376/

27. Jh S, Be L. A RAPID, SIMPLE STAIN FOR FUNGI IN SKIN, NAIL SCRAPINGS, AND HAIRS. Archives of dermatology [Internet]. Ocak 1964 [a.yer 19 Aralık 2022];89. Web adress: https://pubmed.ncbi.nlm.nih.gov/14070843/

28. Et H, O C, D B, Pv M, Je T. Fluconazole levels in plasma and vaginal secretions of patients after a 150-milligram single oral dose and rate of eradication of infection in vaginal candidiasis. Antimicrobial agents and chemotherapy [Internet]. Mayıs 1990 [a.yer 19 Aralık 2022];34(5). Web adress: https://pubmed.ncbi.nlm.nih.gov/2360828/

29. Pg P, Ca K, D A, Dk B, Tf C, Je E, vd. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America [Internet]. 03 Ocak 2009 [a.yer 19 Aralık 2022];48(5). Web adress: https://pubmed.ncbi.nlm.nih.gov/19191635/

30. Jr S, R S, Jk G, Sa S, Sn L, Ma J, vd. Ibrexafungerp Versus Placebo for Vulvovaginal Candidiasis Treatment: A Phase 3, Randomized, Controlled Superiority Trial (VANISH 303). Clinical infectious diseases : an official publication of the Infectious Diseases Society of America [Internet]. 06 Ekim 2022 [a.yer 19 Aralık 2022];74(11). Web adress: https://pubmed.ncbi.nlm.nih.gov/34467969/

31. R S, P N, Ma G, Da D, Ne A, D A, vd. Efficacy and safety of oral ibrexafungerp for the treatment of acute vulvovaginal candidiasis: a global phase 3, randomised, placebo-controlled superiority study (VANISH 306). BJOG : an international journal of obstetrics and gynaecology [Internet]. Şubat 2022 [a.yer 19 Aralık 2022];129(3). Web adress: https://pubmed.ncbi.nlm.nih.gov/34676663/

32. Jd S, W C, V N, D L. Treatment of vaginitis caused by Candida glabrata: use of topical boric acid and flucytosine. American journal of obstetrics and gynecology [Internet]. Kasım 2003 [a.yer 19 Aralık 2022];189(5). Web adress: https://pubmed.ncbi.nlm.nih.gov/14634557/

33. Martens MG, Maximos B, Degenhardt T, Person K, Curelop S, Ghannoum M, vd. Phase 3 study evaluating the safety and efficacy of oteseconazole in the treatment of recurrent vulvovaginal candidiasis and acute vulvovaginal candidiasis infections. Am J Obstet Gynecol. Aralık 2022;227(6):880.e1-880.e11.

34. Pj K, Ca G, Ac S, R SM, D S, We S, vd. Diagnosis and Management of Trichomonas vaginalis: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America [Internet]. 13 Nisan 2022 [a.yer 19 Aralık 2022];74(Suppl_2). Web adress: https://pubmed.ncbi.nlm.nih.gov/35416973/

35. Sexually transmitted infections (STIs) [Internet]. [a.yer 19 Aralık 2022]. Web adress: https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)

36. Jr S, D B. Trichomoniasis. Clinical microbiology reviews [Internet]. Ekim 2004 [a.yer 19 Aralık 2022];17(4). Web adress: https://pubmed.ncbi.nlm.nih.gov/15489349/

37. Ka W, Lh B, Pa C, Cm J, Ca M, I P, vd. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports [Internet]. 23 Temmuz 2021 [a.yer 19 Aralık 2022];70(4). Web adress: https://pubmed.ncbi.nlm.nih.gov/34292926/

38. Centers for Disease Control and Prevention's Sexually Transmitted Diseases Infection Guidelines | Clinical Infectious Diseases | Oxford Academic [İnternet]. [a.yer 19 Aralık 2022]. Web adress: https://academic.oup.com/cid/article/74/Supplement_2/S89/6567949

The Effects of Covid-19 on Musculoskeletal System and Pulmonary Rehabilitation

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Introduction

Coronavirus disease (COVID-19) is a disease which develops with respiratory tract symptoms; it was seen in Wuhan, China in December 2019 for the first time and was defined on January 13, 2020. The outbreak related with the disease first occurred in people in seafood and animal market in Wuhan region. Later on, it spread to other regions, especially Wuhan, to different provinces of China and then to many different countries (T.R. Ministry of Health COVID-19 Information Platform, 2020). Coronavirus (COVID-19), which gradually became a global health problem, affects the daily and social lives of individuals negatively. COVID-19, which initially occurred as a local health problem, became a global epidemic in a short time due to rapidly developing mobility and easy transmission (WHO, 2020). The disease is transmitted among people through the coughs and sneezing of sick individuals. Droplets scattering around as a result of the coughing and sneezing of the sick people and taking hands to eyes, face, nose or mouth without cleaning them after touching surfaces contaminated with respiratory particles are also the causes of virus transmission. Touching the face with contaminated hands increases the contagiousness of COVID-19 (T.R. Ministry of Health COVID-19 Information Platform, 2020). Individuals who have the disease have mild, moderate or serious symptoms. The disease, which usually targets upper respiratory tracts, also brings along musculoskeletal system diseases during and after the disease period. The disease has many side effects. These effects influence individuals and the society severely in health, social and economic areas. COVID-19 pandemic has affected all areas of life and also brought negative consequences in the world and in the field of health (Senol et al., 2021 ; TR. Ministry of National Education, 2020).

What are COVID-19 risk factors?

Not all individuals infected with COVID-19 are affected by the disease to the same degree. Age groups, profession and chronic diseases affect the disease, the course of the disease and its symptoms. 80% of individuals with the disease survive the disease with mild symptoms. 20% of individuals with the disease are treated in health institutions. COVID-19 has more effects on individuals aged 60 and older (T.R. Ministry of Health COVID-19 Information Platform, 2020).

Individuals most affected by the disease and age groups- Individuals older than 60 years of age: Although COVID-19 has affected all age groups, the elderly constitute the majority of individuals with the disease and deaths. According to data published by the United States of America in March 2020, it was reported that more than 80% of the disease-related mortality rate belonged to individuals over the age of 65 (Lee et al., 2020; Aytepe & Efe, 2021).

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Individuals with serious chronic medical conditions: In individuals with chronic disease, the process of surviving the disease and severity of the disease was more difficult than other individuals. Studies conducted showed that the most common comorbidities among chronic diseases were high blood pressure, diabetes, congestive heart failure, asthma, COPD (chronic obstructive pulmonary disease), inflammatory rheumatic disease and coronary artery disease (Günal et al., 2020).

Healthcare professionals: It was inevitable for healthcare professionals, who are at the forefront of the fight against the pandemic, to contact the virus directly. As of April 8, 2020, 22.073 healthcare professionals diagnosed with COVID-19 from 52 different countries were reported to World Health Organization (Pala & Metintaş, 2020).

What are the signs and symptoms of COVID-19?

Common symptoms of COVID-19 are high fever, fatigue and weakness, cough, loss of sense of taste or smell, muscle, joint and back ache. Less common symptoms are headache, diarrhoea, sore throat, skin exfoliation, redness and irritation of the eyes. Severe symptoms are respiratory distress or shortness of breath, confusion, disorientation in movement or speech and chest pain.

What are the post COVID-19 musculoskeletal system complaints?

Low back, back, shoulder and neck pain are common post COVID-19 musculoskeletal system complaints. A large number of studies conducted support these symptoms. The regions with more intense pain in the musculoskeletal system have been found as neck, pain and low back region (Gayretli et al., 2021). Studies conducted have shown that the region where pain occurs most in actively working people is the formations on the spine as the waist, neck and back. In one study, it was stated that waist, back and neck regions had the highest rate of pain during the last year, highest rate of work disability during the last year and the highest rate of pain during the last week (Jang et al., 2014; Briggs et al., 2009). A study by Skovlund et al. (2020) researched musculoskeletal pain in working population and stated that pain occurred mostly on waist and neck regions (Skovlund et al, 2020).

Post COVID-19 Management of Pain in Musculoskeletal System

The main effect in COVID-19 is lung involvement. However, studies conducted have stated that some other organs are also targeted because they have some common tissues with the lungs (ACE 2 receptors). These receptors targeted by the virus can be found in intestines, small vessel endothelium, smooth and striated muscle and synovial tissue. Therefore, when the existing cases are examined, it is possible to encounter not only cough, dyspnoea or high fever, but also symptoms of fatigue, myalgia and arthralgia. The prognosis is no doubt more severe when the patient's motility loss, malnutrition and respiratory problems are added to this picture.

While musculoskeletal system pain is one of the most common acute findings of COVID-19 infection, chronic complications related with moderate and severe COVID-19 have also been reported. In addition to prolonged immobilization and intensive care units processes, pro-inflammatory, cytokines and chemokines and direct damage of SARS-Cov2 to the tissue have been held responsible for the destruction of skeletal muscle, bone, joint, nerve and soft tissues (Huang, 2020). In studies conducted, while the complaint of weakness was the complaint that continued most frequently with a rate of 58% in post COVID-19 patients, joint complaints were found to occur in 19% of the patients (Lopez-Leon, 2021). In addition to this, it was reported that the frequency of muscle weakness or fatigue was 63% in post COVID-19 sixth month (Huang, 2021). Some of the pain felt by patients is related to the pathophysiology. However, the problems in posture and bodily awareness that occur due to lack of movement and spending more time with

technological and electronic devices during the isolation period should not be overlooked. Clinicians should also accept the importance of describing pain phenotypes correctly. For example, it has been suggested that infections may affect peripheral nerve system or central nerve system directly or induce post-viral immune syndrome supporting the development of neuropathic pain. Patients with neuropathic pain usually have negative results when compared with patients who have nociceptive pain. For this reason, evaluating pain phenotypes in patients with permanent COVID-19 symptoms provide important information for healthcare professionals. In addition, the treatment protocol to be applied guides the choice of methods and exercises that will provide holistic healing as well as the muscle and bone structures that are symptomatic (Karateev, 2021). Many problems continue in patients after COVID-19 infection. Patients may have respiratory symptoms such as fatigue, weakness in skeletal and respiratory muscles and atrophy, decreased exercise capacity, fatigue, pain, limitation of functions, dyspnoea during low intensity exercise or daily living activities; psychosocial problems such as anxiety, depression and post-traumatic stress disorder; and prolonged problems such as cognitive disorders, speech and swallowing disorders, deterioration in daily living activities and decreased quality of life. Therefore, COVID-19 patients are candidates for pulmonary rehabilitation (PR) (Zhao et al., 2020; Yang & Yang, 2020).

Methods used in pulmonary rehabilitation for post-COVID patients

Physiotherapy agents

1.Tens (Transcutaneous Electrical Nerve Stimulation): High or low frequency tens is a physiotherapy agent that has been found to be very effective especially in waist and beck pain in studies conducted. Tens can be preferred especially for short-term immediate relief of pain (Oral & Ketenci, 2013).

2.Therapeutic ultrasound: These are devices producing high frequency sound waves. Sound waves can disperse in solid and liquid space. Heat energy is released by reflection in the tissues through which these waves pass. Sound waves have the ability to disperse in solid and liquid space. Heat energy is released by reflection in the tissues through which these waves pass. Micro-massage and warming effect of ultrasound is used in the treatment of pain. The skin is nourished and revitalized by stimulating and working in the capillaries. Ultrasound may be preferred for the recovery of soft tissue disorders and relief of arthralgia symptoms. No treatments are made on bone tissue (Oral & Ketenci, 2013).

3.Hot application: It is the warming of skin and subcutaneous tissue with the help of special fabric bags that do not leak water. A more superficial heat effect is released when compared to ultrasound. The veins expand and circulation relaxes with the effect of increasing heat. It is not preferred in acute inflammation and rheumatological cases and also in cases metastasis may occur (Özdinçler, 2016)

Therapeutic Exercises

Therapeutic exercise is a physical therapy technique used to improve or maintain an individual's physical condition through resistance, endurance, flexibility and balance training. The intensity, volume, progression and type of exercises should be personalized based on the physical condition and tolerance while performing the exercise. When prescribing exercise to patients, exercise type, intensity and frequency suitable for the result of the evaluation should be determined. It has been stated that exercise therapy will be beneficial in the recovery of muscle, bone, joint and connective tissue during the COVID-19 process (Öztürk & Kaya, 2020).

1a. Core stability and balance exercises: The ability of muscles to provide the functional stability of joints by creating synchronized activity and controlled movement is called neuromuscular control. The part of the body known as "core", or the lumbopelvic hip component,

consists of both passive and active structures of the hip and trunk. Stabilization is mainly achieved by co-contraction of the muscles. Core stabilization is very important in maintaining the balance of spine structure. A healthy posture supports the position and functions of the tissue and organs. It also provides the adequacy of joint range of motion in the neck, upper and lower extremities (Yüksel et al., 2019).

1b. Mobilization: According to clinical studies conducted, in and out-of-bed activities performed according to the respiratory and muscular capacity of the individual reduce pain significantly. Transition from lying in bed to sitting position, sitting and short distance walking can be given as examples to these activities (Kırmızı, 2020). Early physiotherapy applications including gradual mobilization and exercise therapy have an important place in reducing complications associated with immobilization, returning to daily life and increasing physical activity tolerance in COVID-19 patients (Kırmızı, 2020).

1c. Joint range of motion exercises: They are the movements performed by the muscle and joint by using the physiological limits of the joint range of motion. They can be performed passively or actively (Yüksel, 2019).

1d. Aerobic exercises: Exercises such as walking, running, swimming and cycling are aerobic exercises; they work the major muscles and have effects such as increasing aerobic capacity, decreasing insulin resistance and inflammation and losing weight. These effects have been found to be effective in decreasing pain intensity in patients with musculoskeletal system pain (Meng, 2015). In patients with continued oxygen support, low intensity aerobic exercises such as walking (<3 MET) are started with close monitoring of blood pressure, SaO_2, heart rate monitorization and newly developed cardiorespiratory symptoms. Duration and intensity of exercise are gradually increased according to the patient's condition by starting 3-4 times a week and for 10-15 minutes (Barker-Davies, 2020).

1e. Strengthening exercises: 1 repetitive maximum (RM) 30-40% mild and moderate intensity 1-3 sets of exercise, 8-15 repetitions of each set with 2 minutes of rest in-between sets should be performed 2 times a week by starting with 3-4 muscle groups in very weak patients. Weight should be lifted for 3-6 seconds without causing pain and discomfort. Valsalva manoeuvre should be created by exhaling while lifting (Yüksel, 2019). Intensity of exercise and number of repetitions are gradually increased according to the tolerance and condition of the patient. While returning to exercise from mild/moderate severity COVID-19, 1 week of low level stretching and mild muscle strengthening activity should be tried before targeted cardiovascular sessions (Barker-Davies, 2020).

1f. Diaphragm exercises: In patients who have had COVID-19, the aim of respiratory exercises is to regulate the respiratory cycle, improve dyspnoea symptoms, regulate respiratory movements, increase oxygenation, decrease secretion in airway, prevent dysfunction, decrease anxiety and depression, provide and improve relaxation, maintain functions and increase quality of life (Arzani, 2020; Zhao, 2020). The objective of PR is not only to improve the physical condition of individuals. It also enables individuals to return more quickly to their environment and the society (Yang & Yang 2020).

With the correct use of diaphragm muscle in individuals who have had COVID-19, the capacity and efficiency of respiration increases, organ mobility is ensured, lymphatic system is activated, spine mobilization is increased with chest mobility and the rate of healing increases in tissues and organs (Yang & Yang 2020).

References

Arzani P, Khalkhali Zavieh M, Khademi-Kalantari K & Akbarzadeh Baghban A. (2020). Pulmonary rehabilitation and exercise therapy in a patient with COVID-19: A Case report. *Medical journal of the Islamic Republic of Iran,* 34: 106.

Aytepe A & Efe M. (2021). Covid-19 Pandemisinde Yaş Ayrımcılığı: Uygulamalar ve Etkileri. *Journal of Geriatric Science,* 4(3):103-112.

Barker-Davies RM, O'Sullivan O, Senaratne KPP, Baker P, Cranley M, DharmDatta S, et al. (2020). The Stanford Hall consensus statement for post-COVID-19 rehabilitation. *Br J Sports Med,* 54(16):949-959.

Briggs AM, Bragge P, Smith AJ, et al. (2009). Prevalence and associated factors for thoracic spine pain in the adult working population: a literature review. *Journal of occupational health*, 51:177.

Gayretli AS, Arslan M & Palalı İ. (2021). COVID-19 Salgınında Sağlık Çalışanlarının Stres Algı Düzeyinin Kas İskelet Sistemi Ağrılarına Etkisi. *MKÜ Tıp Dergisi*, 12(43):106-113.

Günal Ö, Türe E, Bayburtlu M, Arslan U, Demirağ MD, Taşkın MH & Kılıç S. (2020). COVID-19 Tanılı Hastaların Risk Faktörleri Açısından Değerlendirilmesi. *Mikrobiyol Bul*, 2020;54(4):575-582.

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al.(2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet,* 395(10223):497-506.

Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. (2021). 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet*, 397(10270): 220-32.

Jang T-W, Koo J-W, Kwon S-C, et al. (2014). Work-related musculoskeletal diseases and the workers' compensation. *Journal of Korean medical science*, 29:18-23.

Karateev AE, Amirdzhanova VN, Nasonov EL, Lila AM, Alekseeva LI, Pogozheva EY. & Nesterenko VA. (2021). "Post-COVID syndrome": The focus is on musculoskeletal pain. *Nauchno-Prakticheskaya Revmatologiya*, 255-262.

Kırmızı M, Karabay D, Uçurum SG & Kaya ÖD. (2020). COVİD- 19 Hastalarında Erken Dönem Mobilizasyon Uygulamaları. *İzmir Kâtip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi*, 5(2): 83-86

Lee K, Jeong GC. & Yim J. (2020). Consideration of the psychological and mental health of the elderly during Covid-19: a theoretical review. *Int J Environ Res Public Health*, 3;17(21):8098

Meng XG & Yue, SW. (2015). Efficacy of aerobic exercise for treatment of chronic low back pain: a meta-analysis. *Am J Phys Med Rehabil*, 94 (5): 358-365.

Milli Eğitim Bakanlığı. COVİD-19 Salgını Sonrası Dünyada Eğitim [Internet]. Türkiye: Milli Eğitim Bakanlığı; 2020 [cited June 1]. Available from: http://www.meb.gov.tr/covid-19-salgini-sonrasi-dunyadaegitim/haber/20936/tr

Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A. & Villapol, S. (2021). More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. *Scientifc Reports*, 11:16144.

Oral A. & Ketenci, A. (2013). Radiküler Bel Ağrılarının Tedavisinde Fiziksel Tıp ve Rehabilitasyon Yaklaşımları: En Uygun ve Etkin Tedavinin Belirlenmesi Amacıyla Kanıtların Gözden Geçirilmesi ve Güncel Öneriler. *Turk J Phys Med Rehab,* 59:57-68.

Öztürk O. & Kaya, ÖD. (2020). COVI-19'un Kas İskelet Sistemine Etkisi. *İzmir Kâtip Çelebi Üniversitesi Sağlık Bilimleri Fakültesi Dergisi*, 5(2): 179-182.

Pala SÇ. & Metintaş S. (2020). COVID-19 Pandemisinde Sağlık Çalışanları. *ESTÜDAM Halk Sağlığı Dergisi*, 5(COVID-19 Özel Sayısı):156-68.

Senol D, Toy S, Canbolat, M & Pektas M. (2021). Evaluation of Online Anatomy Education Given in Medicine and Dentistry Faculties of Universities During Covid-19 Pandemic with Student. *Konuralp Medical Journal,* 13(1): 30-35.

Skovlund SV, Bláfoss R, Sundstrup E, et al. (2020). Association between physical work demands and work ability in workers with musculoskeletal pain: Cross-sectional study. *BMC Musculoskeletal Disorders*, 21:1-8.

Özdinçler A, Fiziksel Modaliteler ve Elektroterapi, 2.baskı, İstanbul Tıp Kitabevi, İstanbul, 2016

WHO. (2020). Coronavirus disease (COVID-19) pandemic.https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Erişim Tarihi: 29 Aralık 2021.

Yang LL & Yang T. (2020). Pulmonary rehabilitation for patients with coronavirus disease 2019 (COVID-19). *Chronic Dis Transl Med,* 6(2):79-86.

Yüksel İ, Angın E. & Sezerel B. (2019). Spor Yaralanmalarının Rehabilitasyonunda Nöromusküler Yaklaşımlar. Türkiye Klinikleri Fizyoterapi ve Rehabilitasyon, 26-36

Zhao HM, Xie YX & Wang C. 2020. Recommendations for respiratory rehabilitation in adults with coronavirus disease 2019. *Chin Med J*, 133 (13), 1595-1602.

X-Ray Effects on Chondrocyte Volume from Bovine Articular Cartilage

Ekrem ÇİÇEK[1]

Introduction

The amount of ionizing radiation that a person is exposed to has sharply risen over the last decades (Leuraud & et al., 2015). Medical radiation exposure is the largest part of the total amount of radiation. The average yearly medical exposure dose had increased almost 600% (0.5 mGy in 1982 and 30 mGy in 2006) in the United States (Mettler & et al., 2008). The largest parts are coming from nuclear medicine applications and computed tomography scanning. Occupational sources of radiation exposure are also important (Leuraud & et al., 2015; Mettler & et al., 2008).

The effects of X-ray radiation on the mechanical properties of articular cartilage was studied previously. It was found that ionizing radiation caused adverse effects on the function of articular cartilage. The Young's Moduli for the controls were significantly higher than the ionizing radiation exposed samples (Cash & Dean, 2019; Lindburg, Willey & Dean, 2013). The same effects of ionizing radiation found by other studies (Cicek, 2016; Kuzu & Cicek, 2019). Ionizing radiation increased cellular apoptosis both in vivo and in vitro studies (Rocha & et al., 2016).

The effects of ionizing radiation on articular cartilage are characterized as negative effects, no effects and positive effects in the literature (Cash & Dean 2019). Previous studies on cartilage response to ionizing radiation are inconsistent and radiation effects on articular cartilage is unclear (Hugenberg, Myers & Brandt, 1989; Willey & et al., 2013).

Articular cartilage is the smooth connective tissue and is 2 to 4 mm thick (Sophia, Bedi & Rodeo, 2009). The chondrocytes are the only cells in articular cartilage. Chondrocyte shapes are depth-dependent in articular cartilage. There are flat chondrocytes, spherical chondrocytes and elongated chondrocytes in the superficial, middle and radial zone, respectively (Sophia, Bedi & Rodeo, 2009). The injury reaction of the tissue is inefficient since articular cartilage is an avascular tissue (Willey & et al., 2013).

It is important to know ionizing radiation effects to balance medical applications against the risks. The aim of this study was to investigate the effects of ionizing radiation on chondrocyte volume from bovine articular cartilage. The size of the chondrocyte was measured from confocal image of articular cartilage.

Materials and Methods

Explant harvest

Mature bovine knees with joint capsules were obtained from a local abattoir (Bud's Custom Meats, Riverside, IA, USA). Osteochondral explants (2.5 cm x 2.5 cm) were carefully excised from the lateral tibial plateaus of the joints under sterile conditions. Then, they were placed immediately in Hanks balanced salt solution (HBSS). HBSS was supplemented with penicillin/streptomycin and amphotericin B. Explants were incubated in culture medium during the whole study to maintain tissue viability (Ramakrishnan & et al., 2011; Cicek & Arikanoglu, 2014; Goodwin & et al., 2010; Martin & et al., 2012).

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X-ray Irradiation

The explants were exposed with X-ray 150 keV, 15 mA. The distance between X-ray source and samples was 115 cm. The total exposed dose was 100 mGy. Imaging process started 3 hours after the irradiation.

Confocal imaging

The explants were stained with dihydroethidium (DHE, Invitrogen) and calcein AM (Invitrogen) for 30 min. The stained explants were scanned on a BioRad 1024 Confocal Microscope equipped with a Krypton/Argon laser (Biorad Laboratories Inc., Hercules, CA). Images of the osteochondral explants were performed with a 20× water immersion lens. The random sites close to the radial center were imaged from the surface to a depth of ~200 μm in 20μm intervals. 20× image stacks were acquired from randomly selected imaging sites. 20× image stacks were utilized for chondrocyte shapes analysis. More information regarding imaging conditions was described previously (Ramakrishnan & et al., 2011; Cicek & Arikanoglu, 2014; Goodwin & et al., 2010; Martin & et al., 2012).

Image Analysis

The ImageJ version 1.53 was used to analyze live cells from confocal image stacks. We measured the surface area of the chondrocyte, perimeter, long (major) and short axis (minor) inside the cell, height of cell, cell angle, circularity of the chondrocyte and aspect ratio. The size (area) of the chondrocyte was selected between 20 to 80 μm2. The circularity of the chondrocyte was among 0.01-1.00. ImageJ version 1.53 for Windows was obtained from the NIH website (ImageJ, Rasband, W.S., U. S. National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/).

Results

Table 1 presents the chondrocytes parameters for the control group.

Table 2 shows the chondrocytes parameters for the X-ray exposed group.

The p values show the significance between the control group and X-ray exposed group. The volume ($p= 0.001$), area ($p < 0.001$), perimeter ($p < 0.001$), major axis ($p < 0.001$) and minor axis ($p= 0.006$) were significant. The angle of chondrocytes ($p = 0.820$), circularity ($p =0.139$) and aspect ratio ($p =0.343$) were insignificant.

Table 1. The chondrocytes parameters for the control group.

Depth (μm)	Number of cells	For each depth the mean values and standard errors of chondrocytes							
		Volume (μm^3)	Area (μm^2)	Perimeter (μm)	Major axis	Minor axis	Angle	Circularity	Aspect ratio
0	30	1496.92 ± 46.95	86.74 ± 2.51	37.29 ± 0.63	13.32 ± 0.36	8.38 ± 0.23	88.19 ± 8.96	0.79 ± 0.02	1.65 ± 0.08
-40	30	1205.31 ± 67.68	80.79 ± 4.32	35.50 ± 1.13	12.91 ± 0.55	7.96 ± 0.29	100.86 ± 9.39	0.80 ± 0.02	1.69 ± 0.11
-80	30	1247.07 ± 60.74	84.07 ± 4.04	35.66 ± 0.97	12.99 ±0.42	8.17 ±0.24	77.74 ±7.13	0.82 ±0.01	1.62 ±0.06
-120	30	1130.03 ± 51.16	75.17 ± 3.31	33.75 ± 1.01	12.27 ± 0.51	7.84 ± 0.23	80.11 ± 9.00	0.83 ± 0.02	1.61 ± 0.09
-160	30	1168.24 ± 57.58	78.00 ± 3.89	34.57 ± 1.27	12.16 ± 0.53	8.16 ± 0.22	90.38 ± 7.88	0.83 ± 0.02	1.53 ± 0.09
-200	30	1221.57 ± 73.98	73.62 ± 4.10	34.86 ± 1.38	12.66 ± 0.59	7.39 ± 0.23	86.63 ± 8.26	0.77 ± 0.02	1.76 ± 0.10

Table 2. The chondrocytes parameters for the X-ray exposed group.

Depth (μm)	Number of cells	For each depth the mean values and standard errors of chondrocytes							
		Volume (μm³)	**Area (μm²)**	**Perimeter (μm)**	**Major axis**	**Minor axis**	**Angle**	**Circularity**	**Aspect ratio**
		p = 0.001	p < 0.001	p < 0.001	p < 0.001	p = 0.006	p = 0.820	p =0.139	p =0.343
0	30	851.75 ± 49.59	56.93 ± 3.31	29.03 ± 0.97	10.50 ± 0.40	6.79 ± 0.22	103.10 ± 9.55	0.84 ± 0.01	1.57 ± 0.06
-40	30	980.21 ± 56.31	65.64 ± 3.80	31.18 ± 0.96	11.13 ± 0.41	7.40 ± 0.22	105.58 ± 9.14	0.83 ± 0.01	1.52 ± 0.15
-80	30	996.36 ± 51.96	66.92 ± 3.43	32.30 ± 0.97	11.93 ±0.44	7.21 ±0.28	94.80 ±11.64	0.81 ±0.01	1.70 ±0.08
-120	30	938.98 ± 43.59	63.48 ± 2.87	31.17 ± 0.86	11.36 ± 0.39	7.10 ± 0.22	78.82 ± 10.87	0.82 ± 0.02	1.65 ± 0.08
-160	30	999.35 ± 40.22	66.93 ± 2.69	31.94 ± 0.80	11.63 ± 0.42	7.39 ± 0.23	79.22 ± 9.97	0.83 ± 0.02	1.64 ± 0.10
-200	30	1055.16 ± 51.15	70.84 ± 3.41	32.72 ± 0.89	11.47 ± 0.35	7.82 ± 0.26	71.76 ± 9.86	0.82 ± 0.01	1.51 ± 0.07

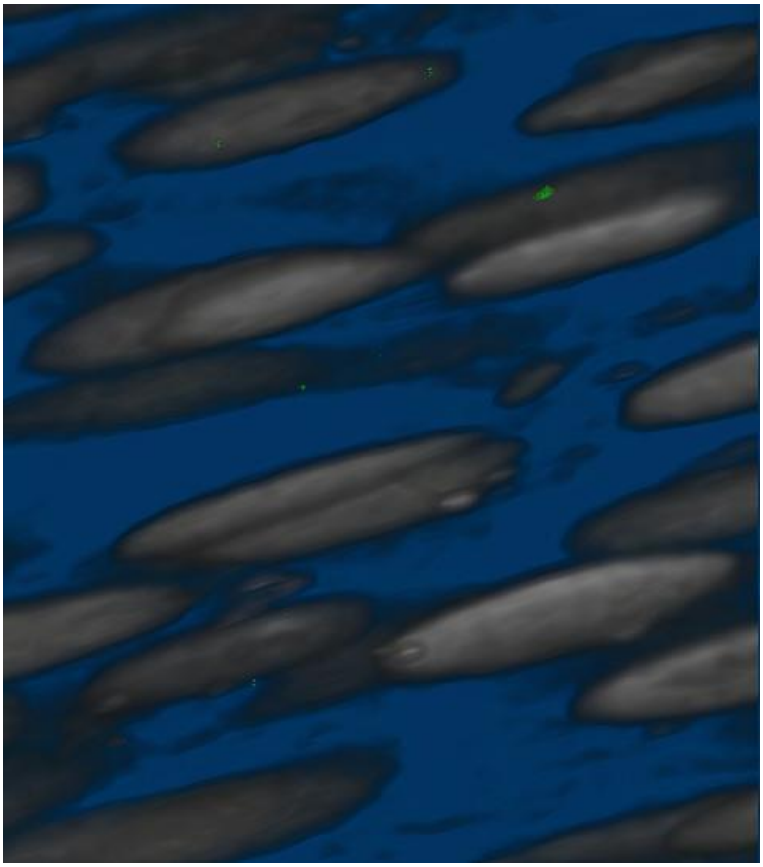

Figure 1. Confocal image from bovine articular cartilage

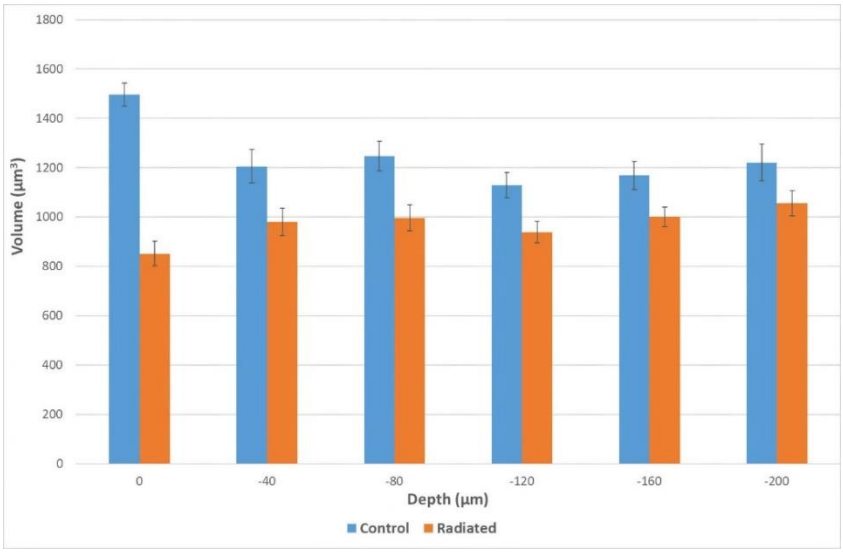

Figure 2. The depth-dependent volume of chondrocyte for control and irradiated groups

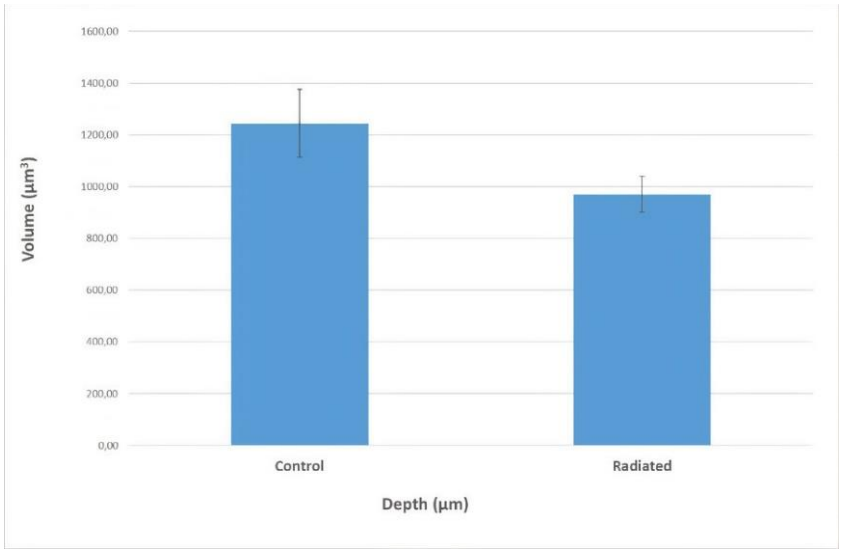

Figure 3. The mean value of chondrocytes volume for control and irradiated groups

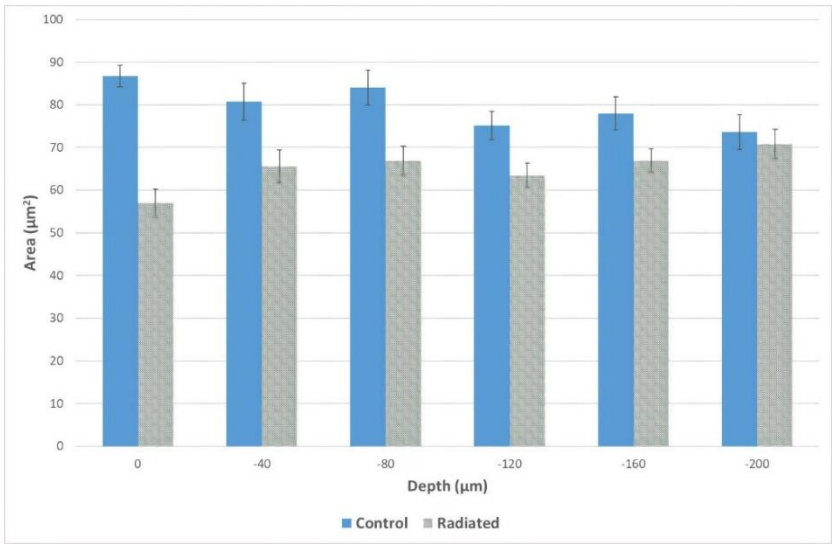

Figure 4. The depth-dependent area of chondrocyte for control and irradiated groups

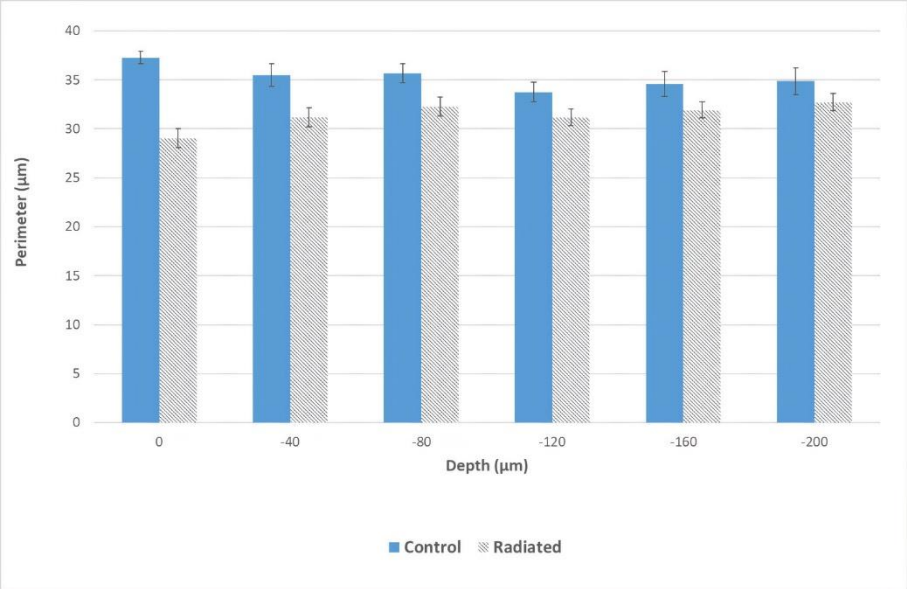

Figure 5. The depth-dependent perimeter of chondrocyte for control and irradiated groups

Figure 1. shows confocal image from bovine articular cartilage. The volume of the chondrocyte can be seen in this image. Figure 2 demonstrates the depth-dependent volume of chondrocyte for control and irradiated bovine articular cartilage. The chondrocyte volume in the control group is higher than the X-ray exposed group for all depth of articular cartilage. The lowest and highest mean volume of chondrocytes were 852 µm3 and 1055 µm3, respectively for the exposed group.

Figure 3 presents the mean value of chondrocytes volume for control and irradiated bovine articular cartilage. The mean value of chondrocyte volume in the control group was 1245 µm3, while it was 970 µm3 in the exposed group. The mean value of chondrocyte volume in the control group was significantly higher than the X-ray exposed group. The mean value of chondrocyte volume was significant between the groups ($p= 0.001$).

Figure 4 shows the depth-dependent area of chondrocyte for control and irradiated bovine articular cartilage. The chondrocyte area in the control group is higher than the X-ray exposed group for all depth of tissue.

Figure 5 demonstrates the depth-dependent perimeter of chondrocyte for control and irradiated bovine articular cartilage. The chondrocyte perimeter in the control group is higher than the X-ray exposed group for all depth of cartilage.

Statistical analyses were performed using the Minitab 19 software. One-way analysis of variance (ANOVA) was used for statistical analysis. A p-value less than 0.05 was considered significant. The depth-dependent measured chondrocyte volume was demonstrated for control and radiated groups. The volume of chondrocytes was decreased in the exposed group. The results were significant ($p= 0.001$).

Discussion

Reduction in matrix metabolism is displayed as the reason of cartilage weakening at articular surfaces in response to irradiation which, due to permanent deterioration of mechanical properties, may contribute to overall joint erosion (Willey & et al., 2013), joint failure (Kolár, Vrabec & Chyba, 1967) and osteoarthritis (Goldring & Marcu, 2009).

It has been shown to induce senescence among young rabbit articular chondrocytes early after radiation exposure (Hong & et al., 2010). It has been reported that radiation exposure of rabbit knee with a total dose of 5 Gy does not affect cartilage mechanical properties, although chondrocyte clustering changes were seen (Öncan & et al., 2013).

Since types and exposure amounts are varying, ionizing radiation effects on articular cartilage is not clear. Degradation of cartilage following exposure may be dependent on the radiation model and radiation dose (Hugenberg, Myers & Brandt, 1989; Jikko & et al., 1996). Ionizing radiation (2 Gy and 10 Gy doses) induced active degradation of matrix and reduced proteoglycan synthesis in human and pig cartilage and chondrocytes. However, the nature of the degradation and impaired production of proteoglycans following ionizing radiation exposure is not clear (Willey & et al., 2013).

A major pathway of active degradation of articular cartilage after radiation exposure may be acute loss of glycosaminoglycans (Cornelissen, Thierens & De Ridder, 1996). The glycosaminoglycan content a week after the irradiation in a study was 55% lower in irradiated explants than controls, in which recovery from the initial loss is not likely (Rojas & et al., 2014). It was shown that 2 Gy X-ray exposure reduces stiffness in a 3D model of porcine articular cartilage. This reduction is related with a large release of glycosaminoglycans from irradiated explant 24 h after exposure (Saintigny & et al., 2005). It is believed that glycosaminoglycan loss is not the only factor affecting the modulus after radiation exposure, although other causes remain undefined. It was not possible to determine if changes in cross-linking or other structural damage are contributing to the reduction in modulus after radiation exposure (Lindburg, Willey & Dean, 2013; Willey & et al., 2013; Hong & et al., 2010).

It was reported that X-ray exposure with 20 Gy decreased type II collagen and glycosaminoglycan production (Cornelissen, Thierens & De Ridder, 1993). They also showed that 50 Gy X-ray dose reduces dramatically extracellular matrix components in 14 days in mature cartilage (Cornelissen, Thierens & De Ridder, 1993a, 1993b). It was shown that type II collagen reduction after X-ray exposure is linked with induction of type I collagen, especially into pericellular spaces of chondrocytes (Muhonen & et al., 2006). It was proposed that radiation-induced changes in oxygen concentrations might increase in type II collagen. Furthermore, it has been suggested that reactive oxygen species (ROS) suppress proteoglycan synthesis. Radiation effect on proteoglycan synthesis might reflect effects of ROS, since free radicals suppress proteoglycan synthesis (Bates, Johnson & Lowther, 1985a, 1985b).

These dramatic failures of fibril organization cause a loss of mechanical properties in mature cartilage (Muhonen & et al., 2006). Moreover, it causes a functional decline of cartilage health, in patients treated with ionizing radiation (Cornelissen, Thierens & De Ridder, 1996; Rojas & et al., 2014). Constant radiation caused changes in extracellular matrix metabolism could deteriorate cartilage mechanical properties after exposure, by extracellular matrix degradation (Saintigny & et al., 2005).

It was reported that lower doses of ionizing radiation may cause a significant decrease in the compressive stiffness of articular cartilage at multiple length scales. Young's modulus reduction in mechanical properties following radiation exposure suggests that alterations are occurring in the matrix. This reduction in Young's modulus after radiation exposure is not solely due to the observed loss of proteoglycans, which are only estimated to be responsible for about 50% of the compressive stiffness of cartilage (Buschmann & Grodzinsky, 1995).

In a previous study, application of X-ray at 4.8 mGy in compressive moduli of equine articular tissue experiments produced similar results with another study (Kuzu & Cicek, 2019), in the decreasing of stiffness (Cicek, 2016).

In a previous study (Bates, Johnson & Lowther, 1985b), in which the differential effect of C-ions versus X-rays was investigated in human articular cartilage, exposure to X-ray and C-ions were not able to induce neither necrosis nor apoptosis in either 2D or 3D culture. The previous studies are supporting the relative radiation resistance of chondrocytes, regardless of the radiation quality in rabbit articular chondrocytes (Hong & et al., 2010), porcine and human articular cartilage (Willey & et al., 2013). However, exposure to ionizing radiation might induce bone differentiation and senescence (Saintigny & et al., 2005), the latter is an important factor of potential cartilage attrition (Hamdi & et al., 2016).

Conclusions

Application and exposure of ionizing radiation is increasing globally. Articular cartilage is the thin and white connective tissue. Chondrocytes are the cells in articular cartilage. The effects of ionizing radiation depend on type and amount of ionizing radiation. In this study, ionizing radiation effects on chondrocyte volume was investigated. We also investigated the changes of chondrocyte shape after ionizing radiation exposure. The results were reported according to the depth of chondrocyte since it displays zonal shape dependency. Further research is necessary to identify potential molecular targets for the functional deficits in cartilage post-irradiation. In conclusion, chondrocyte volume of bovine articular cartilage was found to reduce after exposure to ionizing radiation with X-ray. We demonstrated ionizing radiation (X-ray) effects on chondrocyte volume from confocal image of bovine articular cartilage.

REFERENCES

Leuraud, K, Richardson, D. B., Cardis, E., Daniels, R. D., Gillies, M., O'Hagan, J. A., et al. (2015). Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study. *Lancet Haematology,* 2(7), e276-281. Doi: 10.1016/S2352-3026(15)00094-0

Mettler, F. A. J. R., Thomadsen, B. R., Bhargavan, M., Gilley, D. B., Gray, J. E., Lipoti, J. A., et al. (2008). Medical radiation exposure in the U.S. in 2006: preliminary results. *Health Physics,* 95(5), 502-507. Doi: 10.1097/01.HP.0000326333.42287.a2

Cash, H. & Dean, D. (2019). The effects of low-dose radiation on articular cartilage: a review. *Journal of Biological Engineering,* 7, 13-21. Doi: 10.1186/s13036-018-0125-4

Lindburg, C. A., Willey, J. S. & Dean, D. (2013). Effects of low dose X-ray irradiation on porcine articular cartilage explants. *Journal of Orthopaedic Research,* 31(11), 1780-1785. Doi: 10.1002/jor.22406

Cicek, E. (2016). Effect of X-ray irradiation on articular cartilage mechanical properties. *Acta Physica Polonica A,* 129(2), 200–202. Doi: 10.12693/APhysPolA.129.200

Kuzu, N. & Cicek, E. (2019). Effects of radiopharmaceuticals on articular cartilage's mechanical properties. *Nukleonika,* 64(2), 71-74. Doi: 10.2478/nuka-2019-0009

Rocha, F. S., Limirio, P. H., Zanetta-Barbosa, D., Batista, J. D. & Dechichi, P. (2016). The effects of ionizing radiation on the growth plate in rat tibiae. *Microscopy Research and Technique,* 79(12), 1147-1151. Doi: 10.1002/jemt.22769

Hugenberg, S. T., Myers, S. L. & Brandt, K. D. (1989). Suppression of glycosaminoglycan synthesis by articular cartilage, but not of hyaluronic acid synthesis by synovium, after exposure to radiation. *Arthritis & Rheumatism,* 32(4), 468–474. Doi: 10.1002/anr.1780320417

Willey, J. S., Long, D. L., Vanderman, K. S. & Loeser, R. F. (2013). Ionizing radiation causes active degradation and reduces matrix synthesis in articular cartilage. International Journal of Radiation Biology, 89(4), 268-277. Doi: 10.3109/09553002.2013.747015

Sophia Fox, A. J., Bedi, A. & Rodeo, S. A. (2009). The basic science of articular cartilage: structure, composition, and function. *Sports Health: A Multidisciplinary Approach,* 1(6), 461-468. Doi: 10.1177/1941738109350438

Ramakrishnan, P. S., Pedersen, D. R., Stroud, N. J., McCabe, D. J. & Martin, J. A. (2011). Repeated measurement of mechanical properties in viable osteochondral explants following a single blunt impact injury. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine,* 225(10), 993-1002. Doi: 10.1177/0954411911413063

Cicek, E. & Arikanoglu, A. (2014). Morphological Variations of Chondrocytes in Bovine Articular Cartilage Using Confocal Laser Scanning Microscopy. *Acta Physica Polonica A,*125(4), 898-901. Doi: 10.12693/APhysPolA.125.898

Goodwin, W., McCabe, D., Sauter, E., Reese, E., Walter, M., Buckwalter, J. A. & Martin, J. A. (2010). Rotenone prevents impact-induced chondrocyte death. *Journal of Orthopaedic Research,* 28(8), 1057-63. Doi: 10.1002/jor.21091

Martin, J. A., Martini, A., Molinari, A., Morgan, W., Ramalingam, W., Buckwalter, J. A. & McKinley, T. O. (2012). Mitochondrial electron transport and glycolysis are coupled in articular cartilage. *Osteoarthritis and Cartilage,* 20(4), 323-329. Doi: 10.1016/j.joca.2012.01.003

Saintigny, Y., Cruet-Hennequart, S., Hamdi, D. H., Chevalier, F. & Lefaix, J. L. (2005). Impact of therapeutic irradiation on healthy articular cartilage. *Radiation Research,*183(2), 135–146. Doi: 10.1667/RR13928.1

Kolár, J., Vrabec, R. & Chyba, J. (1967). Arthropathies after irradiation. *The Journal of Bone and Joint Surgery American,* 49(6), 1157–1166.

Goldring, M. & Marcu, K. B. (2009). Cartilage homeostasis in health and rheumatic diseases. *Arthritis Research & Therapy,* 11(3), 224-240. Doi: 10.1186/ar2592

Hong, E. H., Lee, S. J., Kim, J. S., Lee, K. H., Um, H. D., Kim, J. H, et al. (2010). Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase. *Journal of Biological Chemistry,* 285(2), 1283–1295. Doi: 10.1074/jbc.M109.058628

Öncan, T., Demrag, B., Ermutlu, C., Yalçinkaya, U. & Özkan, L. (2013). Effect of low-dose irradiation on structural and mechanical properties of hyaline cartilage-like fibrocartilage. *Acta Orthopaedica et Traumatologica Turcica,* 47(2), 127–133. Doi: 10.3944/AOTT.2013.2709

Jikko, A., Hiranuma, H., Iwamoto, M., Kato, Y., Okada, Y. & Fuchihata, H. (1996). Effects of X irradiation on metabolism of proteoglycans. *Radiation Research,* 146(1), 93–99. Doi: 10.2307/3579401

Cornelissen, M., Thierens, H. & De Ridder, L. (1996). Effects of ionizing radiation on cartilage: emphasis on effects on the extracellular matrix. *Scanning Microscopy,* 10(3), 833–840.

Rojas, F. P., Batista, M. A., Lindburg, C. A., Dean, D., Grodzinsky, A. J., Ortiz, C. & Han, L. (2014). Molecular adhesion between cartilage extracellular matrix macromolecules. *Biomacromolecules,* 15(3), 772-780. Doi: 10.1021/bm401611b

Cornelissen, M., Thierens, H. & De Ridder, L. (1993). Effects of ionizing radiation on the size distribution of proteoglycan aggregates synthesized by chondrocytes in agarose. *Scanning Microscopy,* 7(4), 1263-1268.

Cornelissen, M., Thierens, H. & De Ridder, L. (1993). Radiation effects on the matrix synthesis in non-ossifying embryonic cartilage in vitro: a functional and morphological study. *Tissue Cell,* 25(3), 343-350. Doi: 10.1016/0040-8166(93)90076-W

Muhonen, A., Säämänen, A. M., Peltomäki, T. & Happonen, R. P. (2006). The effect of irradiation and hyperbaric oxygenation (HBO) on extracellular matrix of the condylar cartilage after mandibular distraction osteogenesis in the rabbit. *International Journal of Oral and Maxillofacial Surgery,* 35(1), 79–87. Doi: 10.1016/j.ijom.2005.06.016

Bates, E. J., Johnson, C. C. & Lowther, D. A. (1985). Inhibition of proteoglycan synthesis by hydrogen peroxide in cultured bovine articular cartilage. *Biochimica et Biophysica Acta - General Subjects,* 838(2), 221–228. Doi: 10.1016/0304-4165(85)90082-0

Bates, E. J., Lowther, D. A. & Johnson, C. C. (1985). Hyaluronic acid synthesis in articular cartilage: an inhibition by hydrogen peroxide. *Biochemical and Biophysical Research Communications,* 132, 714–720. Doi: 10.1016/0006-291X(85)91191-X

Buschmann, M. D. & Grodzinsky, A. J. (1995). A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics. *Journal of Biomechanical Engineering,* 117(2), 179–192. Doi: 10.1115/1.2796000

Hamdi, D. H., Chevalier, F., Groetz, J. E., Durantel, F., Thuret, J. Y, Mann, C. & Saintigny, Y. (2016). Comparable senescence induction in three-dimensional human cartilage model by exposure to therapeutic doses of X-rays or C-ions. *International Journal of Radiation Oncology Biology Physics,* 95(1), 139-146. Doi: 10.1016/j.ijrobp.2016.02.014

Rational Use of Medicaments

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Introduction

The definition of rational drug use was first discussed at a meeting held in Nairobi, Kenya in 1985. According to the definition made here, rational drug use; "It is all of the rules that require patients to take the drugs they need to use in accordance with their clinical needs, in an amount that meets their personal needs, in a sufficient time frame, at the lowest cost to themselves and the society" (Holloway & Dijk , 2011).

The use of drugs, which cannot be considered rational, is known as a type of habit that is difficult to correct. Although irrational drug use seems to be a serious problem in all countries, it is more common in underdeveloped or developing countries (Acar & Yeğinoğlu, 2005). In addition to insufficient education level, economic, sociocultural and regulatory mechanisms are important factors in irrational drug use (Kaya et al., 2015).

The issue of rational drug use plays an important role in the interaction of individuals' personal health and social and economic structures and being an effective element of the health care management system (Amin et al., 2011).

Various factors are effective on rational drug use. One of the most important of these is the level of health literacy. It has a direct relationship. These two elements are concepts that complement each other and one improves the other. Individuals with a high level of health literacy are also expected to have a high level of rational drug use (İncesu, 2017).

World Health Organization for rational drug use is guiding in the drug treatments that physicians will recommend to patients and in points such as drug prescribing. This guideline includes criteria such as being effective, safe, appropriate and economical when choosing drugs. These issues are very important in prescribing drugs to patients (Gülhan, 2013; Erden & Göçmez, 2004; Akıcı et al., 2002).

All behavior, except for the rational use of drugs, can be described as irrational drug behavior. Irrational drug use behavior emerges as a result of patients not getting enough benefit from drugs. This situation creates an extremely negative impact on the economies of countries (Ekenler & Koçoğlu, 2016).

Many processes are carried out to ensure the effective, efficient and functional delivery of health services. Due to the scarcity of resources, their efficient use and the implementation of policies aimed at saving have gained importance. Thus, with the increasing health needs, the importance of making rational decisions about using scarce resources more effectively increases (Ateş & Aba, 2019). Living healthy is the most basic right of every human being. For this reason, it is the most natural right of individuals to want to be treated when their health integrity is impaired (Sevim & Nal, 2021). Policies for rational drug use are important for the sustainability of this system.

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Rational Drug Use

The first step in rational drug use is the creation of the Primary Essential Medicines list, which was determined by the World Health Organization (WHO) in 1977 to assist in the preparation of national drug lists to be created in line with the needs of countries. Rational drug use, with its current definition; In a meeting organized by WHO in Kenya in 1985, it was stated as *"The set of rules that require patients to take drugs suitable for their clinical needs, in doses that meet their personal needs, for a sufficient period of time, at the lowest cost for themselves and the society"* . *Following this first step,* the International Network for Rational Drug Use (INRUD) was established in 1989 in order to carry out multicenter research projects from a single center and to support the rational use of drugs (WHO, 2002).

Ministry of Health, rational drug use; It defines it as the planning, execution and monitoring process that enables the use of drugs in an economical, effective and safe manner (TC Ministry of Health, General Coordination of Health Project, 1993). According to the definition of the World Health Organization in 2002, it is the planning process in a way that can provide the rational use of drugs according to the clinical results of the individuals, in the appropriate dose according to their individual characteristics, at the lowest cost for a sufficient period of time (WHO, 2002).

The primary goal in rational drug use is to prevent all the physiological, biological and psychological problems that may arise with the wrong use of drugs, and to try to minimize social and economic damage as much as possible (Ulusoy & Sumak, 2011). While one-third of the world's population struggles to purchase even essential drugs, the ease of access to drugs by some people without a prescription, and the increase in the amount and types of drugs with each passing day show the necessity of the concept of rational drug use (WHO, 2002).

According to the World Health Organization, a drug is defined as *"a substance or product that is used or intended to be used to modify or examine physiological systems or pathological conditions for the benefit of the recipient, and to provide mental or physical well-being in the user"* (WHO, 2002). Medicines are an essential component of health. In this context, it plays a vital role in healing patients and saving lives (Angamo, Wabe & Raju, 2011).

The use of drugs has an important place in the health services offered in terms of the benefits it will provide in the lives of people in the cure of many diseases and in the mortality and morbidity rates caused by many diseases, or vice versa, in the correct and sufficient consumption (Büyükturan & Büyükturan, 2017).

The drug should be used rationally, not randomly. In addition to the economic burden it reflects on individuals and society, unconscious drug use can even result in death. A drug is a product used to protect or regain health, rather than irrational consumption. For this reason, it is necessary to consume the drug consciously and in sufficient quantities (Akıcı et al., 2011; Kurt et al., 2020).

Medicines have an undeniable importance in protecting, maintaining and improving the health of individuals (Memişoğlu, 2016). If drugs and treatments are not managed correctly, they can cause many health problems and death, in addition to their protective and curative effects. Accordingly, the increase in medical costs makes the rational use of drugs important (Pınar, 2012).

all over the world and in Turkey is a concept whose importance is increasing day by day because the country's resources are not wasted. Compared to developed countries, the amount of drugs used in Turkey is stripped of its therapeutic dimension and takes the form of wasting more resources. In this context, the most important cause of waste in drug consumption is over-the-counter and unconscious consumption. Compared to other countries, the share allocated only to

pharmaceuticals in healthcare payments is 25-30% higher in Turkey (Demiroğlu, Polat & Doğan, 2017).

Research shows that more than half of the drugs in the world are prescribed by doctors, but only half of individuals use these drugs correctly in accordance with recommendations. Considering these rates, it is understood how important rational drug use education is (WHO, 2002; Holloway & Dijk , 2011).

Basic Principles of Rational Drug Use

The prescribing guide, organized by the World Health Organization, is a guide in the drug treatments that physicians will recommend to patients and in points such as drug prescribing. This guideline includes criteria such as being effective, safe, appropriate and economical when choosing drugs. Physicians need to create a list of drugs and treatment models that are compatible with the patient, taking into account the prescribing guidelines (Gülhan, 2013; Erden & Göçmez, 2004; Akıcı et al., 2002).

- Effectiveness: Pharmacological properties of drugs
- Being safe: Side effects due to drug use
- Eligibility: Side effects caused by drugs
- Being economical: Presentation price of drugs

Rational Drug Use Criteria

In order to choose an effective, safe, appropriate and economical drug in the principles of rational drug use, a set of criteria must be met in the planning, functioning and follow-up processes. Drug rationality is to ensure that evidence-based information produced by experimentation and observation is effectively included in the diagnosis and treatment process, and is accessible in terms of financing drugs and when necessary. In this context, certain criteria are needed. Criteria for rational drug use; rational choice of drugs, rationality of the pharmaceutical industry, the process of prescribing and responding to prescriptions, rational consumption of drugs, and the rationality of regulations on drug management (The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Rationality of Pharmaceutical Logistics: It consists of purchasing, storage, inspection and distribution of drugs. Rational financing in the purchasing process of the drug, storage and storage in appropriate conditions, delivery to the point of need in a timely and sound manner, and ensuring that all these steps are properly supervised are among the suggestions regarding the rationality of drug logistics (Uslu & Akçadağ, 2012; The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Rational Selection of Medicines: The physician should make a "rational" (rational) drug selection in accordance with the clinical condition of the patient, of the drugs whose safety and effectiveness have been tested and proven in line with the generally accepted parameters. If a rational choice cannot be made in this regard, ineffective treatments will emerge, leading to unnecessary drug consumption by patients (Sağır & Parlakpınar, 2014). It is recommended to consider standardized treatment protocols, epidemiological data obtained with mortality and morbidity numbers, and the distribution of patient burden in determining the priority drug list (The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Rationality of the Prescribing Process: It is the stage where the physician participates in the rational drug use process. In this process, physicians are the party directly responsible. This stage should begin with the correct diagnosis (Yorulmaz, 2003). This process, which is a part of the treatment; It

includes determining the need for medication, evaluating alternative treatment options, arranging the necessary treatment and making appropriate follow-up. The basis of the prescribing process should be the rational decision of the physician, not the pressure made by the patient or the recommendations of the pharmaceutical companies (The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Rationality of the Prescription Response Process: This process is the point where the pharmacist is involved in the rational use of drugs and his role becomes most evident. Reading the prescription written by the physician correctly and communicating with the physician when necessary, responding to the prescription appropriately (preparing the drugs in a timely manner and presenting them to the patient) is followed by providing appropriate communication, including oral or written education of the patient. In this regard, it is very important to do it without commercial concerns (Yılmaztürk, 2013).

Rational Consumption of Drugs: The parties responsible for rational drug consumption are health personnel, mass media groups, pharmaceutical industries and the most important element, society. In this context, knowing the awareness level of the society and its approaches on this issue play an important role in order for the interventions to be applied to the society to be more effective and appropriate. It is a situation where attention should be paid to raising awareness on health literacy by providing training on disease and drug treatment in the society (Ministry of Health, 2011; The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Drug Information Support: It is known that drug information must be accurate, complete, impartial, up-to-date, usable and accessible at all stages of rational drug use. It is extremely important to update and inspect the books, brochures, programs, websites, pharmaceutical companies, guides and guides that are sources of information when necessary (Yorulmaz, 2003; The Alphabet of Rational Drug Use, 1993, www.e-kutuphane.teb.org.tr).

Responsibility of the Parties in Rational Use of Medicines

It is known that there are many factors such as lack of education, socio -cultural, economic, administrative and regulatory mechanisms that lead to Irrational Drug Use . There are three main parties in rational drug use: the supplying party, the demanding party and the supervisory-regulatory mechanisms (Ekenler & Koçoğlu, 2016; Sürmelioğlu et al., 2015) (Figure 1).

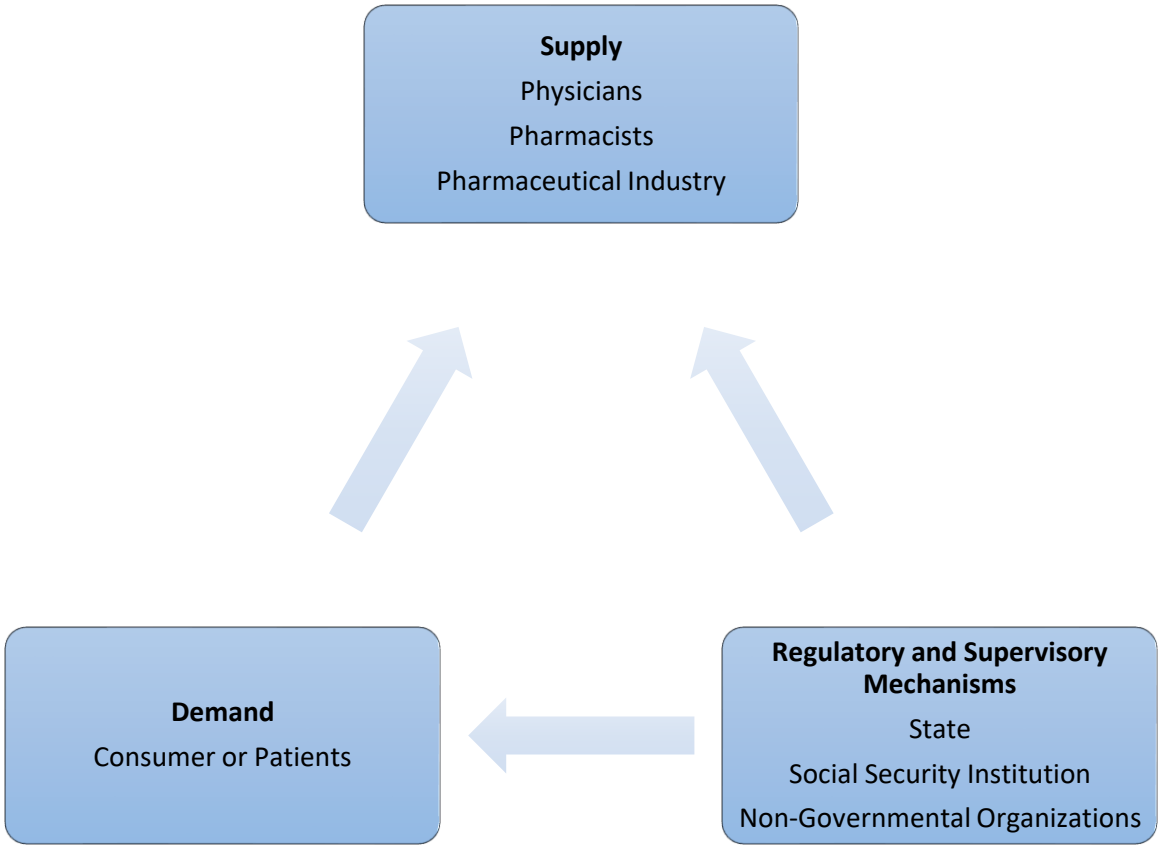

Figure 1. Key stakeholders in rational drug use

Source: (Sürmelioğlu et al, 2015)

Health is an important phenomenon for the individual and society. It is also one of the most basic human rights (Nal, 2021). Supply, demand and regulatory mechanisms have responsibilities for rational drug use in health care.

Responsibility of the Physician: Physicians are known as the occupational group with the highest efficiency in rational drug use. The first step of the triangle of physician, patient and pharmacist in rational drug use can be described as a physician (Saygılı & Özer, 2020).

In addition to making a diagnosis, the physician should fulfill the treatment obligation based on the patient's voluntariness in the process of deciding on the treatment method that is compatible with the patient. The physician, who is in close contact with the patient during the communication process, should inform the patient about the results of the diagnosis and treatment of the disease. The physician should show the necessary care and attention during the treatment applied to the patient (Regulation of Medical Deontology 2015).

In general, physicians, who have great responsibilities in the field of drugs and health services in general, need to be trained on rational drug use and their information on the subject should be kept up-to-date (Ministry of Health, 2011).

Responsibility of the Nurse: In the interaction of physician, nurse and patient, the role of the nurse is primarily communication, education and practice. In this context, physicians and nurses act jointly for the same purpose. Drug treatment to be administered to the patient is a process that starts with the decision of the physician and ends in line with the supervision of the nurse. It is extremely important for the society that nurses maintain the necessary care and awareness in the rational drug use process and maximize the benefit to the health system (Ulupınar & Akıcı, 2015).

The nursing regulation, *"In the implementation of the medical diagnosis and treatment plan; to apply the treatments given by the physician in writing, except for emergencies, to accept the verbal medical request of the treating physician in unexpected or sudden situations that develop in the patient and in the diagnosis and treatment plans that must be applied urgently. In this process, patient and employee safety Statements such as "to monitor the effects of medical diagnosis and treatment interventions on the patient, to keep the necessary records in case of undesirable situations, to notify the physician* and to take the necessary *measures"* are among the duties of the nurse (TC Resmi Gazete, 2010).

Pharmacist's Responsibility: Pharmacists are responsible for presenting drugs to patients and informing patients about the drugs they use. In the rational drug use process, the second responsibility falls on pharmacists (Barutçu, Tengilimoğlu & Naldöken, 2017).

Although it is the physician who provides the treatment of the diagnosed patient, the last person the patient consults before the treatment is usually pharmacists (Toklu & Dülger, 2011). In addition, pharmacists are the first healthcare professionals consulted when a patient wishes to self-medicate. For this reason, it should guide the patient to the correct use of the drug by supplying the drug in the most correct way (Alpdoğan & Altındiş, 2019).

Since the pharmacist is the last step of the treatment process, the role of giving the prescribed drug to the patient, educating the patient about how to use the drug and increasing patient compliance is extremely important in rational drug use (Toklu, 2015).

Patient Responsibility: One of the responsibilities of the society regarding rational drug use is the rational consumption of drugs. This responsibility; should be distributed among the health care workers and mass communicators together with the society (Yorulmaz, 2003).

Rational drug use is the habit that patients acquire during drug use and the process of adherence to the treatment shown accordingly. It is very important to use the prescribed drugs in the amount, time and in accordance with the recommendations made by the physicians. In cases where these items are not fulfilled, the efficiency of other treatment options for the treatments is greatly reduced (Vançelik, Çalıkoğlu & Güraksın, 2006). Misuse or abuse of drugs threatens patient safety and leads to waste of resources in this context (Altındiş, 2020).

The last element of the quartet, which has the most important obligation in rational drug use, is the patient. The use of the prescribed drugs by the patient in accordance with the recommended dose, duration, interval and warnings is considered as patient compliance. The effectiveness of all steps in rational drug use depends on patient compliance. In order to increase compliance, the physician's allocation of sufficient time to the patient, the education of the patient, the delivery of clearly written drug prospectuses, dose scales, and the reinforcement by nurses in clinics and pharmacists, who are the last factor in pharmacies, have a significant impact (State Planning Organization, 2001).

Regulatory and Supervisory Mechanisms: Many organizations and societies such as the Ministry of Health, health institutions, health personnel, policy makers, reimbursement agency, NGOs and the media are responsible parties for rational drug use (Ministry of Health, 2011). Pharmaceutical industry, which is one of the parties to rational drug use, is also the party that has the obligation to provide health workers with accurate information and appropriate training about their current products and clearly inform them about how to use drugs (Pharmaceutical Industry Employers' Union, 2000).

The stages of reaching the consumer of the drug are production, storage and logistics. All processes are under supervision and control in order to offer safe and effective products to consumers, and all processes are controlled to ensure that the quality standards of drugs produced in the country and imported from abroad are met in a certain amount. All stages in the field of inspection are carried out in Turkey by the *"Turkish Medicines and Medical Devices Agency, Drug*

Inspection Department" (Oktay, 2006).

Family Medicine and Rational Drug Use

In Turkey, where drug use habits are high and the share of drug expenditures in health expenditures is high, the necessity of economical and rational drug use has come to the fore once again with the adoption of family medicine practice (Ministry of Health, 2011).

One of the important indicators in the evaluation of the level of rational drug use of family physicians, who play an important role in the diagnosis and treatment of patients in primary health care services, is the prescriptions offered by the physicians to the patients. Recognition of the contents of the prescription written by the family doctor or being aware of the prescription; It can be shown as a guiding resource in identifying the problems experienced in drug use and eliminating the problems in this regard (Aksoy, Alkan & İşli, 2015). One of the rational drug indicators defined by the World Health Organization is the antibiotic prescription rates of physicians (WHO, 2004).

In this context; Yağız (2020) determined the health literacy levels and knowledge levels of university students studying at Kocaeli University in the 2019-2020 academic year on rational drug use. In addition, the relationship between health literacy and rational drug use levels was examined. As a result of the study, it was determined that 87.6% (n:599) of the students had a high level of rational drug use and 12.4% (n:85) had a low level of rational drug use.

In the researches, it is thought that irrational behaviors are common in the society in rational drug use of individuals, therefore it is thought that more studies on the subject will be positive in terms of public awareness.

Rational Drug Use Practices in Turkey and in the World

Depending on the developments in the world in the current century , studies on rational drug use are carried out in Turkey according to WHO recommendations. In this regard, the *"Guide to good The resource named " Prescribing " was* translated as *"Guide to Good Prescribing"* by the Ministry of Health in Turkey and *the "Rational Drug Use Program"* was initiated. The guide also covers rational drug use in the light of the basic principles of general drug treatments (Turkish Medicines and Medical Devices Agency, 2011).

Rational Drug Use Branch Directorate was established in 2010 under the Ministry of Health, General Directorate of Pharmaceuticals and Pharmacy. The Turkish Medicines and Medical Devices Agency, the responsible institution for pharmaceuticals, was established on November 2, 2011 (www.resmigazete.gov.tr, KHK/663). Again, in 2011, a study called "Rational *Prescribing Approach of Physicians"* was conducted by the School of Public Health affiliated to the Refik Saydam Hygiene Center Presidency of the Ministry of Health (Turkey Rational Medicines Bulletin, 2015).

"Regulation on Classification of Medicinal Products for Human Use " that prescription drugs can only be obtained under prescription within the scope of the rationality of the prescription fulfillment process . Prescription drugs within the scope of this regulation are clearly stated as: *"Even if they are used correctly, they pose a direct or indirect health hazard if they are not used under medical supervision, and they are frequently and widely misused, and as a result, they pose a direct or indirect danger to human health"* (Resmi Gazete, 2005, www.resmigazete.gov.tr).

Another study was prepared from the National English Formulary, which includes drug-related formulas, to our British National Formulary - BNF literature in 1999 with a team formed by Prof. Dr. S. Oğuz Kayaalp. The sixth and last edition of the "Turkish Medication Guidelines", which was regularly updated in line with the developments, was published in 2011. (Akıcı, www.tfd.org.tr).

In order to make patients' access to medicines from pharmacies safer, they are monitored in a virtual environment with the "Drug Tracking System". The Pharmaceutical Tracking System and DataMatrix Application was implemented on October 1, 2009 in order to prevent counterfeit medicines by keeping track of the medicines and to prevent counterfeit medicines and to generate data for the policies to be prepared regarding medicines (Pharmaceutical Tracking System, www.its.gov.tr).

In addition to these, Hospital Service Quality Standards; The Guide on Rational Drug Use and the Guide on Rational Drug Use Sessions (issued for drug and product license holders) are published through the Ministry of Health (Turkish Medicines and Medical Devices Agency) in order to involve hospitals in this process and to raise awareness of healthcare professionals and those applying to hospitals. , 2011).

It has found an important research area throughout the country with its studies carried out around the world for thirty years on rational drug use. Activities related to rational drug use started in Turkey in the 1990s. The first step that stands out is *"Problem-Based Rational Treatment Training" in Medical Faculties* It was eliminated with the implementation of the (Akıcı et al., 2011).

"Medium-Term Cooperation Program" that WHO and TR Ministry of Health carried out in coordination starting in 1998, the " Pharmacotherapy Training and Clinical Pharmacology Study Meeting on the Placement of Rational Drug Use Principles in Turkey" was held in 1999 . "Turkish Medicines Guide-TIC" was published in 1999 on the use of physicians and pharmacists. Studies were accelerated in 2003 within the framework of the Health Transformation Program (SDP) (Vançelik, Çalıkoğlu & Güraksın, 2006).

There are 12 basic recommendations put forward by WHO with the aim of promoting rational drug use. These recommendations are shown in table 1:

Table 1. WHO's recommendations for the promotion of rational drug use

1. To create a supervisory and regulatory institution that will monitor the policies regarding drug use.
2. Utilizing clinical findings and treatment guidelines
3. Based on priority treatments in the creation of a list of essential drugs
4. Establishment of drug committees in hospitals and certain districts
5. Ensuring that student physicians studying at medical faculties receive pharmacotherapy training
6. Providing continuous medical education in-service
7. Developing feedback and monitoring systems
8.Using unbiased and complete information data about drugs
9. Informing and educating societies about drugs
10. Prevention of inappropriate financial incentives
11. Implementation of mandatory and appropriate regulations
12. Taking necessary precautions by securing the availability of drugs and staff

Source: (Turkey Bulletin on Rational Use of Medicines, 2015; WHO, 2002).

Rational drug use is a very important health problem all over the world as it is in Turkey. The "National Action Plan for Rational Use of Medicines" was implemented between 2014 and 2017 in order to train the society and health workers on rational drug use, to increase their level of knowledge and awareness, and to ensure the coordination of all activities. The National Action Plan includes a total of 99 activities, consisting of six main titles and twenty basic strategic objectives. One of the most notable activities is the Prescription Information System (RBS), where prescriptions written by physicians can be monitored and evaluated. In addition to this plan, the "Rational Drug Use National Action Plan 2018-2022 Workshop " of the "Rational Drug Use National Action Plan 2014-2017" was held in Ankara in 2018 (Ministry of Health, 2017; Turkish Medicines and Medical Devices Agency, 2018).

Irrational Drug Use

All behavior, except for the rational use of drugs, can be described as irrational drug behavior. Irrational drug behavior emerges as a result of patients not getting enough benefit from drugs. This situation has an extremely negative effect on the economies of the countries. In Turkey, too, irrational drug use behavior causes a higher rate of waste of drugs (Ekenler & Koçoğlu, 2016).

Irrational drug use can lead to serious drug costs, leading to many health problems, including economic problems and even death. In order to find solutions to these important problems, all countries should take direct measures with their health policies and raise the awareness of the society on rational drug use (Sürmelioğlu et al., 2015).

Irrational drug use has become a serious health problem worldwide and in Turkey. Overcoming this problem is through the rational use of personally defined drugs, following the rules set by the WHO, which are universally accepted (Orhaner & Salğın, 2018).

Reasons for Irrational Drug Use

irrational drug use , and among the main problems generally identified in the studies; The antecedents such as prescribing unnecessary amounts of drugs, using unnecessary expensive drugs, not administering drugs correctly, using more antibiotics than necessary or recommending more injections by physicians are among the causes of irrational drug use (WHO, 2002; Sürmelioğlu et al., 2015) . In this context , it is seen that many economic and sociological factors also affect the causes of irrational drug use . It is known that the physician is the basis, but pharmacists, other health personnel, society, non-governmental organizations, the pharmaceutical industry and the state also have important responsibilities (Özata, Aslan & Mete, 2008).

irrational drug use , many factors affect another factor and may complicate the problem. This starts from a low level of education and is based on reasons such as social and economic structure, regulatory and administrative mechanisms (Akıcı et al., 2002).

Unnecessary and inappropriate drug use results are seen as an important public health problem in Turkey and around the world. Irrational drug use may cause decreased adherence to treatment, drug interaction, resistance to certain drugs, recurrence or recurrence of the disease, increased incidence of side effects , and increased treatment costs (Why Rational Drug Use, www.akilciilac.gov.tr/).

Consequences of Irrational Drug Use

Reasons such as irrational drug use, life-threatening danger to the patient, unnecessary consumption of resources, side effects of drugs and the possibility of creating dependency emphasize the necessity of rational use of drugs. If drugs are not used rationally, problems such as disruptions and decreases in patients' adherence to treatment, unexpected results, recurrence of the disease and decreased belief in treatment, increase in the recovery period of the disease, frequent side effects, and increased treatment costs lead to problems. This situation greatly increases the

burden on the economies and health systems of countries. As a result, it causes damage to the trust of the societies in the country's health system (Altindiş, 2017; Özçelikay, 2001; WHO, 1990).

WHO makes recommendations about the irrational use of drugs in order to prevent the negative effects of health on the economy. In this context, it is extremely important to inform physicians, people in the pharmaceutical industry, pharmacists and the public about the rational use of drugs among the recommendations (Sağır & Parlakpınar, 2014; Altındiş, 2017).

Precautions to be Taken Against Irrational Drug Use

In order to prevent or minimize the problems related to irrational drug use, first of all, rational drug use should be ensured. The main points to consider in this regard are;

- The patient's problem must be clearly defined. Undoubtedly, physicians undertake the most important task in this regard (Things to Be Considered, www.akilciilac.gov.tr/).
- In the correct use of drugs, it is extremely important to inform health workers and the community about this issue (Le Grand, Hogerzeil & Haaijer-Ruskamp, 1999).
- to be taken that countries manage their health policies correctly on the rational use of drugs and that physicians and health workers act in a coordinated and conscious manner in this regard (Orhaner & Salğın 2018).
- It is important to increase the amount of lessons related to the problem in addition to the theoretical curriculum during the faculty education period of the physician candidates in terms of reducing the irrational use of drugs (WHO, 2002).
- In order to prevent unnecessary antibiotic use by patients, pharmacies should be directed to physician control before antibiotic sales.
- The society should be kept up-to-date visually and audibly through the media on the rational use of drugs.
- It is important to inform the public that not all drugs on the market will have the same effect on every individual (Ercan & Biçer, 2019).
- Physicians should be prevented from prescribing new and high-priced drugs and making financial profits.
- Increasing the quality of in-service training programs of healthcare professionals and keeping them up to date play an important role in the rational use of drugs.
- In the event that the drug treatment administered by the patient is not completed, waste drugs collection projects should be encouraged in order to prevent child poisoning, drug waste and drug accumulation habits by ensuring the destruction of drugs (Pınar, 2012).

Rational Treatment Process

The rational treatment process begins with the planning of the treatment process after the physician makes the correct diagnosis of the patient in line with the principles of rational drug use. At the stage of diagnosing the patient, the physician sees the patient as a pillar of the treatment process and directs the treatment in the direction it requires. In this context, it starts the treatment process by determining the individual treatment and drug (Akıcı, 2013).

The rational treatment process specified by WHO consists of the following steps (WHO, 1994).

- Determination of the patient's problem by the physician in accordance with the patient's complaint with the correct diagnosis
- therapeutic purpose. At this stage, the goal is "What do you want to achieve with treatment?" is to answer the question. In order to ensure the continuity of treatment compliance between

the physician and the patient, the treatment method should be shared with the patient in a clear language.

- Individualized treatment with the appropriate treatment method and drug selection determined by the physician. While choosing a personalized medicine, principles such as effectiveness, safety, cost and suitability, which are important for rational drug use, should be considered. At this stage, "What are the non-drug treatment options?" search for an answer to the question. It is a priority to provide treatment only by changing the lifestyle. If the change in lifestyle does not have a positive effect in the treatment process, drug therapy should be applied.
- With the drug treatment method, the physician writes a prescription appropriately for the patient and in this context, warns the patient if there is a special situation. Dosage and duration should be determined according to the patient's compliance.
- The last step of the rational treatment process is the physician's evaluation of the treatment applied. At this stage, "Is there a need for the physician to repeat the treatment process with the patient within the scope of the patient's treatment?" The answer to the question is sought (Akıcı, 2013).

Conclusion

The primary goal in rational drug use is to prevent all the physiological, biological and psychological problems that may arise due to the misuse of drugs, and to try to minimize the social and economic damage as much as possible (Ulusoy & Sumak, 2011). While one-third of the world's population struggles to purchase even essential drugs, the ease of access to drugs by some people without a prescription, and the increase in the amount and types of drugs with each passing day show the necessity of the concept of rational drug use (WHO, 2002).

The most basic purpose in rational use is the correct and conscious use of resources. Irrational use causes waste, workload, reductions in service quality and cost increase (Aba, Torun, 2020). The World Health Organization sees it as an extremely important situation to inform physicians, people in the pharmaceutical industry, pharmacists and the public about rational drug use (Sağır & Parlakpınar, 2014; Altındiş, 2017).

Bian et al. (2015) and Demirtas et al. (2018), it has been shown that the level of rational drug use knowledge is higher in young individuals, women, individuals in the high income group, and individuals with high education levels. According to Demirtas et al. (2018), on the other hand, the level of rational drug use was found to be higher in individuals under the age of 30, and it was found to be lower in individuals with regular drug use.

In the study conducted by Kan (2022), it was found that rational drug use differs according to the age of the individuals. In the study, it was observed that the rational drug use scale scores of individuals aged 46 and over were higher than those of other age groups. It was concluded that the presence of chronic disease and regular drug use also affect rational drug use. Considering that age increases the chronic disease state and the associated regular drug use, it can be said that the results of the study are significant. It has been stated that this result may be related to the increase in the incidence of chronic diseases as we age. In addition, it can be considered as an important result considering that 90% of individuals over the age of 65, which is accepted as the old age limit, have at least one chronic disease and 60% of them use at least one drug. In general, the mean score of the rational drug use scale was found to be 38.82±3.90. A score of 35 and above is considered a good result when evaluated as having knowledge of rational drug use. In addition, this result; It was evaluated as a positive result that individuals did not differ between groups according to their age, gender, educational status, income status, occupation, chronic disease status and regular drug use.

It is important when considering the reasons such as irrational drug use, patient's life danger, unnecessary use of resources, side effects of drugs and the possibility of creating addiction accordingly. If drugs are not used rationally, problems such as disruptions and decreases in patients' adherence to treatment, unexpected results, recurrence of the disease and decreased belief in treatment, increase in the recovery period of the disease, frequent side effects, and increased treatment costs lead to problems. This situation greatly increases the burden on the economies and health systems of countries. As a result, it causes damage to the trust of the societies in the country's health system (Altındiş, 2017; Özçelikay, 2001; WHO, 1990).

There are some actions that can be taken to prevent or minimize the problems that occur in irrational drug use. One of the most important of these is that physicians define the patient's problems clearly. Another important issue is raising awareness of the society on the correct use of drugs. Making this awareness by all health professionals, especially physicians, pharmacists and nurses, is important in achieving success. In addition, it can be said that the results can be achieved more easily with the applications to be made within the scope of social marketing.

REFERENCES

Aba, G. & Torun, M. T. (2020). Sağlık hizmetlerinin akılcı kullanımında sevk sisteminin önemi: KBB polikliniğinde bir araştırma. Erdal Eke (Ed.), *Sağlık yönetiminde güncel tartışmalar* içinde (111-128). Ankara: Nobel Akademik Yayıncılık.

Acar, A., Yeğenoğlu S. (2005). Akılcı ilaç kullanımı açısından farmakoekonomi ve hastane formülleri. Ankara Eczacılık Fakültesi Dergisi, 34(33), 207-215.

Akıcı, A. (2013). Akılcı tedavi sürecinde hekimlere yol gösterecek pratik yaklaşımlar. Turkish Family Physician, 4(2).

Akıcı, A. İlaç Formüleri ve Bu Bilgi Kaynağının Akılcıl İlaç Kullanımına Katkısı. http://tfd.org.tr/sites/default/files/Klasor/Dosyalar/ebultenler/ktcg/57_3_a_akici.pdf.

Akıcı, A., Gelal, A., Erenmemişoğlu, A., Melli, M., Babaoğlu, M. & Oktay, Ş. (2011). Akılcı ilaç kullanımı eğitimi uygulama sürecinde Türkiye'deki Tıp Fakültelerinde farmakoloji anabilim dallarının durumunun incelenmesi. Tıp Eğitimi Dünyası, 29(29),11–20.

Akıcı, A., Uğurlu, M.Ü., Gönüllü, N., Oktay, Ş. & Kalaça, S. (2002). Pratisyen hekimlerin akılcı ilaç kullanımı konusunda bilgi ve tutumlarının değerlendirilmesi. Sürekli Tıp Eğitimi Dergisi,11(7),253-257.

Akılcı İlaç Kullanımının Alfabesi, TEB Haber, 1993, 12-14, https://e-kutuphane.teb.org.tr/pdf/tebhaberler/eylul93/8.pdf (Erişim tarihi: 10. 02. 2022).

Aksoy, M., Alkan, A. & İşli, F. (2015). Sağlık Bakanlığı'nın akılcı ilaç kullanımını yaygınlaştırma faaliyetleri. Türkiye Klinikleri Dergisi, 3(1),19–26.

Alpdoğan, C. & Altındiş, S. (2019). Eczacıların akılcı ilaç kullanımı ile ilgili yaklaşımları. Sakarya Tıp Dergisi, 9 (1), 103-112.

Altındiş, S. (2017). Akılcı ilaç kullanımına sistematik bir bakış. J Biotechinol Strateg Heal Res., 1(2),34–38.

Altındiş, S. (2020). Salgınları önleme ve kontrolde sağlık okuryazarlığının katkısı. Sağlık Düşüncesi ve Tıp Kültürü Dergisi, 56, 29-30.

Amin, A., Khan, M.A., Azam, S. M. F. & Haroon, U. (2011). Review of prescriber approach towards rational drug practice in hospitalised patients. J Ayub Med Coll Abbottabad, 23(1).

Angamo, M. T., Wabe, N. T. & Raju, N. J. (2011). Assessment of patterns of drug use by using World Health Organization's prescribing, patient care and healt facility indicators in selected health facilities in Southwest Ethiopia. Journal of Applied Pharmaceutical Science,1(7),62-66.

Ateş, Y. & Aba, G. (2019). Patoloji laboratuvar rutininde akılcı laboratuvar kullanımı: Tetkik istemlerinin retrospektif değerlendirmesi. Süleyman Demirel Üniversitesi Vizyoner Dergisi, Yıl: 2019, Cilt: 10, Sayı: 25, ss.612-630.

Barutçu, A., Tengilimoğlu, D. & Naldöken, Ü. (2017). Vatandaşların akılcı ilaç kullanımı, bilgi ve tutum değerlendirmesi: Ankara ili metropol ilçeler örneği. Gazi Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 19(3), 1062-1078.

Bian, C., Xu, S., Wang, H., Li, N., Wu, J., Zhao, Y., Li, P. & Lu, H. (2015) A study on the application of the information-motivation-behavioral skills (IMB) model on rational drug use behavior among second-level hospital outpatients in Anhui, China. Plos One. 10(8), e0135782.

Büyükturan, Ö. & Büyükturan, B. (2017). Bir grup hasta ve hasta yakınlarının ilaç kullanımı ile ilgili tutum ve davranışları. Sakarya Tıp Dergisi,7(4), 211-216.

Demirtaş, Z., Dağtekin, G., Alaiye, M., Sağlan, R., Önsüz, M. F., Işıklı, B., Kılıç, F. S. & Metintaş, S. (2018) Akılcı İlaç Kullanımı Ölçeği geçerlilik ve güvenilirliği.' ESTÜDAM Halk Sağlığı Dergisi, 3(1), 12-23.

Demiroğlu, T., Polat, Y. & Doğan, U. (2017). Kilis devlet hastanesinde yatan yetişkin hastaların ilaç kullanımına yönelik davranış ve alışkanlıklarının belirlenmesi. Gümüşhane Ünivesitesi Sağlık Bilimleri Dergisi, 6(1),93-98.

Devlet Planlama Teşkilatı (2001). Sekizinci Beş Yıllık Kalkınma Planı İlaç Sanayii Özel İhtisas Komisyonu Raporu. Ankara: Devlet Planlama Teşkilatı.

Dikkat Edilmesi Gerekenler. http://www.akilciilac.gov.tr/?page_id=85 (Erişim tarihi: 26.05.2022).

Ekenler, Ş. & Koçoğlu, D. (2016). Bireylerin akılcı ilaç kullanımıyla ilgili bilgi ve uygulamaları. Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi, 3(3),44-55.

Ercan, T. & Biçer, D. F. (2019). Tüketicilerin akılcı ilaç kullanımına yönelik bilgi düzeyleri ve davranışlarını etkileyen faktörlerin değerlendirilmesi: Sivas ili örneği. Business & Management Studies: An International Journal, 7(2),998-1021.

Erden, F. & Göçmez, S. (2004). Kanıta dayalı tıp ve ilaç seçimi. Sürekli Tıp Eğitimi Dergisi,13(4),134-136.

Gülhan, R. (2013). Yaşlılarda akılcı ilaç kullanımı. Okmeydanı Tıp Dergisi, 29(2), 100-101.

Holloway, K. & Dijk, L. V. (2011). The World Medicines Situation 2011-Rational Use of Medicines. WHO:Geneva.

İlaç Endüstrisi İşverenler Sendikası. İEİS İlaç Tanıtım İlkeleri ve Sağlık Mensuplarıyla İlişkiler Hakkında Yönetmelik. Erişim adresi: https://www.ieis.org.tr/ieis/tr/ieis/40/ieis-tanitim-ilkeleri (Erişim tarihi:29.03.2022).

İlaç Takip Sistemi Nedir? Erişim adresi: https://www.its.gov.tr/ (Erişim tarihi:10.02.2022).

İncesu, E. (2017). Akılcı ilaç kullanımında sağlık okuryazarlığı: Bir kamu hastanesi yatan hastaların üzerine bir araştırma. Sağlık Akademisyenleri Dergisi, 4(1).

Kan, Z. E. (2022). Sağlik okuryazarliği ile akilci ilaç kullanim arasindaki ilişkilerin incelenmesi. Bandirma Onyedi Eylül Üniversitesi Sağlik Bilimleri Enstitüsü. (Yayımlanmamış Yüksek Lisans Tezi). Balıkesir.

Kaya, H., Turan, N., Keskin, Ö., Tencere, Z., Uzun, E., Demir, G. & Yılmaz, T. (2015). Üniversite öğrencilerinin akılcı ilaç kullanma davranışları. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi, 18(1), 35-42.

Kurt, O., Oğuzöncül, A. F., Deveci, S. E. & Pirinççi, E. (2020). Bir sosyal bilimler meslek yüksekokulu öğrencilerinin akılcı ilaç kullanımı konusunda bilgi ve davranışlarının değerlendirilmesi. ESTÜDAM Halk Sağlığı Dergisi, 5(1), 62-72.

Le Grand, A., Hogerzeil, H. V. & Haaijer-Ruskamp, F.M. (1999). Intervention Research in Rational Use of Drugs: A Review. Health Policy and Planning, 14(2), 89-102.

Memişoğlu, D. (2016). Bir kamu politikası analizi örneği: Sağlıkta dönüşüm programı. Yasama Dergisi 34,62-93.

Nal, M. (2021). Factors effecting the demand for healthcare services. Violla Makhzum (Ed.), *Contemporary studies on management and organization* içinde. (49-70). Ankara: Iksad Publications.

Neden Akılcı İlaç Kullanımı. http://www.akilciilac.gov.tr/?page_id=81 (Erişim tarihi: 15.05.2022).

Oktay, Ş. (2006). Akılcı ilaç kullanımının genel ilkeleri. Türk Geriatri Dergisi, özel sayı,15-18.

Orhaner, E. & Salğın, E. (2018). Akılcı ilaç kullanımında aile hekiminin rolü: Bitlis ilinde bir uygulama. Uluslararası Sağlık Yönetimi ve Stratejileri Araştırma Dergisi, 4(3).

Özata, M., Aslan, Ş. & Mete, M. (2008). Rasyonel ilaç kullanımının hasta güvenliğine etkileri: Hekimlerin rasyonel ilaç kullanımına etki eden faktörlerin belirlenmesi. Selçuk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, 20,529–542.

Özçelikay, G. (2001). Akılcı ilaç kullanımı üzerinde bir pilot çalışma. Ankara Eczacılık Fakültesi Dergisi, 30(2),9-18.

Pınar N. (2012). Ülkemizde ilaç harcamaları. İnönü Üniversitesi Tıp Fakültesi Dergisi, 19(1),59-65.

Sağır, M. & Parlakpınar, H. (2014). Akılcı ilaç kullanımı. İnönü Üniversitesi Sağlık Bilimleri Dergisi, 3(2),32-35.

Sağlık Projesi Genel Koordinatörlüğü (1993). Rasyonel İlaç Kullanımı Bölümü: Akılcı İlaç Kullanımının Alfabesi. TEB Haberler, 3-10.

Saygılı, M. & Özer, Ö. (2020). Sağlık çalışanlarında ekip çalışması tutumlarının incelenmesi. Süleyman Demirel Üniversitesi Vizyoner Dergisi, 11 (27), 444-454.

Sevim, E. & Nal, M. (2021). Türkiye'de Özel Sağlık Sigortacılığı Sisteminin Mevcut Durum Analizi. Journal of Social Security. 11(1). 157-174.

Sürmelioğlu, N., Kıroğlu, O., Erdoğdu, T. & Karataş, Y. (2015). Akılcı Olmayan İlaç Kullanımını Önlemeye Yönelik Tedbirler. Arşiv Kaynak Tarama Dergisi, 24(4),452–62.

T.C. Bakanlar Kurulu. Sağlık Bakanlığı ve bağlı kuruluşlarının teşkilat ve görevleri hakkında kanun hükmünde kararname. Karar sayısı: Khk/663. Resmi Gazete, 2 Kasım 2011; 28103, https://www.resmigazete.gov. tr/eskiler/ 2011/11/20111102M1-3.htm (Erişim tarihi: 10. 02. 2022).

T.C. Resmi Gazete (2010). T.C. Sağlık Bakanlığı Hemşirelik Yönetmeliği. Sayı: 27515, 08.03.2010, Ankara: Başbakanlık Basımevi.

T.C. Sağlık Bakanlığı (2011). İlaç ve Eczacılık Genel Müdürlüğü Akılcı İlaç Kullanımı Şube Müdürlüğü. Hastane Hizmet Kalite Standartları; Akılcı İlaç Kullanımı İle İlgili Kılavuz,2011. http://www.akilciilac.gov.tr/?p=431 (Erişim tarihi: 4.01. 2022).

T.C. Sağlık Bakanlığı (2011). Refik Saydam Hıfzıssıhha Merkezi Başkanlığı Hıfzıssıhha Mektebi Müdürlüğü "Birinci Basamakta Akılcı Reçete Yazımı" Araştırma Serisi 7. Ankara.

T.C. Sağlık Bakanlığı (2011). Refik Saydam Hıfzısıhha Merkezi Başkanlığı Hıfzısıhha Mektebi Müdürlüğü "Toplumun Akılcı İlaç Kullanımına Bakışı" Araştırma Serisi 9. Ankara.

T.C. Sağlık Bakanlığı (2017). Akılcı İlaç Kullanımı Ulusal Eylem Planı 2014- 2017. Ankara.

T.C. Sağlık Bakanlığı Sağlık Projesi Genel Koordinatörlüğü (1993). Akılcı İlaç Kullanımı'nın Alfabesi, Nisan.

T.C. Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü (2011). Sağlığın Teşviki ve Geliştirilmesi Sözlüğü. Bakanlık Yayın No: 814. Ankara: Anıl Matbaacılık.

T.C. Sağlık Bakanlığı Türkiye İlaç ve Tıbbi Cihaz Kurumu (2011). Hastane Hizmet Kalite Standartları; Akılcı İlaç Kullanımı ile İlgili Kılavuz. Erişim: http://www.akilciilac.gov.tr/?p=431. (Erişim tarihi: 30.04.2022).

T.C. Sağlık Bakanlığı Türkiye İlaç ve Tıbbi Cihaz Kurumu (2015). Türkiye Akılcı İlaç Kullanımı Bülteni. Türkiye Kllinikleri Farmakoloji Özel Dergisi, 2(9).

T.C. Sağlık Bakanlığı. Beşeri tıbbi ürünlerin sınıflandırılmasına dair yönetmelik. Resmi Gazete, 17 Şubat 2005; 25730. https://www.resmigazete. gov. tr/eskiler/2021/12/20211211-5.htm (Erişim tarihi:14.02.2022).

Tıbbi Deontoloji Tüzüğü. (2015). https://www.ttb.org.tr/mevzuat/index. php?option= com_content&task=view&id=52&Itemid=31, 12 Ocak 2005 (Erişim tarihi:18.02.2022).

Toklu, H. Z. (2015). Eczacılık uygulamalarında akılcı ilaç kullanımı eczacılık uygulamasında akılcı ilaç kullanımı. Türkiye Klinikleri J Pharmacol, 3(1), 74-83.

Toklu, H. & Dülger, A. G. (2011). Akılcı ilaç kullanımı ve eczacının rolü. Marmara Pharmaceutical Journal, Cilt 15, Sayı 3, 89 – 93.

Türk, S. (2018). Akılcı ilaç kullanımı. Mustafa Kemal Üniversitesi Tıp Dergisi, 9(33), 20-28.

Türkiye İlaç ve Tıbbi Cihaz Kurumu (2018). Akılcı İlaç Kullanımı Ulusal Eylem Planı 2018-2022 Çalıştayı. Ankara. 2018. https://www.titck.gov. tr/haber/akilci-ilac-kullanimi-ulusal-eylem-plani-2018-2022-calistayi-27122018172841 (Erişim tarihi: 10. 02. 2022).

Ulupınar, S. & Akıcı, A. (2015). Hemşirelik uygulamalarında akılcı ilaç kullanımı. Turkiye Klinikleri J Pharmacol-SpecialTopics Dergisi, 3(1), 84-93.

Ulusoy, H. B. & Sumak, T. (2011). Kayseri'de pratisyen hekimlere verilen groningen modeli akılcı ilaç kullanımı eğitiminin değerlendirilmesi. Erciyes Tıp Dergisi, 33(4),309–316.

Uslu, Ş. & Akçadağ, M. (2012). İlaç sektöründe tersine lojistik ve dağıtımın rolü: bir uygulama. Niğde Üniversitesi İİBF Dergisi, 5(1),149-158.

Vançelik, S., Çalıkoğlu, O. & Güraksın, A (2006). Pratisyen hekimlerin reçete yazımını şekillendiren faktörler ve akılcı ilaç kullanım kriterlerini önemseme durumları. Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi, 26(2),65-75.

World Health Organization (WHO) (1990). The Role Of The Pharmacist In The Health Care System. https://apps.who.int/iris/handle/10665/61679 (Erişim tarihi: 24.05.2022).

World Health Organization (WHO) (1994). Guide to Good Prescribing, Geneva, Action Programme on Essential Drugs https://apps.who.int/iris/bitstream/handle/10665/59001/WHO_DAP_94.11.pdf (Erişim tarihi: 10.02.2022).

World Health Organization (WHO) (1998). Health Promotion Glossary. https://www.who.int/healthpromotion/about/HPR%20Glossary%201998.pdf (Erişim tarihi:16.02.2022).

World Health Organization (WHO) (2002). Promoting Rational Use of Medicines: Core Components. https://www.who.int/medicines/publications/policyperspectives/ppm05en.pdf (Erişim tarihi:16.02.2022).

World Health Organization (WHO) (2004). The World Medicines Situation: Chapter 8. Rational use of medicines.

https://apps.who.int/iris/bitstream/handle/10665/68735/WHO_EDM_PAR_2004.5.pdf (Erişim tarihi:16.02.2022).

Yağız, E. (2020). Kocaeli Üniversitesi Öğrencilerinin Sağlık Okuryazarlığı Düzeyleri ve Bu Durumun Akılcı İlaç Kullanımıyla Olan İlişkisinin İncelenmesi. (Yayımlanmamış tıpta uzmanlık tezi). Kocaeli Üniversitesi, Kocaeli.

Yılmaztürk, A. (2013). Türkiye'de ve Dünyada akılcı ilaç kullanımı. Kastamonu Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 2(2),42-49.

Yorulmaz, F. (2003). Reçeteleme kusurları halk sağlığını olumsuz etkiliyor. TTB Sürekli Tıp Eğitim Dergisi, 12(6), 218-221.

Remineralization Efficiency of Herbal Products

Elif ALKAN[1]
Dilek TAĞTEKİN[2]

Introduction

Dental caries is one of the most common chronic diseases in the world and might cause tooth loss when not treated properly. The concept of modern dentistry aims to prevent the progression of caries with early diagnosis, and to improve the aesthetics and function of the tooth with the remineralization process. Thus, caries lesions without cavity are treated with a non-invasive method (Nagarathana et al., 2015). Demineralization occurs through the leaving of mineral ions in dental hard tissues from hydroxyapatite crystals. Restoring these mineral ions to hydroxyapatite crystals is called remineralization. Both processes take place on the tooth surface and a large number of mineral ions can be lost from hydroxyapatite without deterioration of tooth integrity. As a result of the deterioration of the hydroxyapatite structure, cavities are formed. Demineralization is a reversible process; therefore, partially demineralized hydroxyapatite crystals in teeth can be remineralized (Higham, 2016). Enamel caries diagnosed at the initial stage can be treated by regulating diet, controlling plaque formation, and applying appropriate antibacterial agents, thus, demineralized dental tissue can become remineralized (Guerrieri et al., 2012).

Formation of Initial Enamel Caries

The caries formation process is a gradual process that requires periods of repeated and prolonged exposure of the enamel to acidic attacks, with the environment dropping below the critical pH (pH 5.5) and returning to the intermediate medium's plaque pH (pH 7.0). In the development of dental caries, the stage before cavitation is called the initial caries lesion. It is characterized by subsurface demineralization areas that occur under an intact enamel surface (Kidd & Fejerskov 2004) (Figure 1). The mineral content of the affected area is reduced, which affects the translucent nature of enamel and the color of these areas appears more opaque white. For this reason, initial enamel lesions or flat surface caries are also called white spot lesions and are the first visible signs of caries formation (Øgaard et al., 1988). It is important to distinguish the etiology of these lesions clinically, because white lesions can be caused by caries or be hereditary. When the tooth surface is air-dried, caries lesions appear opaque and chalky. This appearance is due to the loss of its translucent feature due to subsurface demineralization of the enamel tissue in this area. In contrast, developmental lesions are less or unaffected by air drying (Fejerskov et al., 2015).

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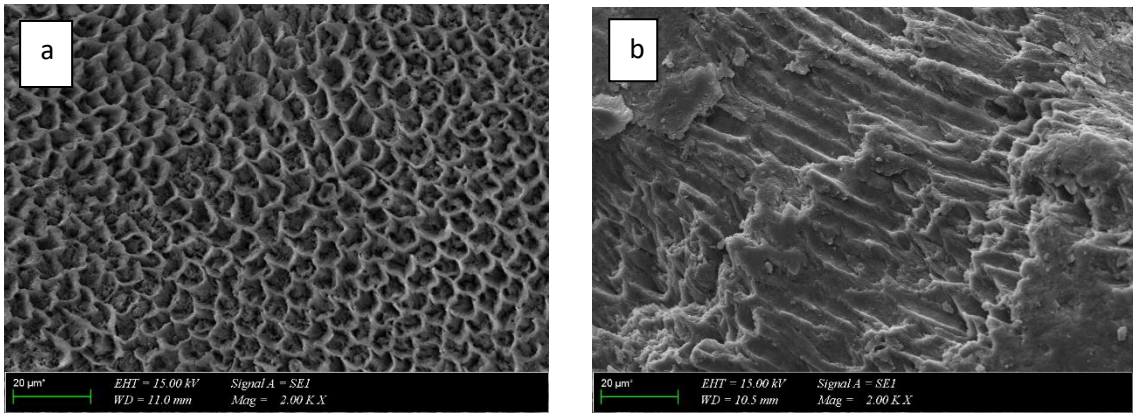

Figure 1. *Demineralized enamel tissue: a)coronal section b) saggital section. (Alkan, 2022)*

Treatment of Initial Carious Lesions

Various options are available for the treatment of initial carious lesions. Recent approaches are listed in the following sections.

Providing Oral Hygiene

Dental caries is an important problem that affects not only the individual, but also social institutions and the health economy. Untreated caries affects millions of people worldwide. Such a big problem also causes huge cost. Raising awareness about individual oral hygiene and daily brushing with fluoride toothpaste is one of the solutions for tooth decay. Providing personal oral hygiene with tooth brushing and flossing, removing the bacterial plaque and thus changing the initial carious lesion formation process are the most effective methods that patients can apply individually (Hicks et al., 2005).

Diet Regulation

Apart from the presence of bacterial plaque, another factor affecting caries formation is diet. The foods taken can act as an inhibitor of caries formation and can be among the causes of caries formation. For example, some foods with a hard and fibrous structure help mechanical cleaning, while others show a caries preventive effect by increasing the amount and flow rate of saliva with their taste and smell. Food and beverages such as cheese, milk, cocoa, tea and proteins have a bacteriostatic effect by changing the metabolism of cariogenic bacteria. However, fermented carbohydrates can cause caries when consumed frequently (Kalender, 2017).

Use of Antimicrobial Agents

The use of chlorhexidine was first offered to the public in the USA as a 0.12% mouthwash for periodontal treatment of high-risk patients. It reduces the number of Streptococcus mutans and accelerates remineralization. It is recommended to use a chlorhexidine mouthwash for 30 seconds just before going to bed, as the reduced salivary flow rate at night helps chlorhexidine to bind to the structures in the mouth more easily. It has been reported that when chlorhexidine mouthwash is used in this way for 2 weeks, it reduces the number of Streptococcus mutans below the potential for caries formation, and the effect of this reduction lasts between 12 and 26 weeks (Roberson et al., 2006).

Remineralization of Initial Carious Lesions

Initial caries lesions can be remineralized by preventing the factors that cause caries formation and increasing the duration of the protective factors in the mouth. In general, remineralization is a natural repair process and many methods have been proposed to improve it. The dynamics of formation and prevention of the initial caries lesion occurs between demineralization, which is the process of removing mineral ions from the hydroxyapatite crystals of enamel, and remineralization, which is the process of restoring these lost mineral ions, particularly calcium and phosphate ions, into the cavities in the hydroxyapatite crystals (Figures 2,3).

An ideal remineralizing agent (Walsh, 2009):

• It should provide optimum level of calcium and phosphate ions, thus increasing remineralization and preventing the formation of tartar.

• It should be able to penetrate deeply into the subsurface demineralized area.

• It should be in active form even below the critical pH and thus prevent demineralization.

• It should have antimicrobial activity against cariogenic microorganisms.

• It should show a synergistic effect with the remineralization capacity of saliva.

• It should be effective even in cases where there is a decrease in salivary flow rate and in xerostomia patients.

• It should not cause any negative effects.

• It should be easily accepted by the society.

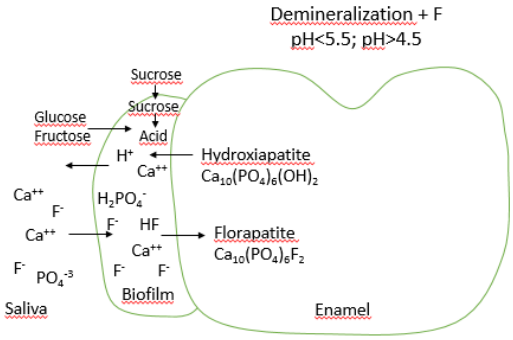

Figure 2. *Enamel demineralization in the presence of F in dental biofilm. Carbohydrates (sucrose, glucose, fructose) are converted into organic acids by bacteria in the biofilm. When the pH drops below 5.5, the saturation of hydroxyapatite (HA) in the biofilm decreases, causing mineral dissolution. However, if the pH is higher than 4.5 and F is present, the biofilm Florapatite (FA) becomes supersaturated and mineral re-precipitation occurs in the enamel. As a result, demineralization is reduced (Cury & Tenuta 2008).*

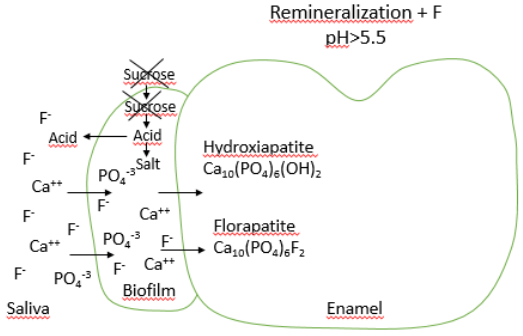

Figure 3. *Enamel remineralization in the presence of F in dental biofilm. After the intake of carbohydrates has ceased, the acids in the biofilm are cleared by saliva and converted into salts. As a result, the pH increases and at 5.5 or higher the biofilm becomes supersaturated with HA and FA. Thus, Ca and P lost by the enamel can be recovered more efficiently if F is still present in the biofilm (Cury &Tenuta 2008).*

Fluoride Applications

Fluoride shows its effect against caries by three different mechanisms. First, the presence of fluoride greatly enhances the formation and accumulation of fluorapatite, which is formed by the combination of calcium and phosphate ions in saliva. Florapatite is resistant to dissolution; therefore, it replaces salts containing manganese and carbonate, which are easily dissolved and lost due to demineralization, making the enamel more resistant to acids. The second mechanism is remineralization of the initial cavity lesion with florapatite crystals. The third and final mechanism is the antimicrobial activity of fluoride ions. Low fluoride concentrations inhibit the production of the glycosyltransferase enzyme. The glycosyltransferase enzyme enhances bacterial adhesion and provides glucose for extracellular polysaccharide formation. Topical fluoride applications at high concentrations (12,000 ppm) have a direct toxic effect on oral microorganisms, including mutans streptococci (Roberson et al., 2006). Fluoride applications can be classified as systemic and topical.

Systemic Applications

Systemic applications are effective methods, especially in individuals with a high risk of caries formation and in societies where fluoride use is low. Fluoridation of water, which is one of the systemic applications, shows not only systemic but also topical effects (Roberson et al., 2006). The World Health Organization (WHO) has reported that consuming 1 mg of fluoride per day is beneficial for health. Systemic applications of fluoride are the fluoridation of drinking water, salts and milk, and the addition of fluorinated tablets or drops to the diet.

Topical Applications

Systemic fluoride intake was thought to be effective for a long time before the application of fluoride directly to the tooth surface was initiated. However, it is now accepted that the use of topical fluoride is more beneficial during tooth development and maturation. Topical fluoride in high concentrations (12,000 ppm), often professionally applied in dental clinics, has a direct toxic effect on oral microorganisms, including Streptococcus mutans (Roberson et al., 2006). Fluoride varnishes have been developed to prolong the contact time with the tooth, to hold onto the enamel for a longer time, and thus to prevent rapid fluoride loss after application. Varnishes act as a reservoir for slow release and facilitate further fluoride uptake (Øgaard et al., 1994). In the guidelines of the American Academy of Pediatric Dentistry, it is recommended to apply fluoride varnishes at a concentration of 5% (22,600 ppm) at least twice a year for primary teeth and two or four times a year for permanent teeth (de Sousa et al., 2019).

Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP–ACP) Applications

ACP is a tricalcium phosphate containing calcium and phosphate ions in an amorphous structure. When ACP enters a solution, it rapidly transforms into a stable structure such as octacalcium phosphate or apatite. Casein is a phosphoprotein that makes up 80% of the proteins found in cow's milk. The most important feature of casein is the retention of calcium and phosphate ions in protein complexes. These ions break down into smaller peptides like CPP, making them highly durable. Casein molecules act as a carrier, providing calcium and phosphate ions that tissues such as teeth or bones can use for remineralization. Mendes et al. reported that the use of CPP-ACP is a good alternative for remineralization of initial carious lesions (Mendes et al., 2018). The remineralization effect can be improved when this product is applied together with fluoride.

Nanohydroxyapatite

Today, nanotechnology is developing by offering various caries preventive and therapeutic applications. Nanotechnology has sought to replicate the nanostructural properties of human

enamel and offer biology-inspired strategies for remineralization and caries treatment (Chandki et al., 2012).

In dentistry, nanoparticle application is used in two ways as preventive dentistry and restorative dentistry (Maman et al., 2018). In recent years, various hydroxyapatite types of calcium carbonate in nanometric sizes have been applied in the prevention and treatment of incipient caries lesions. In dentistry, the sizes of hydroxyapatite nanocrystals vary between 50-1000 nm; being so small in size increases the surface area they can bind to, so they can bind to proteins, biofilm and bacteria when present in the toothpaste. Likewise, thanks to their small size, they can repair small cavities and indentations in the enamel surface. Nanohydroxyapatite, a bioactive and biocompatible material, contributes to the remineralization process with high amounts of calcium and phosphate ions from oral fluids, increasing crystallization by directly filling the micropores of the initial caries lesions. It has been reported that a 10% nanohydroxyapatite concentration is optimal for remineralization of initial enamel caries (Huang et al., 2009).

Sodium Calciumphosphosilicate (Bioactive Glass)

When bioactive glass comes into contact with saliva, it rapidly releases sodium, calcium and phosphorus ions available for remineralization of the tooth surface into the saliva and increases the pH. The released ions directly form hydroxycarbonate apatite (HCA). They also attach to the tooth surface and continue to release ions and remineralize the tooth surface after the first application. These particles have been shown to convert to HCA for up to 2 weeks by releasing ions (Du et al., 2004). Novamin, a commercial form of bioactive glass, adheres to the exposed dentin surface and forms a mechanically strong and acid-resistant mineralized layer. There is a sustained release of calcium that maintains its protective effects on dentin over time (Burwell et al., 2010).

Natural Remineralization Systems

Plant extracts have been used as medicine in traditional medicine since ancient times. Today, these plant extracts are of particular interest because they are not chemical or synthetic. If the effect of herbal products is supported by evidence as a result of scientific-based studies, they may be better and safer alternatives for treatment (Shaheen et al., 2015). In recent years, the desire to return to natural products has also affected the view against the use of herbal agents as antimicrobial agents in dentistry. Antimicrobial properties of essential oils obtained from plants instead of artificial molecules are being investigated. Besra et al. investigated the antimicrobial properties of various plant extracts on caries pathogens and concluded that the plants can be used alone or in combination in the treatment of dental caries (Besra & Kumar 2018). Studies have been conducted in which herbal extracts are used as antimicrobial plaque agents to prevent dental caries and reduce gingivitis (Poureslami, 2012).

Some natural plant extracts have antimicrobial properties and have the potential to prevent demineralization and provide remineralization of enamel (Philip, 2019). Among the remineralization systems, fluoride-free and natural systems will reduce the risk of fluoride toxicity. Because plant-based phytochemicals and bioflavonoids can provide remineralization of the initial caries lesion, they will be more acceptable to the public than artificial chemical derivatives and fluoride-based systems used for dental caries prevention. Common oral health problems such as dental caries, periodontitis, microbial dental plaque and gingivitis can be prevented and treated if appropriate herbal treatments are used consistently over a period of time (Bilgin Göçmen et al., 2020). Also, most of them are natural foodstuffs that show no toxicity and are "generally recognized as safe" (GRAS) by the US Food and Drug Administration (FDA) (Annamalai et al., 2020). Some of the natural and herbal extracts are listed below.

Cocoa Bean

Theobromine is an alkaloid found in cocoa beans (Theobroma coca) and chocolate. It is a white crystalline powder from the methylxanthine family (3.7 dimethylxanthine). Theobromine has been shown to increase crystal growth, resistance to acid attack, and microhardness of enamel (Amaechi et al., 2013; Syafira et al., 2013). In another in vitro study, it was reported that theobromine has a remineralization capacity similar to that of fluoridated toothpastes (Taneja et al., 2019).

Xylitol

Xylitol is a naturally occurring five-carbon sugar polyol (pentylol) found naturally in fruits, vegetables, and berries. It is mainly derived from xylan-rich plant materials such as birch and beech (Bar, 1988). It is used instead of sugar. It has been reported that the use of chewing gum containing xylitol increases the salivary flow rate, improves the protective properties of saliva and helps remineralization. It has been suggested that caries-associated mutans streptococci are unable to use xylitol in their metabolism and therefore reduce acid formation in the oral biofilm. It has been reported that xylitol significantly reduces the incidence of caries and increases tooth remineralization (Hildebrandt & Sparks, 2000; Ritter et al., 2013).

Ginger

Ginger (Zingiber officinale) is one of the most widely used herbs in traditional medicine. Numerous pharmacological activities such as antioxidant, antibacterial, anti-inflammatory, antinociceptive, antimutagenic and hepatoprotective have been shown in many studies (Abdel-Azeem et al., 2013; Jeena et al., 2013; Jeena et al., 2014). In addition, there are studies showing the antibacterial effect of ginger against many microorganisms, including mutans streptococci (Fatima et al., 2000; Khan et al., 2010; Islam et al., 2012).

Ginger content; It contains approximately 50% carbohydrates, 9% protein and free amino acids, 6-8% fatty acids and triglycerides, 3-6% ash and 3-6% crude fiber (on the basis of dry matter), although it may vary depending on the type and geography of cultivation (Tang & Eisenbrand, 1992). Ginger is a good source of essential micronutrients such as potassium, magnesium, copper and manganese. Ginger rhizome also contains small amounts of vitamins A, E, and some B- and vitamin C (PR & Prakash, 2010). Ginger is a non-toxic natural product and is considered "generally safe" (GRAS) by the U.S. Food and Drug Administration (FDA). The pungent oil component in it consists of a series of polyphenolic ketones with many pharmacological activities (Ravindran & Babu, 2016). The bioactive components gingerol and shagelol obtained from the ethnolic extracts of ginger exhibit antifungal and antibacterial activity. It is thought that ginger's remineralization capacity is due to its antimicrobial properties and high fluoride content (Al-Duboni et al., 2013).

Ginger is one of these herbal extracts with its antibacterial properties. Ginger essential oil and oleoresin have shown significant antioxidant and antimicrobial activities (Bellik, 2014). Ginger; It has been included in oral and dental care products as an accepted and renewable natural product due to fewer side effects, better patient tolerance, relatively cheaper, long history of use (Ody, 2000; Al-Achi, 2001; Abebe, 2003; Barrett, 2004).

Bilgin et al. conducted two studies with a herbal mixture consisting of chocolate, ginger and honey, and in both in vitro and in situ studies, they showed that tooth cream containing ginger and honey had very positive effects on remineralization as a result of both microhardness and fluorescence evaluations compared to alternative normal fluoride toothpaste. (Bilgin et al., 2016; Bilgin Göçmen et al., 2016).

Rosemary

Rosemary (Salvia rosmarinus) is a small aromatic shrub composed of terpenoids, flavonoids, phenols and essential oils with antimicrobial, antifungal and antioxidant properties. Al-Duboni et al. reported that methanolic extract of rosemary (30g/100mL) had inhibitory effects on mutans streptococci, and stated that rosemary extract was effective in remineralization as a result of fluorescence and microhardness evaluations on enamel (Al-Duboni et al., 2013). Bilgin et al. reported the in vitro remineralization capacity of a mixture of rosemary, ginger extract and honey (Bilgin et al., 2016).

Hesperidin

Hesperidin is a member of the flavanone group of flavonoids isolated in large quantities from the peels of citrus fruits such as lemons, oranges, grapes and tangerines. Hesperidin has been shown to have antioxidant, anti-inflammatory and anti-carcinogenic properties. It has been reported that Hesperidin inhibits demineralization, increases remineralization in dentin, and preserves collagen even under fluoride-free conditions (Islam et al., 2012; Li & Schluesener 2017).

Grape Seed Extract

Grape (Vitis vinefera) seed extract contains a powerful antioxidant polyphenol, proanthocyanidin. It strengthens collagen-based tissues by increasing collagen cross-links. Proanthocyanidin has been shown to inhibit root caries by interacting with microbial cell membrane proteins and lipids, leading to cell membrane disruption. Proanthocyanidin inhibits the enzymatic activity of ATPase and amylase. At the same time, it inhibits the glucosyl transferase enzyme produced by mutans streptococci, preventing bacterial adhesion to the tooth surface, which shows anticariogenic properties. It has been shown that grape seed extract can be used as a substitute for fluorides in the prevention of root caries in elderly patients. An in vitro study reported that mouthwash containing grape seed extract limits dentin matrix degradation and increases remineralization. A recent systematic review concluded that the caries prevention of grape seed extract compared with fluoride inhibits the proliferation of bacterial biofilms on the tooth surface and promotes remineralization (Xie et al., 2008; Khaddam et al., 2014; Delimont & Carlson, 2020).

Galla Chinensis

Galla chinensis is a traditional Chinese herb that has been thought to have anti-caries effect in recent years. It has been stated that Galla Chinensis prevents demineralization and contributes to remineralization and increases the effect of fluorine. According to a hypothesis that emerged as a result of the studies, they stated that Galla chinensis can affect the remineralization of the caries lesion, and that it does this by combining with the organic matrix in the enamel. The possible effect of Galla chinensis is thought to be through its polyphenols, which combine with the organic matrix in the enamel and block the ion diffusion pathways. Polyphenol compounds have been reported to act as calcium ion carriers to the lesion body (Cheng et al., 2010; Zhang et al., 2016; Philip, 2019).

Honey

Honey is a natural food product produced by honey bees (Apismellifera), consisting of nectar secreted from flowers and substances secreted by insects. Due to its chemical composition, honey has been used for many years in the treatment of burns and infected wounds (Medhi et al., 2008; Mandal & Mandal, 2011). Honey has been found to have promising potential as an alternative agent in the management of disease conditions such as dental caries and gingivitis following orthodontic treatment (Atwa et al., 2014).

Honey, one of the agents traditionally used with herbal medicines, has a bacteriostatic effect on pathogens and has a pH of about 3.9. Studies have shown its effectiveness on oral pathogenic

bacteria (Al-Hasani, 2018; Sateriale et al., 2020). Honey has antibacterial effects on S.mutans, L. acidophillus, A.viscosus, P.aeroginosa, V.alcaligens and S.aureus. Honey has a pH of 3.9 so it is acidic and can inhibit the growth of pathogens as most pathogenic microorganisms thrive at pH 4.0-4.5. Diluting honey with saliva will increase the pH and reduce its effect. This contributes to its antibacterial activity by increasing the enzyme activity and the effect of glucose oxidase enzyme and the production of hydrogen peroxide, which is an oxidizing agent (Molan, 1992; Brudzynski et al., 2011). The glucuronic acid produced by glucosoxidase in honey helps calcium absorption (Eteraf-Oskouei & Najafi, 2013). In addition, it has been reported that orally taken honey reduces prostaglandin levels and has prebiotic and antibacterial effects (Al-Waili et al., 2011; Al-Waili et al., 2011).

Conclusion

Although modern chemicals are widely used today, the demand for herbal/natural products has increased in recent years. Plant extracts, which are frequently used in traditional medicine, gain importance as an alternative treatment for dental health. As a result of the researches and achievements in the field of herbal medicines and formulations, many problems of oral health might be prevented and treated. Today, there are many herbal medicines and derivatives, toothpastes, mouthwashes, etc., which are related to oral and dental health, sold with or without a prescription exist. Natural products are potential and valuable resources for the development of medicines and products to manage oral diseases. Although some progress has been made in this area, there is still limited information about the safety and effectiveness of these products. Thus dentists should be skeptical about the utilization of products that do not have sufficient scientific studies and proven efficacy, and should pay attention not to mislead the society.

Further clinical research is needed to develop drugs and products containing active plant extracts or phytochemicals that can be offered as a supplement to existing treatment or as an alternative treatment. Dentists should be knowledgeable about these products as they are natural, safe and economical. They should also choose to prescribe and recommend these products in their treatments.

REFERENCES

Abdel-Azeem, A. S., A. M. Hegazy, K. S. Ibrahim, A.-R. H. Farrag and E. M. El-Sayed (2013). Hepatoprotective, antioxidant, and ameliorative effects of ginger (Zingiber officinale Roscoe) and vitamin E in acetaminophen treated rats. *Journal of dietary supplements, 10*(3): 195-209.

Abebe, W. (2003). An overview of herbal supplement utilization with particular emphasis on possible interactions with dental drugs and oral manifestations. *Journal of Dental Hygiene: JDH 77*(1): 37-46.

Al-Achi, A. (2001). A current look at ginger use. *US Pharmacist 26*(9): HS13.

Al-Duboni, G., M. T. Osman and R. Al-Naggar (2013). Antimicrobial activity of aqueous extracts of cinnamon and ginger on two oral pathogens causing dental caries. *Research Journal of Pharmaceutical, Biological and Chemical Sciences 4*(3): 957-965.

Al-Hasani, H. M. H. (2018). Study antibacterial activity of honey against some common species of pathogenic bacteria. *Iraqi Journal of Science*: 30-37.

Alkan, E. Tağtekin, D., Korkmaz, N., Yanıkoğlu, F. (2022). Bitkisel Diş Macunlarının Başlangıç Mine Çürükleri Üzerine Remineralizasyon Etkisinin Değerlendirilmesi. *26. Türk Diş Hekimleri Birliği Uluslararasi Dişhekimliği Kongresi*, 8-11 Eylül 2022, İstanbul, 629-630.

Al-Waili, N., K. Salom and A. A. Al-Ghamdi (2011). Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. *TheScientificWorldJournal 11*: 766-787.

Al-Waili, N. S., K. Salom, G. Butler and A. A. Al Ghamdi (2011). Honey and microbial infections: a review supporting the use of honey for microbial control. *Journal of medicinal food 14*(10): 1079-1096.

Amaechi, B., N. Porteous, K. Ramalingam, P. Mensinkai, R. C. Vasquez, A. Sadeghpour and T. Nakamoto (2013). Remineralization of artificial enamel lesions by theobromine. *Caries research 47*(5): 399-405.

Annamalai, S., S. Ballal and N. Arani (2020). Remineralization of white spot lesion in the natural way–A review. *Annals of the Romanian Society for Cell Biology*: 1197-1202.

Atwa, A.-D. A., R. Y. AbuShahba, M. Mostafa and M. I. Hashem (2014). Effect of honey in preventing gingivitis and dental caries in patients undergoing orthodontic treatment. *The Saudi dental journal 26*(3): 108-114.

Bar, A. (1988). Caries prevention with xylitol: a review of the scientific evidence. *World review of nutrition and dietetics*.

Barrett, M. L. (2004). Handbook of clinically tested herbal remedies, Haworth Medical Press.

Bellik, Y. (2014). Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of Zingiber officinale Roscoe. *Asian Pacific Journal of Tropical Disease 4*(1): 40-44.

Besra, M. and V. Kumar (2018). In vitro investigation of antimicrobial activities of ethnomedicinal plants against dental caries pathogens. *3 Biotech 8*(5): 1-8.

Bilgin, G., F. Yanıkoğlu and D. Tagtekin (2016). Remineralization potential of herbal mixtures: an in situ study. *Indian journal of research 5*(2): 264-268.

Bilgin Göçmen, G., F. Yanıkoğlu, D. Tağtekin, G. K. Stookey, B. R. Schemehorn and O. Hayran (2016). Effectiveness of some herbals on initial enamel caries lesion. *Asian Pacific Journal of Tropical Biomedicine 6*(10): 846–850.

Bilgin Göçmen G., Arslantunalı Tağtekin D., Yanıkoğlu F. (2020) Bitkisel diş macunları. Dilek Arslantunalı Tağtekin (Ed.), *Diş Macun ve Kremleri* (p. 97-106). Ankara: Türkiye Klinikleri.

Brudzynski, K., K. Abubaker and A. Castle (2011). Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. *Frontiers in microbiology 2*: 213.

Burwell, A., D. Jennings and D. C. Greenspan (2010). NovaMin and dentin hypersensitivity--in vitro evidence of efficacy. *The Journal of clinical dentistry 21*(3): 66-71.

Chandki, R., M. Kala, K. N. Kumar, B. Brigit, P. Banthia and R. Banthia (2012). Nanodentistry': Exploring the beauty of miniature. *Journal of Clinical and Experimental Dentistry 4*(2): e119.

Cheng, L., J. Li, Y. Hao and X. Zhou (2010). Effect of compounds of Galla chinensis on remineralization of enamel surface in vitro. *Archives of oral biology 55*(6): 435-440.

Cury, J. and L. Tenuta (2008). How to maintain a cariostatic fluoride concentration in the oral environment. *Advances in dental research 20*(1): 13-16.

de Sousa, F. S. d. O., A. P. P. Dos Santos, P. Nadanovsky, P. Hujoel, J. Cunha-Cruz and B. H. de Oliveira (2019). Fluoride varnish and dental caries in preschoolers: a systematic review and meta-analysis. *Caries Research 53*(5): 502-513.

Delimont, N. M. and B. N. Carlson (2020). Prevention of dental caries by grape seed extract supplementation: A systematic review. *Nutrition and Health 26*(1): 43-52.

Du, M., B. Tai, H. Jiang, J. Zhong, D. Greenspan and A. Clark (2004). Efficacy of dentifrice containing bioactive glass (NovaMin) on dentine hypersensitivity. *J Dent Res 83*(abstract, special issue A): 1586.

Eteraf-Oskouei, T. and M. Najafi (2013). Traditional and modern uses of natural honey in human diseases: a review. *Iranian journal of basic medical sciences 16*(6): 731.

Fatima, S., A. Farooqi, R. Kumar, T. Kumar and S. Khanuja (2000). Antibacterial activity possessed by medicinal plants used in tooth powders. *Antibacterial activity possessed by medicinal plants used in tooth powders. 22*(4a): 187-189.

Fejerskov, O., B. Nyvad and E. Kidd (2015). Dental caries: the disease and its clinical management, John Wiley & Sons.

Guerrieri, A., C. Gaucher, E. Bonte and J. Lasfargues (2012). Minimal intervention dentistry: part 4. Detection and diagnosis of initial caries lesions. *British dental journal 213*(11): 551-557.

Hicks, J., F. Garcia-Godoy and C. Flaitz (2005). Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2). *Journal of clinical pediatric dentistry 28*(2): 119-124.

Higham, S. (2016). webpage on the Internet. Caries Process and Prevention Strategies: Demineralization/Remineralization.[Accessed June 23, 2016]. Available from: http://www. dentalcare. com/media/en-US/education/ce372/ce372. pdf.

Hildebrandt, G. H. and B. S. Sparks (2000). Maintaining mutans streptococci suppression: with xylitol chewing gum. *The Journal of the American Dental Association 131*(7): 909-916.

Huang, S., S. Gao and H. Yu (2009). Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. *Biomedical materials 4*(3): 034104.

Islam, M. S., N. Hiraishi, M. Nassar, R. Sono, M. Otsuki, T. Takatsura, C. Yiu and J. Tagami (2012). In vitro effect of hesperidin on root dentin collagen and de/re-mineralization. *Dental materials journal*: 2011-2203.

Islam, T. H., A. H. B. Azad, S. Akter and S. Datta (2012). Antimicrobial activity of medicinal plants on Streptococcus mutans, a causing agent of dental caries. *J Eng Res Tech 1*: 121-126.

Jeena, K., V. B. Liju and R. Kuttan (2013). Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. *Indian J Physiol Pharmacol 57*(1): 51-62.

Jeena, K., V. B. Liju, R. Viswanathan and R. Kuttan (2014). Antimutagenic potential and modulation of carcinogen-metabolizing enzymes by ginger essential oil. *Phytotherapy Research 28*(6): 849-855.

Jeon JG, Rosalen PL, Falsetta ML, Koo H. (2011). Natural products in caries research: current (limited) knowledge, challenges and future perspective. *Caries Res. 45*(3):243-63.

Kalender, B. (2017). Başlangıç Çürük Lezyonlarının Tedavisi. *Turkiye Klinikleri J Restor Dent-Special Topics 3*(2): 58-65.

Khaddam, M., B. Salmon, D. Le Denmat, L. Tjaderhane, S. Menashi, C. Chaussain, G. Y. Rochefort and T. Boukpessi (2014). Grape seed extracts inhibit dentin matrix degradation by MMP-3. *Frontiers in physiology 5*: 425.

Khan, R., M. Zakir, S. H. Afaq, A. Latif and A. U. Khan (2010). Activity of solvent extracts of Prosopis spicigera, Zingiber officinale and Trachyspermum ammi against multidrug resistant bacterial and fungal strains. *The Journal of Infection in Developing Countries 4*(05): 292-300.

Kidd, E. and O. Fejerskov (2004). What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *Journal of dental research 83*(1_suppl): 35-38.

Li, C. and H. Schluesener (2017). Health-promoting effects of the citrus flavanone hesperidin. *Critical reviews in food science and nutrition 57*(3): 613-631.

Maman, P., M. Nagpal, R. M. Gilhotra and G. Aggarwal (2018). Nano era of dentistry-an update. *Current drug delivery 15*(2): 186-204.

Mandal, M. D. and S. Mandal (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific journal of tropical biomedicine 1*(2): 154-160.

Medhi, B., A. Puri, S. Upadhyay and L. Kaman (2008). Topical application of honey in the treatment of wound healing: a metaanalysis. *JK Sci 10*(4): 166-169.

Mendes, A. C., M. Restrepo, D. Bussaneli and A. C. Zuanon (2018). Use of casein amorphous calcium phosphate (CPP-ACP) on white-spot lesions: Randomised clinical trial. *Oral Health Prev Dent 16*(1): 27-31.

Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee world 73*(1): 5-28.

Nagarathana, C., B. Sakunthala and P. Naveena Preethi (2015). An update on current remineralizing agent. *OHDM 14*(4): 183-187.

Ody, P. (2000). The Complete Guide Medicinal Herbals. London: Dorling Kindersley: 75.

Øgaard, B., G. Rølla and J. Arends (1988). Orthodontic appliances and enamel demineralization: Part 1. Lesion development. *American Journal of Orthodontics and Dentofacial Orthopedics 94*(1): 68-73.

Øgaard, B., L. Seppä and G. Rolla (1994). Professional topical fluoride applications—clinical efficacy and mechanism of action. *Advances in Dental Research 8*(2): 190-201.

Philip, N. (2019). State of the art enamel remineralization systems: the next frontier in caries management. *Caries research 53*(3): 284-295.

Poureslami, H. (2012). The effects of plant extracts on dental plaque and caries. *Contemporary approach to dental caries 20*: 395-402.

PR, S. A. and J. Prakash (2010). Chemical composition and antioxidant properties of ginger root (Zingiber officinale). *Journal of Medicinal Plants Research 4*(24): 2674-2679.

Ravindran, P. and K. N. Babu (2016). Ginger: the genus Zingiber, CRC press.

Ritter, A., J. Bader, M. Leo, J. Preisser, D. Shugars, W. Vollmer, B. Amaechi and J. Holland (2013). Tooth-surface-specific effects of xylitol: randomized trial results. *Journal of dental research 92*(6): 512-517.

Roberson, T., H. O. Heymann and E. J. Swift Jr (2006). Clinical significance of dental anatomy, histology, physiology and occlusion. Sturdevant's art and science of operative dentistry, Elsevier Health Sciences: 17-64.

Sateriale, D., S. Facchiano, R. Colicchio, C. Pagliuca, E. Varricchio, M. Paolucci, M. G. Volpe, P. Salvatore and C. Pagliarulo (2020). In vitro synergy of polyphenolic extracts from honey, myrtle and pomegranate against oral pathogens, S. mutans and R. dentocariosa. *Frontiers in microbiology*: 1465.

Shaheen, S. S., P. Reddy, S. R. Hemalatha, D. Doshi, S. Kulkarni and M. Kumar (2015). Antimicrobial efficacy of ten commercially available herbal dentifrices against specific oral microflora–In vitro study. *Journal of Clinical and Diagnostic Research: JCDR 9*(4): ZC42.

Syafira, G., R. Permatasari and N. Wardani (2013). Theobromine effects on enamel surface microhardness: in vitro. *Journal of Dentistry Indonesia 19*(2): 32-36.

Taneja, V., S. Nekkanti, K. Gupta and J. Hassija (2019). Remineralization potential of theobromine on artificial carious lesions. *Journal of International Society of Preventive & Community Dentistry 9*(6): 576.

Tang, W. and G. Eisenbrand (1992). Zingiber officinale (Willd.) Rosc. Chinese Drugs of Plant Origin, Springer: 1011-1015.

Walsh, L. J. (2009). Contemporary technologies for remineralization therapies: A review. *Int Dent SA 11*(6): 6-16.

Xie, Q., A. K. Bedran-Russo and C. D. Wu (2008). In vitro remineralization effects of grape seed extract on artificial root caries. *Journal of dentistry 36*(11): 900-906.

Zhang, T.-T., H.-J. Guo, X.-J. Liu, J.-P. Chu and X.-D. Zhou (2016). Galla chinensis compounds remineralize enamel caries lesions in a rat model. *Caries Research 50*(2): 159-165.

The Relationship of Exapolysaccharides with Biofilm and Quarum Sensing

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Introduction

Probiotics, derived from the Greek and meaning "for life", is a term used to define living non-pathogenic organisms which have a demonstrated beneficial effect on the host when ingested in adequate amounts (FAO/WHO, 2001). The most commonly used probiotics are represented by lactobacilli, bifidobacteria and non-pathogenic yeasts. They must be capable of surviving the passage through the stomach to enter the intestinal tract, where they exert helpful effects on the gut microbiota, and of resisting to the stressful environments during industrial processes. The most important and known beneficial effects of probiotics include the prevention of diarrhea, constipation, and food allergies; other factors, such as a reduction of gas production, changes in bile salt conjugation, anti-bacterial, anti-inflammatory and anti-viral effects, may result relevant to the benefits of specific probiotics (Barrett et al., 2008).

Biofilms and Quorum Sensing

Biofilms form a heterogeneous matrix consisting of microbial communities and their secreted extracellular polymeric substances (EPSs) (Wingender et al., 1999) and in sediments incorporate particles within the matrix. Microorganisms that inhabit sediments and form biofilms are highly adapted for this habitat and secrete adhesive polymers. The EPS is primarily composed of organic molecules such as polysaccharides, proteins, lipids, and nucleic acids (Stoodley et al., 2002). EPS affects sediment dynamics by, directly enhancing the cohesive forces between sediments, binding them together, and coating sediment particles and changing the micromorphology of individual grains (Paterson et al., 2009). Bacteria in natural habitats predominantly exist as matrix-encased species-rich communities, either as surface associated biofilms or surface independent floccular aggregates. This is in contrast to their planktonic single species population counterparts commonly studied in laboratory conditions. The formation and dispersal of structured bacterial biofilms or aggregates occur in response to a range of environmental cues and signals, such as changes in nutrient concentrations, oxygen, temperature, as well as chemicals and predatory stresses (Matz et al., 2004).

Bacterial biofilm formation is often regulated by quorum sensing (QS) which is a population density-based cell-cell communication process via signaling molecules. Numerous signaling molecules such as acyl homoserine lactones, peptides, autoinducer-2, diffusion signaling factors, and α-hydroxyketones have been studied in bacteria. Genetic alteration of QS machinery can be useful to modulate vital characters valuable for environmental applications such as biofilm formation, biosurfactant production, exopolysaccharide synthesis, horizontal gene transfer, catabolic gene expression, motility, and chemotaxis (Fig 1). These qualities are imperative for bacteria during degradation or detoxification of any pollutant. QS signals can be used for the fabrication of engineered biofilms with enhanced degradation kinetics (Mangwani et al., 2016).

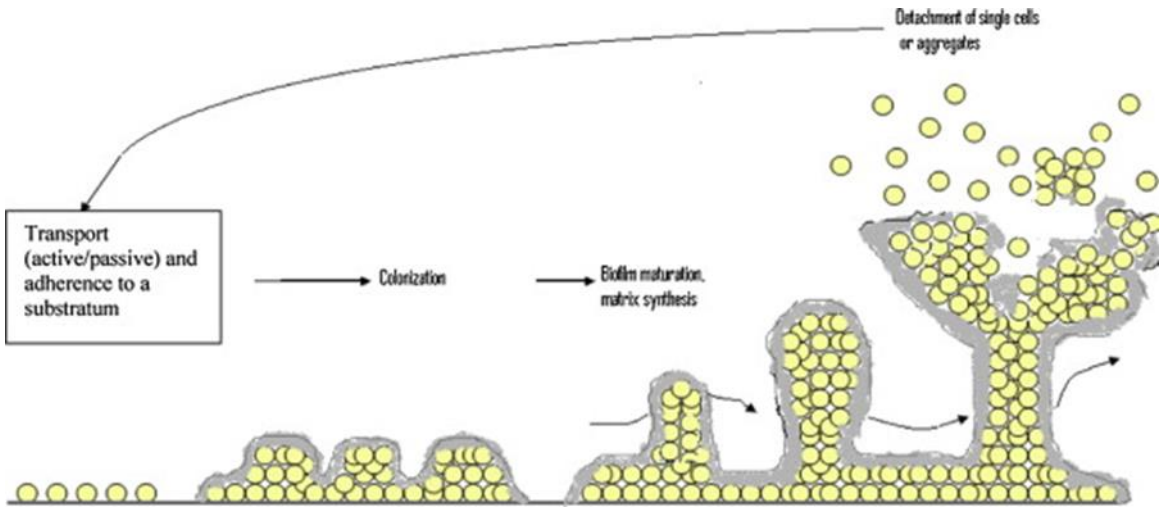

Fig. 1. The cyclic evolution of a biofilm at cellular level, from single cells, sedimented and adherent to a conditioning pellicle on a substratum, cellular multiplication and colonization of substratum, formation of a multilayer biofilm with columns and mushroom-like structures, and finally the detachment of single cells or aggregates and their dissemination (Lazar, 2011).

QS also regulates many biological behaviors of the mature biofilm. For example, the expressionof several extracellular virulence factors, including exotoxin A, elastase, pyoverdin and rhamnolipid (Kirisits and Parsek, 2006). QS factors are critical to establishing infection in the host and contribute antibiotic resistance properties, as well; therefore, inhibition of the QS system is believed to be a promising strategy to attenuate production of virulence factors and protect against infection. Secretion of an extracellular polysaccharide substances that acts as a physical protection barrier for the encased bacteria. there is pressing demand for targeted therapies that are capable of killing cancer cells selectively without afecting normal healthy cells or at least to act as adjuvants to lower the therapeutic doses and increase efciency of conventional anticancer drugs. Synthesis of EPSs by LAB is a well-known phenomen on which exists as a cell-bound EPS, adhering closely to the bacterial surface, or releases EPS into the surrounding medium (Paulo et al., 2012). The polysaccharide structure may affect bacterial adhesion to mucus. Other authors have claimed that, in some cases, the EPS envelope covering the producing strains hinders bacterial adhesion (Nikolic et al., 2012).

Lactobacillus EPSs have been reported to contain several functional groups, for instance, carbonyl, phosphate, andhydroxyl groups, which were suggested to play an essential role in exerting the antimicrobial and antioxidant effects of EPS (Riaz Rajoka et al., 2020). These polymers showed in vitro or in vivo inhibition of Gram-positive and Gram-negative pathogens with different degrees of resistance. The Gram-positive bacteria cell wall includes numerous structural components, which are essential components involved in the interaction between bacterial cells and various receptors on other surfaces (AlKassaa et al., 2014). The cell surface of bacteria is a vital elementin cell-to-cell and cell-to-host communications (Dertli et al., 2015). Many of the studies reviewed in this report did not examine the electrical charge on the polymer. For instance, negatively charged EPS from the Lactococcus lactis F-mou strain revealed higher inhibitory action against Gram-positive than Gram-negative pathogens, with Bacillus cereus ATCC 10702 demonstrating the greatest inhibition (Nehal et al., 2019). There is no definitive mechanism to explain the antibacterial

action of the EPS produced by the LAB against Gram-positive and -negative bacteria. Attempts to investigate a potential mechanism(s) to explain the observed antibacterial activity are continuing. It was thought that because EPS was able to disrupt the structure of the bacterial cell envelope, especially the peptidoglycan layer, it was proposed as a potential inhibitory mechanism (Sivasankar et al., 2018). These biofilm communities containing beneficial organisms such as lactobacilli may play an essential role as protective agents (Aoudia et al., 2016).

Biofilm formation is a complex and dynamic process comprising five steps including: initial attachment, irreversible attachment, initial growth of the biofilm structure, maturation, and dispersion (Spanò et al., 2016). Bacteria release chemical autoinducers (AIs) through the action of QS that accumulate within the bacterial environment (Fig 2). Through detecting changes in AI concentrations, bacteria exchange information. A range of signaling molecules related to bacterial QS has been discovered. Dependent on the type of signaling and sensor systems, the QS of bacteria can be classified, we summarize the pathways associated with each QS system and its distribution in bacteria (Ran et al., 2016). İnhibition of signaling molecule generation: the QS process is inseparable from the generation and participation of signaling molecules. By inhibiting related enzymes in the signaling molecule synthesis pathway, the generation of signal molecules can be blocked and QS can be inhibited. For example, Triclosan can inhibit enoyl-ACP reductase an important protein in the acyl-homoserine lactone (AHL) generation process (Fischer et al., 2015).

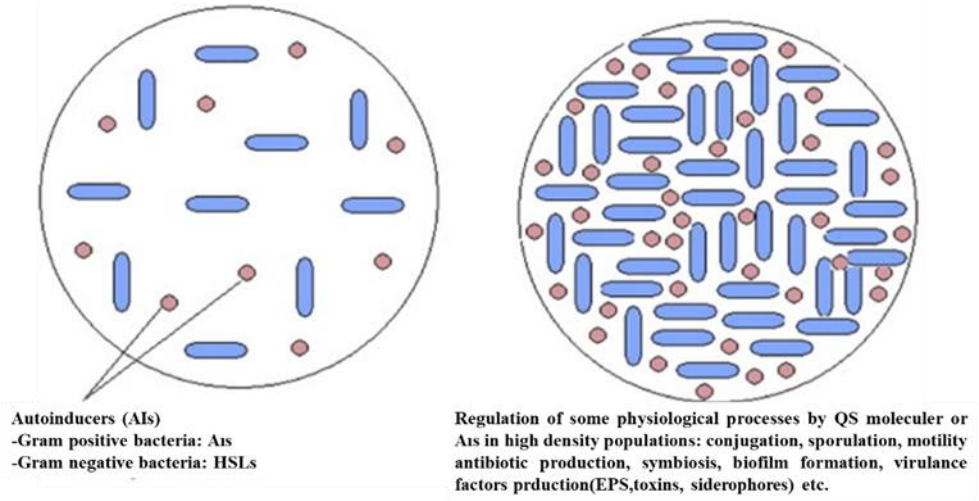

Fig 2. Cell to cell communication by QS mechanism, mediated by small molecules or autoinducers (Lazar, 2011).

REFERANCE

Al Kassaa, I., Hober, D., Hamze, M., Chihib, N. E., and Drider, D. (2014). Antiviral potential of lactic acid bacteria and their bacteriocins. Probiotics Antimicrob. Proteins 6, 177–185.

Amiri, S., Rezaei Mokarram, R., Sowti Khiabani, M., Rezazadeh Bari, M., Alizadeh Khaledabad, M. (2019). Exopolysaccharides production by Lactobacillus acidophilus LA5 *and Bifidobacterium animalis* subsp. lactis BB12: Optimization of fermentation variables and characterization of structure and bioactivities. *Int. J. Biol. Macromol.* 123, 752–765.

Aoudia, N., Rieu, A., Briandet, R., Deschamps, J., Chluba, J., Jego, G., et al. (2016). Biofilms of *Lactobacillus plantarum* and *Lactobacillus fermentum*: effect on stress responses, antagonistic effects on pathogen growth and immunodulatory properties. *Food Microbiol.* 53,51-59.

Badel, S., Bernardi, T., Michaud, P. (2011) New perspectives for *Lactobacilli* exopolysaccharides. *Biotechnol Adv* 29(1):54–66

Barrett, J.S., Canale, K.E., Gearry, R. B., Irving, P. M., Gibson, P.R. (2008) Probiotic effects on intestinal fermentation patterns in patients with irritable bowel syndrome. World J. *Gastroenterol.* 14:5020–5024

Castro-Bravo, N., Wells, J. M., Margolles, A., Ruas-Madiedo, P. (2018) Interactions of surface exopolysaccharides from *Bifidobacterium* and *Lactobacillus* within the intestinal environment. *Front. Microbiol.* 9:2426.

Dertli, E., Mayer, M. J., and Narbad, A. (2015) Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. BMC Microbiol. 15:8.

El-Newary, S.A., Ibrahim, A. Y., Asker, M. S., Mahmoud, M. G., El Awady, M. E. (2017) Production, characterization and biological activities of acidic exopolysaccharide from marine Bacillus amyloliquefaciens 3MS 2017. *Asian Pac. J. Trop. Med.* 10(7):652-662.

FAO/WHO Experts' Report. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Cordoba, Argentina, 1-4 October 2001.

Fischer, T. L., White, R. J., Mares, K. F., Molnau, D. E., Donato J. J. (2015) FabV requires functional reductase activity to confer reduced Triclosan susceptibility in Escherichia coli, *Journal of Molecular Microbiology and Biotechnology*. 25(6):394–402,

Gaspar, P., Carvalho, A. L., Vinga, S., Santos, H., and Neves, A. R. (2013). From physiology to systems metabolic engineering for the production of biochemicals by lactic acid bacteria. *Biotechnol. Adv.* 31, 764–788.

Kirisits, M.J., and M.R, Parsek. (2006). Does Pseudomonas aeruginosa use intercellular signalling to build biofilm communities? Cell Microbiol. 8:1841-1849.

Kusada, H. Tamaki, H., Y., Kamagata, S., Hanada, and N. Kimura. (2017) A novel quorum-quenching N-acylhomoserinelactone acylase from Acidovorax sp. strain MR-S7 mediates antibiotic resistance, *Applied and Environmental Microbiology*. 83(13):13.

Lazar, V. (2011) Quorum sensing in biofilms- How to destroy the bacterial citadels or their cohesion/power? *Anaerobe* 17(6):280-285.

Mangwani, N., Kumari, S., Das, S. (2016) Bacterial biofilms and quorum sensing: fidelity in bioremediation technology. *Biotechnol Genet Eng Rev.* 32(1-2):43-73.

Matz, C., Bergfeld, T., Rice, SA., Kjelleberg, S. (2004) Microcolonies, quorum sensing and cytotoxicity determine the survival of Pseudomonas aeruginosa biofilms exposed to protozoan grazing. Environ Microbiol 6: 218–226

Nehal, F., Sahnoun, M., Smaoui, S., Jaouadi, B., Bejar, S., and Mohammed, S. (2019). Characterization, high production and antimicrobial activity of exopolysaccharides from *Lactococcus lactis* F-mou. Microb. *Pathogen.* 132, 10–19.

Nguyen, A.T.-B., Picart-Palmade, L., Nigen, M., Jimenez, L., Ait-Abderahim, H., Marchesseau, S. (2019). Effect of mono or co-culture of EPS-producing Streptococcus thermophilus strains on the formation of acid milk gel and the appearance of texture defects. *Int. Dairy J.* 98:17-24.

Nikolic, M., López, P., Strahinic, I., Suárez, A., Kojic, M., Fernández-García, M., Topisirovic, L., Golic, N., Ruas-Madiedo, P. (2012) Characterisation of the exopolysaccharides (EPS)-producing Lactobacillus paraplantarum BGCG11 and its non-EPS producing derivative strains as potential probiotics. *Int J Food Microbiol.* 158:155–162.

Nwodo, U., Green, E., Okoh, A. (2012). Bacterial exopolysaccharides: functionality and prospects. Int. J. Mol. Sci. 13:14002–14015.

Paterson, D. M., Aspden, R. J., & Black, K. S. (2009) Intertidalflats: Ecosystem functioning of soft sediment systems. In G. M. E. Perillo, et al. (Eds.) Coastal Wetlands: An Integrated Ecosystem Approach 317–338.

Paulo, E., Bofo, E., Branco, E., Valente, E., Melo, E., Ferreira, A., Roque, M., Assis, S. (2012). Production, extraction and characterization of exopolysaccharides produced by the native Leuconostoc pseudomesenteroides R2 strain. *An Acad Bras Cienc.* 84:495-508.

Riaz Rajoka, M. S., Wu, Y., Mehwish, H. M., Bansal, M., and Zhao, L. (2020). Lactobacillus exopolysaccharides: new perspectives on engineering strategies, physiochemical functions, and immunomodulatory effects on host health. *Trends Food Sci. Technol.* 103:36–48.

Ruas-Madiedo, P. (2014) Biosynthesis and bioactivity of exopolysaccharides produced by probiotic bacteria. In: Moreno F. J., Sanz L. M., editors. Wiley; *Food Oligosaccharides. Hoboken*, NJ, USA. 118-133.

Sivasankar, P., Seedevi, P., Poongodi, S., Sivakumar, M., Murugan, T., Sivakumar, L., et al. (2018) Characterization, antimicrobial and antioxidant property of exopolysaccharide mediated silver nanoparticles synthesized by Streptomyces violaceus MM72. *Carbohydr. Polym.* 181, 752–759.

Spanò, A., Laganà, P., Visalli, G., Maugeri, T. L., and Gugliandolo, C. (2016) In vitro antibiofilm activity of an exopolysaccharide from the marine thermophilic Bacillus licheniformis T14. Curr Microbiol. 72, 518–528.

Stoodley, P., Sauer, K., Davies, D. G., & Costerton, J. W. (2002) Biofilms as complex differentiated communities. *Annual Review of Microbiology.* 56(1): 187–209.

T. Ran, C., Zhou, L., Xu, et, al. "Initial detection of the quorumsensing autoinducer activity in the rumen of goats in vivoand in vitro," Journal of Integrative Agriculture, vol. 15,no. 10, pp. 2343–2352, 2016.

Wingender, J., Neu, T., Flemming, H. (1999) Microbial extracellular polymeric substances: Characterization, structures and function. Berlin: Springer.

Y. Chang, P. C., Wang, H. M., Ma, et, al. (2019) Design, synthesis and evaluation of halogenated furanone derivatives as quorum evaluation of halogenated furanone derivatives as quorum sensing inhibitors in Pseudomonas aeruginosa, *European Journal of Pharmaceutical Sciences*,140, 105058,

Zannini E., Waters D.M., Coffey A., Arendt E.K. (2016). Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl. *Microbiol. Biotechnol.* 100:1121-1135.

The Problems Experıenced By Infertil Couples and the Nursing Approach

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Introduction

Infertility is defined as the inability to conceive or maintain a pregnancy despite having regular sexual intercourse (two or three times a week) for at least one year without using any contraception method. It is reported that 10-15% of couples of reproductive age in the world have infertility problems and more than 80 million people are affected by infertility. In our country, it has been determined that the rate of married women between the ages of 15-49 who have no children and who are not able to have children is 11.2%. According to the 2018 data of the Turkey Demographic and Health Survey (TNSA), the rate of married women between the ages of 15-49 who have no children and stated that it is not possible to have a child is 5%. Approximately 40% of infertility cases, which is one of the important questions of our age and affects many couples, are women, 40% are men, and the remaining 20% are combined causes.

Infertility diagnosis and treatment process is a difficult and long process that affects couples physically, psychologically and financially. In this process, couples may experience stress, panic and intense emotional pressure. At the same time, in societies where femininity is equivalent to motherhood and men's productivity, fertility is accepted as an indicator of an individual's sexual identity. The society's ostracism of the woman without children and the questioning of her husband's masculinity may negatively affect the mental health and quality of life of the couple, leading to depression, anxiety, decreased sexual desire and deterioration in marital relations. In many studies, it has been determined that infertile couples experience psychological, social and sexual problems. Although both spouses are affected during the diagnosis and treatment of infertility, women feel more stress, guilt and responsibility than men due to the effect of interventions on the female body.

In this section, it is aimed to understand the problems experienced by infertile couples by examining the socio-cultural, psychosocial, economic, sexual problems and quality of life of infertile couples in line with the literature.

Sociocultural Problems in Infertile Couples

Marriage brings with it having children and problems may arise in marriages where there is no child. Birth not only increases the respect for the woman, but also strengthens the place of the woman in the family, relatives and group. The father, on the other hand, not only looks to the future with confidence, but also gains prestige among his friends and relatives. Because no matter how despised the infertile woman is by her relatives because she cannot give birth, the man also feels the social and mental oppression of the pressure coming from the environment and the 'not being put in the place of a man'. A woman who does not have children is not treated well in the family, and a woman who is called "infertile" can be described as "unlucky". As in many countries, the words woman and mother are used synonymously in Turkey, and childlessness in such a

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cultural structure is quite a degrading situation for women. In our country, couples who do not have children in rural areas are excluded by the society, but more blame falls on the shoulders of women, and the woman has to accept the marriage in order for her husband to have a child. One of the basic instincts in humans is the reproductive instinct. It may become inevitable for sexuality to go beyond the reproductive function, to experience feelings of failure and inadequacy at this stage, and to create problems that spread to many areas of life. Inability to reproduce often creates a social stigma and is perceived as a shameful inadequacy.

Cultural, ethnic or religious factors also impose new burdens on the infertile couple. For example, there is a lot of superstition and misinformation about infertility, especially in vitro fertilization (IVF) treatment in Turkish culture. Kamacı (2003) found in his study that 28% of women and 8% of men visit the hodja-mausoleum-çeşme, 7% of women and 36% of men use herbal medicine, and about half of the couples resort to traditional methods.

Childbirth is perceived as a divine reward, while infertility is perceived as divine punishment. In some religions, assisted reproductive techniques are considered a sin, and if the woman is infertile, her marriage is perceived as inadequacy.

Psychosocial Problems in Infertile Couples

Although the reactions of individuals to infertility show individual differences, it is stated that there are similar aspects. For couples, the common feelings of not being able to have children are the feeling of frustration and the lack of parental roles in society.

Since infertility is a multidimensional problem, it negatively affects the psychological well-being of couples. D'Souza et al., in their study, stated that the psychological well-being of women who received infertility treatment was low. In Klemetti's study; states that the psychological well-being of infertile women and men is very low and that this situation is related to anxiety and depression. In addition, it has been reported that the emotional stress experienced during the diagnosis and treatment of infertility may cause couples to think that they cannot have children and lead them to a sense of hopelessness. Kaya et al., in their study, found that infertile women experience stigma and hopelessness. determined that hopelessness increased as social support decreased in infertile women.

Depression and anxiety levels were compared among infertile couples in Poland in 2009. According to Beck depression scale; Depression level was found to be 28.6% in infertile women, 18.4% in fertile women, 9.0% in infertile men, and 11.0% in fertile men (Drosdzol and Skrzypulec 2009). In the study of El Kissi et al. (2013) with couples who received infertility treatment, it was determined that women experience more anxiety and depression than their spouses. In addition, women's self-esteem was found to be lower than their spouses [16]. In Yusuf's study (2016), it was found that 70% of infertile women experience varying degrees of anxiety and 58% experience moderate-to-severe anxiety.

Psychosexual Problems Experienced by Infertile Couples

Evaluation of infertility is a condition that is emotionally disturbing and also sexually disturbing to individuals. For example, when taking the history of the couples, the couples are asked questions about their sexual performance such as copulation techniques, sexual desire and responses, history of abortion, discomforts during intercourse, knowledge of sex and reproduction. Such questions may be seen as threatening, disturbing and inappropriate by couples. In some cases, questions focusing on sexual competence can lead to sexual failures or changes in sexual behavior over time.

Infertile couples frequently experience sexual problems such as sexual reluctance, anorgasmia, decreased sexual satisfaction, and dyspareunia as sexuality becomes an act to have children. In the study of Tao et al. (2011) it was stated that infertility affects the sexual activities of

individuals, and there is a tendency to decrease in the frequency of sexual intercourse in individuals experiencing fertility stress. In the study of Duman and Koçak (2013), it was determined that the most common sexual problem in infertile women was arousal at a rate of 20.5% and orgasmic disorder in 19.5% of women. In the study of Ergin et al. (2018), women reported that they did not find themselves attractive because sexuality was seen as a duty. In the process of infertility, couples who adapt to the change in sexual life increase mutual communication, attachment and satisfaction with marriage. It is stated that infertile women have lower marital adjustment than infertile men (Egelioğlu Çetişli et al., 2014; Bodur et al., 2013) and that as dyadic adjustment increases in infertile couples, there is a decrease in anxiety and depression levels (Bodur et al., 2013).

Economic Problems in Infertile Couples

Since the drugs used in the treatment of infertility and the treatment process are quite expensive, they also cause economic difficulties for couples. Working couples are constantly dismissed from work due to treatment.

They have to get permission. Especially women quit their jobs or give up on making a career. In a study conducted by Koçyiğit in 2012, it was reported that the average expenditure for infertility treatments reached approximately three times the average household budget (Koçyiğit, 2012). The economic difficulties experienced can cause domestic economic violence. In a study, it was stated that 41 out of 122 infertile women were subjected to domestic violence and 29.2% were exposed to economic violence. In his study, Can (2005) found economic problems at a rate of 16.4% among the causes of stress experienced by infertile women during treatment. In the study of Kamacı (2003) it was determined that 84% of women and 85% of men stated that infertility treatment forced their family budgets economically.

Quality of Life of Infertile Couples

For most couples, infertility is a major life crisis. Studies on the relationship between the psychological reaction to infertility and gender have shown that women experience higher levels of anxiety, depression and loss of self-confidence compared to their spouses. However, psychological factors such as anxiety, depression and anxiety may affect physiological factors by reducing the probability of pregnancy. Psychosocial effects in the diagnosis and treatment of infertility can significantly affect the overall quality of life. In addition, spouses may react differently to infertility.

On the one hand, infertility itself, on the other hand, examination and treatment approaches applied for reproductive assistance, strain the coping skills and social support resources of the individual and the couple, and consume their physical and emotional energy, causing sexual dysfunction, depression, anxiety and deterioration in the couple's relationship. In the Rashidi and Montazeri study in Iran (2008), when the quality of life of women and men who receive IVF treatment is examined, according to the findings obtained, men lead a better and better quality life than women. In general, the relationship between being young, being female, low education level and quality of life was found to be significantly significant; No relationship was found between the duration of infertility, previous infertility treatment, or the causes of infertility and quality of life. It is understood that while the studies carried out to date mostly focus on documenting the physiological causes of infertility, less attention is paid to the individual's quality of life.

Johansson et al., examining the quality of life in couples 4–5.5 years after failed IVF treatment, found that childless couples had a lower quality of life than couples with children. Khayata et al. reported that female factor infertility adversely affects quality of life in infertile women compared to male and combined factor infertility.

Infertility Nursing

Nurses have an important role in supporting the communication of infertile couples, providing counseling for psychological problems, supporting coping resources and raising

awareness of the society. In addition to the roles of infertility nurses in medical care, there are roles of providing information, coordinator-manager, researcher, patient rights advocate, educator-counseling and psychological support. Within the scope of these roles, nurses often take responsibility in practices that require special knowledge and skills, such as evaluating the infertile couple, planning the determined treatment, assisting in assisted reproductive treatment procedures, preventing complications, case management, infertility counseling and coordinating. In addition, infertility nurses can provide information, explanation and decision-making counseling (the type of counseling in which the effect of starting or ending infertility treatment on the couple's life is explained), support counseling (the type of counseling given with therapeutic counseling for stress reduction) and therapeutic counseling (individual counseling, couple counseling). , group therapy, individual behavioral groups, emotional interactive groups, groups created using technology), telephone counseling and self-help groups are recommended. Nurses should be aware of the problems and effects caused by infertility, which creates a crisis in the lives of couples. The communication of the infertile couple should be supported, stress, anxiety and depression symptoms should be aware of the psychological problems that the couple may experience, risk factors should be questioned, and cooperation should be made with a psychologist or psychiatric nurse. The family should be supported in coping with the crisis situation, approaches that increase women's self-esteem and strengthen the relationship between couples should be adopted to reduce hopelessness, and public awareness should be raised in order to reduce social pressure on the couple.

Due to their changing and developing roles in the team, infertility nurses need to be aware of the economic and ethical problems and develop themselves in counseling for sexual health. Effectively fulfilling the roles of nurses will help couples to cope with problems and become stronger in coping with the process. Continuous trainings to be organized in this field will also contribute to the empowerment of infertility nurses.

References

Afshani AS, Abooei A, Abdoli MA. Self-compassion training and psychological well-being of infertile female. *Int J Reprod Biomed* 2019, 17(10): 757-62.

Anokye R, E. Acheampong, W.K. Mprah, J.O. Ope, T.N. Barivure, "Psychosocial effects of infertility among couples attending St. Michael's Hospital, Jachie-Pramso in the Ashanti Region of Ghana," BMC Research Notes, 10(1):690-694, 2017.

Bayraktar. E. (2018) Toplumsal cinsiyet, kültür ve şiddetin infertilite ile ilişkisi, *Sağlık Bilimleri Dergisi*, 27(3): 234-238.

Beji NK. İnfertilite. Hemşire ve Ebelere Yönelik Kadın Sağlığı ve Hastalıkları Nobel Tıp Kitapevleri İstanbul: İletişim, 2016

Can G (2005). Yardımcı üreme tekniklerine başvuran kadınların anksiyete ve depresyon düzeylerinin incelenmesi. Mezuniyet tezi. Ege Üniversitesi Hemşirelik Yüksekokulu, Bornova, İzmir

Darbandi S, M. Darbandi, H.R.K. Khorshid, M.R. Sadeghi, "Yoga can improve assisted reproduction technology outcomes in couples with infertility", Reproductive Health, 1, 2, 2018.

Deka PK, Sarma S. Psychologicalaspects of infertility. British Journal of MedicalPractitioners.2010; 3(3): 336- 8.

Demirci H (2001). İnfertilitenin Çiftler Üzerinde Psikososyal ve Psikoseksüel Etkileri. İnfertilite Sorunu, Yardımcı Üreme Teknikleri ve Hemşirelik Yaklaşımı. Editör: NK Beji, İstanbul. 104-117

Demirci N, Çoşkuner D.P, "İnfertilitede temel kavramlar, etiyolojisi ve risk faktörleri", İnfertilite Hemşireliği El Kitabı, Nobel Tıp Kitabevi, İstanbul, 11-36, 2017.

Demirci N, Gün Ç, Potur DC, Koyucu R. İnfertilite hemşirelerinin yaşadığı güçlükler ve iş doyumu. Türkiye Klinikleri Doğum-Kadın Sağlığı ve Hastalıkları Hemşireliği Özel Dergisi 2016; 2 (3): 34-39.

Drosdzol, A., & Skrzypulec, V. (2008). Evaluation of marital and sexual ınteractions of Polish infertile couples. The European Journal of Contraception and Reproductive Health Care, 1-11

D'Souza V, Noronha JA, Kamath S. Psychosocial wellbeing and coping strategies of infertile women seeking infertility treatment. *J. Infertil. Reprod. Biol.* 2015, 3(2): 176-80.

Duman, D.Y. Koçak, "İnfertilitenin cinsel sağlığa etkileri ve profesyonel hemşirelik yaklaşımları", *Turkiye Klinikleri Obstetricwomen's Health and Diseases Nursing-special Topics*, 2(1):51-58, 2016.

El KissiY, RomdhaneAB, Hidar S, Bannour S, AyoubiIdrissi K, Khairi H, Ben Hadj Ali B. General psychopathology, anxiety, depressionand self-esteem in couplesundergoingınfertilitytreatment: a comparativestudybetween men andwomen. EuropeanJournal Of Obstetrics&GynecologyandPeproductiveBiology 2013; 167(2): 185-189

Emül TG. İnfertil çiftlerin yaşadığı cinsel sorunlarda hemşirenin rolü. E-sağlık Hemşirelik Dergisi 2018: 9(33)

Ergin N, Polat A, Kars B, Öztekin D, Sofuoğlu K, Çalışkan E, "Social stigma and familial attitudes related to infertility" *Turkish Journal of Obstetrics and Gynecology*, 15(1):46-49, 2018.

Fieldsend M, Smith JA. 'Either stay grieving, or deal with it': the psychological impact of involuntary childlessness for women living in midlife. *Hum Reprod* 2020, 35(4): 876-85.

Gözüyeşil E, Yikar SK, Nazik E. An analysis of the anxiety and hopelessness levels of women during IVF-ET treatment. *Perspect Psychiatr Care* 2020, 56(2): 338-46.

Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü Türkiye Nüfus ve Sağlık Araştırması. Ankara, Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü, 2013

Johansson, M., Adolfsson, A., Berg, M., Francis, J., Hogström, L., Janson P.O., Sogn, J., Hellström, A.L., (2009). Quality of life for couples 4-5.5 years after unsuccessful IVF treatment. Acta Obstetricia et Gynecologica. 88; 291- 300.

Kamacı S (2003). Primer İnfertil çiftlerde infertilitenin aile yaşamına etkisinin incelenmesi. Mezuniyet tezi. Ege Üniversitesi Hemşirelik Yüksekokulu, Bornova, İzmir.

Khayata G.M., Rizk D.E., Hasan M.Y., (2003). Factors infl uencing the quality of life of infertile women in United Arab Emirates. International Journal of Gynaecology & Obstetrics, 80(2); 183–88.

Kaya Z, Oskay U. Stigma, hopelessness and coping experiences of Turkish women with ınfertility. J Reprod Infant Psychol 2019, 1-12.

Klemetti R, Raitanen J, Sihvo S, Saarni S, Koponen P. Infertility, Mental Disorders and Well-Being: A Nationwide Survey. Acta Obstetricia et Gynecologica Scandinavica 2010; 89: 677-682.

Kırca N, PasinlioğluT. İnfertilitetedavisinde karşılaşılan psikososyal sorunlar. Psikiyatride Güncel Yaklaşımlar 2013; 5(2): 162-178.

Miner S.A, Robins S, Zhu Y.J, Keeren K, Gu V., S.C. Read, P. Zelkowitz, "Evidence for the use of complementary and alternative medicines during fertility treatment: a scoping review", BMC complementary and alternative medicine, 18(1), 158, 2018.

Rashidi, B., Montazeri, A., Ramezanzadeh, F., Shariat, M., Abedinia, N., & Ashrafi,M. (2008). Health-related quality of life in infertile couples receiving IVF or ICSI treatment. BMC Health Serv Res., 8; 1-6.

Slade P, O'Neill C, Simpson AJ, LashenH. T h e r e l a t i o n s h i p b e t w e e n p e r c e i v e d s t i g m a , disclosurepatterns, supportanddistress in newattendees at an ınfertility clinic. Human Reproduction2007; 22(8):2309-2317

Seymenler S, Siyez D.M., "İnfertilite psikolojik danışmanlığı", Current Approaches in Psychiatry/Psikiyatride Güncel Yaklaşımlar, 10(2), 2018.

Tao P, R. Coates, B. Maycock, "The impact of infertility on sexuality: a literature review," *AMJ,* 4(11):620-627, 2011.

Türkiye Nüfus ve Sağlık Araştırması (TNSA) 2018. Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü Türkiye Nüfus ve Sağlık Araştırması, Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü, Sağlık Bakanlığı Ana Çocuk Sağlığı ve Aile Planlaması Genel Müdürlüğü, Başbakanlık Devlet Planlama Teşkilatı Müsteşarlığı ve TÜBİTAK, Ankara, Türkiye, 2018.

Vural PI, Beji NK. İnfertilite sorununun psikoseksüel etkileri. Androloji Bülteni 2014; 16 (57): 135-8.

Yağmur Y, Oltuluoğlu H. Social support and hopelessness in women undergoing ınfertility treatment in Eastern Turkey. *Public Health Nursing* 2011, 29(2): 99–104.

Yanıkkerem E, Kavlak O, Sevil Ü, "İnfertil çiftlerin yaşadıkları sorunlar ve hemşirelik yaklaşımı," *Atatürk Üniversitesi Hemşirelik Yüksekokulu Dergisi*, 4:112-121, 2008.

Yusuf L. Depression, anxietyandstressamongfemalepatients of ınfertility; a casecontrolstudy. Pakistan Journal Of MedicalSciences 2016; 32(6):1340-1343.

Zare Z, Amirian M, Golmakani N, Mazlom R, Ahangar ML. Sexualdysfunction in infertilewomen. International Journal of ReproductiveBioMedicine 2016; 14(2), 89- 94.

Zeren E, Gürsoy E, "İnfertil çiftlerde çift uyumu ve yaşam kalitesinin önemi", Hemşirelikte Eğitim ve Araştırma Dergisi,16(1):68-72, 2019.

Quality of Life and Nursing Care in Menopause

Fatma BAŞAR[1]

Introduction

Menopause, which is a natural part of the aging process in women, is defined as the loss of reproductive ability due to regression in ovarian functions. WHO defines menopause as "the permanent cessation of menstruation as a result of loss of ovarian activity". In the studies, it is seen that the age of menopause varies between 49.3-51.4 years in developed countries, while this age range is 43.5-49.4 years in developing countries. When the TDHS data is examined, it has been determined that the age of Turkish women at menopause is concentrated in the 46-49 age range. According to the TDHS 2018 results, the age group with the highest menopause rate is the 48-49 age group with a rate of 45.1%. While the rate of women entering menopause in the 46-47 age range is 31.2%, the rate in the 44-45 age range is 15.9%.

In some women, the menopause process begins earlier than the expected age range. This process, called premature menopause; It is defined as "the loss of ovarian function of the ovary before the age of 40 (cessation of menstruation)". According to the 2013 results of TDHS, the rate of women between the ages of 30-39 who entered menopause is 1.9%. According to the 2018 results of TDHS, the proportion of women between the ages of 30-39 who entered menopause increased to 2.6%.

As in every life period, many factors are effective on the time of menopause. In the fact that the age of menopause differs from society to society, even in different cultures of the same society; It is thought that individual factors such as genetic and environmental factors, nutrition and routine habits also have an effect. age of menopause; It is not affected by race, socio-economic status, number of pregnancies, oral contraceptive use, education, physical characteristics, alcohol consumption, age, or date of last pregnancy. It has been determined that smoking definitely accelerates the consumption of follicles and causes early menopause. Menopause directly affects both work and family life and social life of women.

Menopausal period; women experience intense hormonal changes; It is a period in which physiological, psychological, sexual and social changes occur depending on this change. The main reason for these changes is the decrease in estrogen level. During this period, symptoms related to different body systems may occur, from vasomotor symptoms to mental symptoms, from sexual function changes to osteoporosis. Symptoms such as menstrual irregularities, vaginal dryness, flora changes, sleep disorders, headache, dizziness, shortness of breath, heart palpitations, tremors, and anxiety can also be seen.

It is among the most important responsibilities of the nurse to determine their quality of life and the factors affecting it, while giving care to women in the menopausal period, and to use the results to improve women's health. Nurses should have knowledge about the risk factors that

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may develop by determining the changes that may occur in women in this period and know how this will affect their quality of life; will provide support for women to gain coping skills with menopausal complaints. In addition, nurses; Prioritizing the quality of life while giving care to women in the menopausal period will enable them to provide higher quality care and counseling services in order to increase the quality of life of women. In this section, the effects of menopausal problems on quality of life and nursing approach are discussed.

Changes Seen During The Menopuse Period

With the menopause period, the activity of the ovaries begins to decrease and there is a decrease in the estrogen level to women; physiological, psychological, sexual and social etc. changes in many ways. These changes, which are thought to occur due to estrogen deficiency, cause many problems in the early and late periods. In the early period, vasomotor symptoms such as hot flushes, sweating, palpitations, headache, insomnia, muscle-bone pains, depression, loss of attention, forgetfulness, decreased libido, vaginal atrophy and urinary problems are. In the long term, there is an increase in the incidence of osteoporosis, cardiovascular diseases and cancers.

Cycle Changes

One of the most obvious differences experienced by women in the menopausal period is menstrual cycle changes. The termination of estrogen production, which is considered to be the main cause of menopausal changes, does not occur suddenly. Estrogen continues to be secreted in small amounts, so menstrual bleeding in women may continue for a while. This is most common in the premenopausal period. During this period, menstruation is of the anovulatory type. That is, the luteal phase has disappeared. Due to the decrease in the activity of the ovaries, enough estrogen is not secreted and the endometrial cycle cannot occur because the amount of estrogen in the blood decreases. Due to the anovulatory cycle, estrogen is constantly secreted from the follicles, and since this excess is not met by progesterone, bleeding can be intense and continuous during this period. As a result of all these changes, menstruation in women; It may occur as polymenorrhea, amonorrhea, hypomenorrhea, and hypermenorrhea. This type of bleeding can be a normal physiological process as well as a harbinger of various diseases. Therefore, women in the menopausal period should be considered as a risky group starting from the premenopausal period and the care to be given should be planned correctly.

Vasomotor Changes

Vasomotor changes are one of the most common symptoms, the severity of which varies by most women during the climacteric period. Vasomotor symptoms begin to appear from the premenopausal period and their incidence varies between 60% and 85%. vasomotor changes; Hot flashes are defined as an uncomfortable feeling of warmth that starts in the face, head or chest and progresses in any direction, sometimes spreading to the whole body. In the study of Ege et al. (2014) on women in the menopausal period; 69.5% experienced hot flashes, 55.3% had night sweats and 53.7% had insomnia problems; According to the study of Gözübebek and Başer (2016), it was seen that these vasomotor problems experienced by women negatively affect their daily lives.

Emotional Changes

Each life period has its own characteristics and stress factors. The menopausal period is also a period that includes many changes in women's lives. Therefore, it is important for women to be able to adapt to these changes and not to deteriorate their quality of life. The degree to which the woman is affected by each physiological event seen in this period and the meaning attributed to it varies from person to person. Mental problems are very common in the menopausal phase of women's life, especially in the perimenopause period. In the first place, thoughts that anxiety, depression and sexual dysfunctions increase are frequently expressed. Studies conducted to determine the emotional changes of women in the menopause period have also revealed that

women in this period experience emotional problems. In the study of Simon and Reape (2009), 91% of women in the climacteric period had irritability, irritability, 86% depression, 82% loss of concentration, 81% personality change, 77% sleep disorder, 77% It was determined that there was lack of motivation in .75%, memory deficit in 75%, hot flashes in 37%, headache in 19% and excessive sweating in 18%. According to the research results of Bozkurt and Sevil (2016); 44.2% of the women participating in the study had menopause after distress-stress, followed by "relaxation (20.8%)", "inability to bear children (12.5%), "aging (8.3%)", "a natural event (7.5%)" and "loss". (6.7%)". In Taiwan, a study was conducted with 3359 women aged 40-55 years to determine the level of depression occurring during the menopause, and 145 of the women had high-grade depression symptoms related to menopause.

Structural Changes in Bone

Another change that occurs as a result of long-term estrogen deficiency is osteoporosis. osteoporosis; It is a chronic and progressive musculoskeletal problem that begins with a decrease in the volume, density and quality of bone mass and continues with increased susceptibility to fracture. 50% of individuals aged 50 and over living in Turkey have osteopenia and 25% have osteoporosis. While this rate is 12.9% for women over 50 years old, it is 7.5% for men. Since 80% of people with osteoporosis are women, osteoporosis is an important gynecological problem. The national osteoporosis organization states that there are 200 million people in the world suffering from osteoporosis. When we look at the rate of women experiencing this problem, 30% of women with osteoporosis are postmenopausal women, and one out of every three women over the age of 50 experiences bone fractures caused by osteoporosis.

Cardiovascular Changes

Menopause is accepted as a risk factor for cardiovascular system diseases. Especially in terms of coronary artery disease, women with surgical menopause and early menopause are at higher risk than women who enter menopause in the normal age range. Since the positive effect of estrogen will disappear earlier in women who enter menopause early, these women are at higher risk for cardiovascular diseases. In the study of Ebong et al. (2014), the risk of heart failure in women with early menopause was found to be 66% higher than in women with normal menopause. Physiologically, estrogen decreases the LDL cholesterol level and increases the HDL cholesterol level, thus regulating the serum cholesterol level. In postmenopausal women, where estrogen levels are not sufficient, the cholesterol level gradually rises and the risk of atherosclerotic disease increases. Also, from menopause, women are more susceptible to weight gain and fat distribution due to changes in estrogen levels. This is another risk factor that puts women at risk for cardiovascular diseases.

Urogenital Changes

Another change that occurs due to a decrease in estrogen is urogenital changes. Urogenital changes; They are changes in the vulva, vagina, cervix, uterus, tuba uterine, pelvis and urinary system. The main reason behind these changes is the decrease in estrogen level. Among the most important effects of estrogen on the urogenital system of women; By increasing sexual motivation, it improves secondary sex characters and gives a feminine identity. Especially in women with early menopause, the absence of estrogen stimulation for a long time with menopause adversely affects the reproductive organs and regression is observed in the reproductive organs. This regression mostly occurs in the uterus, vagina, vulva and urethra. The vulva shows very rapid atrophy in the face of estrogen deficiency. Therefore, dystrophies are one of the most common problems in menopausal women in the future. Dystrophies cause excessive stenosis of the vulva and are accompanied by itching. In addition, atrophy seen in the vulva can cause cancer formation. With the decrease in estrogen level, some changes occur in the color, texture and flexibility of the vagina. These changes are more; It manifests itself as a narrowing in the vagina, a decrease in its color and

flexibility. In menopause, the vaginal wall becomes thinner and holds less moisture. This causes vaginal dryness. Vaginal dryness is seen at low rates in women with regular menstrual cycles; The incidence of vaginal dryness is increasing in perimenopause, postmenopause and late postmenopause periods. Dyspareunia is seen with vaginal dryness. In order to prevent dyspareunia, it is recommended to use water-soluble oils to eliminate vaginal dryness, and not to use insoluble oils such as vaseline. Because insoluble oils can clog the vaginal glands and cause infection. In addition, with the decrease of Estrogen, alkaline PH occurs in the vagina and the vaginal flora deteriorates. Estrogen deficiency changes the vaginal pH from 3.5-4.5 to 6-8. The alkaline environment makes the vagina susceptible to the invasion of a large number of pathogenic bacteria. This directly makes women more susceptible to infections. As a result of the loss of flexibility of the vagina after menopause, the infrastructure of the urethra shortens and narrows. Accordingly, especially in the following years, women; problems such as urination difficulties, true stress incontinence and urethral syndrome.

Vaginal disorders mentioned in postmenopausal women negatively affect sexual function. Levine et al. (2008). reported that sexually active women with sexual dysfunction and in the postmenopausal period were approximately four times more likely to have vulvovaginal symptoms than those without sexual dysfunction. In the same study, it was determined that 40% of women with vulvovaginal symptoms experienced general sexual dysfunction, 24% aversion, 34% difficulty in arousal, and 19% difficulty in orgasm [95]. In the study of Süt and Küçükkaya (2018), in which they investigated the sexual quality of life of women in the menopause period, it was determined that the most intense vaginal dryness complaint was in the perimenopausal period, and the complaint of dyspareunia was in the postmenopausal period, and it was reported that these symptoms reduced the quality of life of women [96]. Simon et al. (2014) in a study of 1000 postmenopausal women to investigate the effects of vulvovaginal atrophy, they reported that 64% of women experienced painful intercourse and loss of libido [89]. As can be seen, it is noteworthy that the quality of sexual life decreases in most of the women in the menopause period. Although these symptoms are not life-threatening, they can be progressive. All these urogenital system changes, in fact, seriously affect the sexual life of women and their quality of life. Women should develop some healthy lifestyle behaviors and evaluate treatment options in order to be least affected by these changes.

Dermatological Changes

One of the areas most affected by estrogen deficiency is the skin. Along with the decrease in estrogen level, the aging of the skin accelerates, collagen connective tissue and elastic fibers decrease, and the epidermis becomes thinner. Depending on the decreased collagen, epidermal changes such as decrease in the thickness of the epidermis, decrease in the secretion of the sebaceous glands, loss of flexibility, and decrease in blood flow are experienced. Studies show that HRT has a positive effect on skin dryness and skin thickness. It has been observed that there is a 10%-20% reduction in skin thinning and skin dryness in women taking HRT in the postmenopausal period (Aboobac. The decreasing estrogen level since menopause increases the testosterone level by decreasing the SHBG level, which leads to hair growth in the mustache and chin area and thinning of the hair in the pelvic and axillary region. Although all these skin changes are not actually a disease or a deterioration in physiological function, conditions such as thinning of the skin, wrinkling of the skin, deterioration in the appearance of the skin, sagging of the breasts, etc. can negatively affect the self-perspective in women, as they may cause a feeling of loss of attractiveness. and can damage self-esteem.

Menopause and Quality Of Life

It is very difficult to define quality of life and there is no agreed definition at present. The reason for this is that quality of life is a multidimensional concept used in all areas of life. According to the World Health Organization, health is "a state of complete physical, mental and social well-

being and not merely the absence of disease or infirmity". Based on the "well-being" in this definition, WHO defines quality of life as "the patient's personal perception of his/her situation in life, both in the context of the cultural structure and value system in which he/she lives, and in terms of his/her own goals, expectations, standards and concerns".

During menopause, women experience many symptoms and their daily living activities are significantly affected. Although the reasons for the emergence of menopausal symptoms are not known exactly, it is generally thought to be caused by the deficiency of ovarian hormones. These symptoms are; cycle disorders, vasomotor symptoms (VMS), emotional changes, sleep disorders, genitourinary problems, sexual problems, cardiovascular diseases and osteoporosis. All these changes bring about problems that negatively affect women's quality of life. Although the symptoms of menopause differ in each woman, it is estimated that 80-85% of women experience these symptoms and this reduces their quality of life. Physical, psychological and social changes that negatively affect the quality of life during menopause are quite complex. The nursing approach in this period includes careful diagnosis, support and education. Therefore, women's health nurses should provide care and counseling in order to increase the quality of life of women and to ensure that they spend this period in the best way possible. There are many studies in the literature to determine the symptoms and quality of life experienced by women during menopause. Ertem (2010) stated in his study that 79% of women in the menopause period experienced hot flashes and their quality of life decreased during this period. Asadi et al. (2012) found that the symptoms experienced by women during menopause were hot flashes (59.5%), mood changes (42.6%), vaginal dryness (41.1%), sleep problems (40.4%), night sweats (38.2%), memory loss (42.6%). 32.3%), urinary symptoms (18.3%), palpitations (6.6%), anxiety (5.8%), joint and muscle pain (59.9%), depression (4.4%), and irritability (3.6%). In the study conducted by Tümer and Kartal (2018) with 152 women to determine the relationship between women's attitudes towards menopause and their menopausal complaints, it was found that women experienced the most psychological problems. In another study, it was determined that 80% of women experience hot flashes and 31.3% of them experience night sweats. In an international study conducted in five European countries, it was reported that 55% of menopausal women and 75% of women living in the United States experienced hot flashes. In a study in many developed countries, it was reported that 75% of menopausal women experience hot flashes and 39% experience sweating. In the study conducted by Kharbouch and Hotun Şahin (2007), it was determined that women who have been in the postmenopausal period for five years or more have low quality of life scores in psychosocial, physical and sexual areas. All these study results show us that women may encounter many health problems during menopause, which is a normal process of their life stage, and their quality of life may be adversely affected.

Menopausal Period and Nursing Approach

Menopause is a period in which many important changes and related problems are experienced. The nurse, who is a member of the multidisciplinary team, helps women in the menopausal period to cope with these problems; practitioner, manager, trainer, consultant and researcher. Nurses, who are professional members of the health team, should play an active role in determining the health problems of women specific to the menopausal period, planning and implementing the interventions. Researches made; It has been shown that many women do not know what menopause is and what changes are taking place in their bodies. Based on the idea that many women have little knowledge about menopause, they should be told what changes will occur in menopause and how to take precautions, and whether these changes are normal.

It is among the important responsibilities of nurses to determine the quality of life of women in the menopausal period and the factors affecting them, and to use the results to improve women's health. The fact that nurses have knowledge about the quality of life of women in this period and are aware of the risks that affect their quality of life will enable them to provide more

support to women in coping with menopausal complaints. The nursing approach in this period includes careful diagnosis, support and education. Nurses should provide quality care and counseling services to improve the quality of life of menopausal women.

Health professionals should have up-to-date information about the menopausal period, menopausal symptoms, complications and their effects on quality of life. It is recommended to educate women on the management of symptoms related to menopause and how to prevent complications, to provide counseling to increase women's quality of life, and to carry out experimental studies that will lead to a personalized healthy lifestyle, considering that individual characteristics may affect menopausal complaints.

References

Abay H, Kaplan S. Menopozal dönem yaşam kalitesini nasıl etkiliyor?. Ankara Sağlık Bilimleri Derg. 2014;4:1–24. [CrossRef]

Altundağ H, Şahin S. Menopozal Donemdeki Kadınlarda Huzursuz Bacak Sendromunun Gorulme Sıklığı, Yaşam ve Uyku Kalitesi Uzerine Etkisi. Sağlık Bilimleri ve Meslekleri Derg. 2019;6:62–73.

Armand, M., Ozgoli, G., Giti, R. H., Majd, H. A. (2017). Effect of acupressure on early complications of menopause in women referring to selected health care centers. Iranian Journal of Nursing and Midwifery Research, 22 (3), 237

Arslan, H., Altınsoy, N. (2004). Klimakterik dönemde vazomotor bozukluklar ve cinsel işlevleri ile ilgili yakınmalarda hemşirelik danışmanlığının etkisi. Androloji Bülteni, 19, 360- 363.

Asadi, M., Jouyandeh, Z., Nayebzadeh, F. (2012). Prevalence of menopause symptoms among Iranian women. Journal of Family and Reproductive Health, 1-3.

Avis, N. E., Crawford, S. L., McKinlay, S. M. (1997). Psychosocial, behavioral, and health factors related to menopause symptomatology. Women's Health (Hillsdale, NJ), 3 (2), 103- 120.

Bawar, S. Sadaf, F. Rahim, R. Faiz, N. R. (2013). Comparison of vasomotor symptoms in postmenopausal women with different socio-economic status. Gomal Journal of Medical Sciences, 11(2), 95-98.

Bekmezci, E., Altuntuğ, K. (2020). Menopoz ile İlişkili Semptomlara Yönelik Kanıta Dayalı Uygulamaların İncelenmesi. Genel Sağlık Bilimleri Dergisi, 2(3), 167-174

Bener, A. and Falah, A. (2014). A measurement-specific quality-of-life satisfaction during premenopause, perimenopause and postmenopause in Arabian Qatari women. J Midlife Health, 5, 126- 134.

Bozkurt, Ö. D., Sevil, Ü. (2016). Menopoz ve cinsel yaşam. Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi, 3 (4), 497-50

Ceylan B, Ozerdoğan N. Menopausal symptoms and quality of life in Turkish women in the climacteric period. Climacteric. 2014;17:705–12. [CrossRef]

Daan, N.M. and Fauser, B.C. (2015). Menopause prediction and potential implications. Maturitas, 82, 257-265.

Ebong, I.A., Watson, K.E., Goff, D.C. (2014). Age at Menopause and İncident Heart Failure: The Multi-Ethnic Study of Atherosclerosis. Menopause, 21:585–591.

Ege, E., Kal, H.E., Altuntuğ, K. (2014). The Use Of Alternatıve Methods In Reducıng Menopausal Complaints İn Turkey. Afr J Tradit Complement Altern Med,11(2):295- 300.

Erkin, Ö., Ardahan, M. ve Kert, A. (2014). Menopoz döneminin kadınların yaşam kalitesine etkisi. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi, 3, 1095-1113.

Ertem, G. (2010). Kadınların menopoz sonrası yaşam kalitelerinin incelenmesi. Uluslararası İnsan Bilimleri Dergisi, 7, 469-483.

Fredman, R. R. (2005). Hot flashes: Behavioral treatments, mechanisms and relation to sleep. The American Journal of Medicine, 118(12):1245-1305.

Gao, J., Bai, D. X., Wu, C. X., Yang, X. Y., & Zhang, Q. (2016). Effects of compound essential oil acupressure on quality of life in perimenopausal syndrome patients. Chin J Nurs, 1, 53-56. [39].

Gözüyeşil E, Başer M. [1]. The effect of foot reflexology applied to women aged between 40 and 60 on vasomotor complaints and quality of life. Complement Ther Clin Pract. 2016;24:78–85. [CrossRef]

Hacettepe Üniversitesi Nüfus Etütleri.(2013). Türkiye Nüfus ve Sağlık Araştırması. http://www.hips.hacettepe.edu.tr/TNSA_2013_ana_rapor.pdf Erişim Tarihi: 27.04.2015

Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü. (2019). 2018 Türkiye Nüfus ve Sağlık Araştırması (TNSA), T.C. Kalkınma Bakanlığı ve TÜBİTAK, Ankara, Türkiye.

Herman, J., Rost Roszkowska, M., Skotnicka Graca, U. (2013). Skin Care During the Menopause Period: Noninvasive Procedures of Beauty Studies. Postep Derm Alergol,30(6):388–395

Kao, Y. H., Huang, Y. C., Chung, U. L., Hsu, W. N., Tang, Y. T., & Liao, Y. H. (2017). Comparisons for effectiveness of aromatherapy and acupressure massage on quality of life in career women: a randomized controlled trial. The Journal of Alternative and Complementary Medicine, 23(6), 451-460

Kharbouch, S. B., Şahin, N. H. (2007). Menopozal dönemlerdeki yaşam kalitesinin belirlenmesi. Florence Nightingale Hemşirelik Dergisi, 15 (59), 82-90.

Kim, H. K., Kang, S. Y., Chung, Y. J., Kim, J. H., & Kim, M. R. (2015). The recent review of the genitourinary syndrome of menopause. Journal of Menopausal Medicine, 21(2), 65-71.

Levine, K. B., Williams, R. E., Hartmann, K. E. (2008). Vulvovaginal atrophy is strongly associated with female sexual dysfunction among sexually active postmenopausal women. Menopause, 15(4), 661-666.

Miranda, J.S., Ferreira Mde, L., Corrente, J.E. (2014). Quality of life of postmenopausal women attended at Primary Health Care. Rev Bras Enferm, 67, 803-809

Reid, R., Abramson, B.L., Blake, J., Desindes, S., Dodin, S., Johnston, S., Fortier, M. (2014). Managing Menopause Abstract and Summary Statement. Journal Of Obstetrics And Gynaecology Canada, 36(9):1- 72.

Royal College of Nursing, 2020. Menopause RCN guidance for nurses, midwives and health visitors. Second edition. Erişim tarihi: 10.12.2020

Simon, J. A., Nappi, R. E., Kingsberg, S. A., Maamari, R., Brown, V. (2014). Clarifying Vaginal Atrophy's Impact on Sex and Relationships (CLOSER) survey: emotional and physical impact of vaginal discomfort on North American postmenopausal women and their partners. Menopause, 21(2), 137-142

Süt, K., Küçükkaya, B. (2018). Premenopoz, Perimenopoz ve Postmenopoz Dönem Kadınlarında Cinsel Yaşam Kalitesi. Kadın Sağlığı Hemşireliği Dergisi, 4(1): 51-68

Timur, S., Hotun Şahin, N. (2010). Menopoz ve uyku. Maltepe Üniversitesi Hemşirelik Bilim ve Sanatı Dergisi, 3 (3), 61-67.

Tümer, A. Kartal, A. (2018). Kadınların menopoza ilişkin tutumları ile menopozal yakınmaları arasındaki ilişki. Pam Tıp Dergisi, 11(3):337-346

Zıvdır P, Sohbet R. Menopozdaki kadınların sucluluk ve utanç duygularının yaşam kalitesine etkisi. Med Sci. 2017;12:1–9.

The Relationship Between Long-Term Care Expenditures and Health Status

Gülay EKİNCİ[1]

Introduction

Long-term care (health) consists of a set of medical and personal care services consumed for the purpose of relieving pain and suffering and managing deterioration in health status in patients with long-term dependence (OECD, 2011).

LTC includes services for patients with persistent or recurring illnesses and increased levels of addiction. These diseases include a wide range of services such as recurrent long or short-term care needs such as substance abuse, psychiatric disorders, management of chronic diseases such as cancer, end-of-life care, and care needs of individuals with physical disabilities.

Since long-term health services are provided informally (by family or relatives in the home environment) in low- and middle-income countries, data on these services are limited to upper-income countries where they were formal structured.

The need for long-term care is increasing day by day due to changes in demographic indicators such as the prolongation of life expectancy, the increase and structural changes in the burden of diseases, and the transformation in the work and employment structure.

The aging phenomenon, which develops due to the prolongation of life expectancy, increases the need for care support due to chronic diseases and mobility weakness due to aging. At the same time, the prevalence of chronic diseases such as high blood pressure, diabetes, muscle and bone diseases, alzheimer's, demenias increases the need for long-term care.

Long-term care services within the scope of "medical or nursing care" includes to prevent further deterioration of the patient's state of health, chronic disease management, rehabilitative care to restore functionality

Personal care services included supported self-care primarily due to disability and illness. These services are administered directly or under the supervision of nursing staff. These services provide help with activities of daily living (ADL) such as: eating, dressing, bathing, washing, getting in and out of bed, getting to and from the toilet and managing incontinence. These services are included within the health care boundary because the purpose of this type of care is linked to survival and the maintenance of health status (OECD, 2011).

Health status is an indicator of an individual's health level. Health status can be evaluated objectively and subjectively. The concept of perceived health status is a subjective concept.

In the perceived health status, the individual may have a positive or negative attitude towards the disease they have, or they may remain indifferent. Therefore, there are three types of assessments on perceived health status. It is possible for an individual to feel good, to feel bad, or to evaluate himself as neither good nor bad.

In this context, three types of evaluations are made under the heading of the individual's perceived health status in the OECD database. These perceived health status were "Good/very good health, total aged 15+ % of population (crude rate) "; "Fair (not good, not bad) health, total

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aged 15+ % of population (crude rate)"; "Bad/very bad health, total aged 15+ % of population (crude rate)".

Health status can be measured statistically and objectively expressed with various indicators and methods developed (Goodacre vd.,2010) These indicators are life expectancy at birth (LEAB), mortality rates, morbidity rates.

The need for long-term care is closely related to life expectancy and morbidity from a health status perspective.

Average Life Expectancy is one of the most common measures used to obtain information about the health of a population. Average life expectancy is a rough measure of how long a person will live. This measure shows how long a person is likely to live at a given age unless there is a trend change in death rates (Ulutürk, 2015).

Morbidity is another measure of the health status of the population. It gives the ratio of individuals with impaired health to the population of the society. The most important concept used within the scope of morbidity is disease burden. The burden of disease measures the difference between the current life status and the ideal life status, where each individual can live free from diseases and disabilities in later life, to see the effect of diseases and disabilities (Ulutürk, 2015). The most frequently used indicators of disease burden from the perspective of health economics;

- Quality Adjusted Life Year (QALY) is defined as the expected life expectancy of the patient and the quality of life in these years. QALY value is between 1 and 0. "1" represents perfect health while "0" represents death (Rudmik ve Drummond, 2013). For example, if a heart disease that has a negative impact on quality of life reaches a disability score of 0.8, the quality of life score will be 0.2.
- Disability Adjusted Life Year (DALY) is a measure of the overall disease burden, expressed as the number of years lost due to loss of health, disability, or premature death.
- Year Lost Life (YLL) expresses the number of deaths occurring at each age multiplied by the estimated remaining life years according to life expectancy. It is based on the calculation of life years lost due to death.
- Year Lost Disability (YLD); the number of cases resulting from injury or illness is based on disability-adjusted life years calculation.

Diseases are considered at three basic levels in the calculation of disease burdens:

a. Noncommunicable diseases (NCD) are a group of noncommunicable diseases that occur in the long term due to one or more risk factors, for example; Alzheimer's, diabetes, cancer diseases etc.

b. Communicable diseases–CD is a type of disease that has a disease agent in its etiology, occurs in the short or long term, and shows contagious features, for example; AIDS, syphilis, tuberculosis, influenza etc.

c. Injuries are the negative changes that occur in the health level of the individual by any sharp object injury, fall, drowning, poisoning, accident, terrorism, etc. For example; traffic accidents, drowning, etc.

At a global level in 2019, DALY for non-communicable diseases was 1.6 billion/year, DALY for communicable diseases was 668 million/year, and DALY for injuries was 249 million years was calculated. DALY of non-communicable diseases has a high share of 64% in the total disease burden. This calculation shows that the years of life spent due to diseases related to chronic diseases or lost due to premature death are at a very high level. Therefore, chronic diseases appear as an issue that should be evaluated in terms of health economics.

According to the World Health Organization (WHO), chronic diseases are defined as diseases that are not contagious, persist for 6 months or more, generally progress slowly, and many risk factors play a role in their development.

According to the Commission on Chronic Illness, chronic diseases are defined that are generally non-infectious in nature, in which socioeconomic, personal characteristics, and genetic

factors play a role in the formation of slowly progressive, permanent, and usually permanent disabilities, in which the recovery is not usually seen completely (Aywater, 1949; Bilir, 2006).

The group of non-communicable diseases consists of chronic diseases. Among these diseases, mental diseases such as cancer, cardiovascular system diseases, diabetes, Alzheimer's, and dementia have an important place. Chronic diseases involve high costs due to the long-term diagnosis, treatment, and rehabilitation processes. In addition, increases in life expectancy and the incidence-prevelance of chronic diseases brought about by these increases also increase health expenditures.

In a study, the annual cost per patient was calculated as $48293 for HIV/AIDS disease; $38551 for kidney diseases, $37155 for CVD, and $25016 for respiratory disorders (Hajat et al, 2021).

These diseases involve high costs depending on their duration and whether they are comorbid with additional diseases.

In the same study, in the evaluation of chronic diseases per patient; the Annual health expenditure of patients with 2 chronic diseases was 4385 dollars, 5851 dollars in 3 chronic diseases, 10088 dollars in 5 chronic diseases, 19856 dollars in 8 chronic diseases, 33874 dollars in 11 chronic diseases (Hajat et al, 2021).

In another study, in the percentage of Health Care Total Spending by Number of Cronic Conditions, it was determined that; 16.2% of expenditures are spent on 0 chronic diseases, while 18.4% on 1 chronic disease; 17.2 % was spent on 2 chronic diseases, 15 % on 3 chronic diseases, 11.1 % on 4 chronic diseases, and 22.1 on 5 or more (Gerteis et al. 2014).

Countries make health expenditures to manage the health needs for individuals. Health expenditures are generally considered as total and current health expenditures. However, demographic changes and changing health needs cause the services offered to diversify or to be handled in detail. Contrary to the general approach to health expenditures, WHO has published a more detailed guide and discussed health expenditures in 9 categories. One of these expenditures is long-term maintenance expenditures. Long-term care expenditures are the expenditures made to ensure the well-being of the individual for conditions such as the management of chronic diseases, the need for palliative care services, age-related health deficiencies, and substance abuse.

In this context, long-term care expenditures are handled in two dimensions health expenditures and social expenditures. Long-term care expenditures-social are expenditures made to meet the social and economic needs of individuals (retirement, incapacity payments, sickness payments, services to support daily activities, etc.) in long-term deterioration in the health status of the individual. Long-term care expenditures-health are health-related expenditures made for the management of long-term deterioration in the health status of the individual (OECD, 2011). Long-term care-health; i) inpatient long-term care-health, ii) Day long-term care-health, iii) Outpatient longterm care-health, iv) Home-based longterm care-health.

Countries determine their health expenditures and investments by taking into account the health services needed by the population. This study, it was aimed to evaluate the relationship between long-term care expenditures, life expectancy at birth, and perceived health status.

Methods

Descriptive methods were used in the analysis. The analysis was done in three stages. In the first stage, descriptive information about the variables was given; in the second stage, the increase-decrease relationship of variables was examined according to years; in the third stage, the correlation relationship between the variables was examined; in addition, the relationships between the variables were evaluated through graphics.

Variables

Only 29 countries had regular long-term care expenditures data between 2010-2020. These countries were Austria, Belgium, Canada, Croatia, Czech Republic, Denmark, Estonia, Finland,

France, Germany, Greece, Hungary, Iceland, Ireland, Korea, Latvia, Lithuania, Luxemburg, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United States. The data on long-term care expenditures according to the PPP$ (Per capita, current prices, current PPPs) for 2010-2020 was used.

The variables used in the study were long-term care expenditures (LTCE), life expectancy at birth (LEAB), three separate data given under the heading of Perceived health status as the well-being of the population in terms of morbidity. These variables of Perceived health status were "Good Health: Good/very good health, total aged 15+ % of population (crude rate) "; "Fair Health: Fair (not good, not bad) health, total aged 15+ % of population (crude rate)"; "Bad Health: Bad/very bad health, total aged 15+ % of population (crude rate)".

Limitations Of This Study

The limitation of this study was considered to be the number of countries and year range. The other limitation of this study is Turkey does not have data on long-term care expenditures, so it could not be included in the analysis. In addition, disease burdens were not evaluated as a variable in this study.

Statistical Analysis

Statistical analyzes were made using Eviews 10 program (Eviews 10, IHS Global Inc., 4521 Campus Drive, #336, Irvine, CA 92612).

Results

Results were made in three stages such as; i) descriptive analysis of the variables, ii) results on the relationship between long-term care expenditures and variables , iii) results on the relationship between life expentancy at birth and variables.

a. Descriptive analysis of the variables

According to Table 1; the mean of LEAB of 29 countries was 80.01 ± 2.71 (min: 73.10; max: 84.00); the mean of LTCE was 595.26 ± 494.08 PPP$ (min: 6.42; max: 1946.49); the mean of Good Health Status was 67.02 ± 13.11 (min: 29.50; max: 89.00); the mean of Fair Health Status was 23.16 ± 8.86 (min: 8.00; max: 53.50); the mean of Bad Health Status was 9.86 ± 4.96 (min: 2.50; max: 26.90).

Tablo 1. *Descriptive Analysis of The Variables*

	LTCE*	LEAB	Good Health	Fair Health	Bad Health
Mean	595.2613	80.01379	67.01661	23.16426	9.858934
Median	636.3710	81.10000	69.50000	21.70000	8.700000
Maximum	1946.494	84.00000	89.00000	53.50000	26.90000
Minimum	6.423000	73.10000	29.50000	8.000000	2.500000
Std. Dev.	494.0802	2.706282	13.10574	8.864702	4.964747
Skewness	0.487303	-0.802500	-0.637659	0.964884	0.692728
Kurtosis	2.168927	2.449334	3.000417	4.239605	3.111243
Jarque-Bera	21.80549	38.27010	21.61803	69.92253	25.67768
Probability	0.000018	0.000000	0.000020	0.000000	0.000003

Source: Prepared by the author, * Per capita, current prices, current PPPs

Long-term care expenditures have increased by an average of 1.02 times (102%) at the level of 29 countries in 11 years. Countries with the highest increase in Long-Term Care Expenditures

in 2020 compared to 2010 were Croatia; Czech, Estonia, Korea, Greece, Poland, Germany (an increase of 1x or more); decreases were experienced in Luxemburg and Romania.

Life Expectancy At Birth has increased by an average of 1.3% at the level of 29 countries in 11 years. Countries with the highest increase in Life Expentancy At Birth in 2020 compared to 2010;Korea, Estonia, Latvia, Denmark, Finland, Lithuania, Norway, Ireland, Romania (an increase of 2% or more); decreases were experienced in United States (respectively %0.27; %2.1)

Good Health Status has increased by an average of 2.7 % at the level of 29 countries in 11 years. Countries with the highest increase in Good/Very Good Health total aged 15+ in 2020 compared to 2010; Croatia, Hungary, Slovenia, Estonia (an increase of 10-30 %); the highest decreases were experienced in Korea, Lithuania (decreases respectively 16%, 12%).

Fair Health Status has increased by an average of 1.08 % at the level of 29 countries in 11 years. Countries with the highest increase in Fair (Not GoodNot Bad) Health total aged 15+ in 2020 compared to 2010; United States, Luxemburg, Sweden, Korea, Lithuania, Portugal (an increase of 10-30 %); the highest decreases were experienced in Slovenia, Austria, Switzerland, Ireland, Germany (decreases respectively 15-10 %).

Bad Health Status has decreased by an average of 10.7 % at the level of 29 countries in 11 years. Countries with the highest increase in Bad/very bad Health total aged 15+ in 2020 compared to 2010; Germany, Ireland, Norway, Iceland, United States, Switzerland, (an increase of 2.8-52 %); the highest decreases were experienced in Croatia, Hungary, Portugal, Greece, Poland, Slovenia, Romania, Czech, Estonia, Finland, Latvia, Austria, Spain, Lithuania, Belgium, Netherlands, Denmark, Korea, Slovak Republic, Sweden, Canada, Luxemburg (decreases respectively 2.5-44 %); besides this there was no increase or decrease in France.

Tablo 2. *Descriptive Analysis of The Series, % Change, 2010-2020*

Countries	LTCE	LEAB	GOOD HEALTH	FAIR HEALTH	BAD HEALTH
Germany	1,0276	0,0075	-0,0215	-0,1049	0,5185
Ireland	0,3329	0,0223	0,0109	-0,1143	0,2188
Norway	0,4298	0,0259	-0,0287	0,0581	0,1538
Iceland	0,4689	0,0147	-0,0154	0,0353	0,1346
United States	0,4176	-0,0216	0,0034	0,1847	0,1000
Switzerland	0,4480	0,0048	0,0221	-0,1267	0,0286
France	0,5491	0,0049	0,0178	-0,0504	0,0000
Luxemburg	-0,0605	0,0173	-0,0239	0,1131	-0,0250
Canada	0,4965	0,0037	0,0102	-0,0889	-0,0345
Sweden	0,2520	0,0098	-0,0242	0,1235	-0,0370
Slovak Republic	0,4036	0,0185	0,0252	-0,0398	-0,0435
Korea	2,3092	0,0411	-0,1622	0,1584	-0,0495
Denmark	0,2811	0,0290	0,0028	0,0095	-0,0506
Netherlands	0,5585	0,0049	0,0000	0,0353	-0,1000
Belgium	0,4204	0,0062	0,0329	-0,0615	-0,1429
Lithuania	0,8456	0,0246	-0,1175	0,2990	-0,1818
Spain	0,2752	0,0000	0,0167	0,0149	-0,1852
Austria	0,3803	0,0074	0,0647	-0,1315	-0,1935
Latvia	0,8054	0,0328	0,0397	0,0548	-0,2171

Finland	0,1248	0,0224	0,0220	0,0041	-0,2222
Estonia	2,9224	0,0382	0,1082	-0,0679	-0,2349
Czech	5,3974	0,0077	0,0193	0,0664	-0,2377
Romania	-0,1314	0.0068	0,0641	-0,0963	-0,2396
Slovenia	0,6274	0,0100	0,1258	-0,1365	-0,2857
Poland	1,1378	0,0013	0,0657	0,0299	-0,2987
Greece	1,4271	0,0099	0,0384	0,0068	-0,2990
Portugal	0,9169	0,0125	0,0407	0,1639	-0,3448
Hungary	0,4292	0,0134	0,1273	-0,0106	-0,4036
Croatia	6,1942	0,0143	0,3181	-0,1424	-0,4424
	1,0237	**0,0131**	**0,0270**	**0,0108**	**-0,1074**

*Year ranges used in the calculation of countries data: LTCE: Ireland 2011-2020, Croatia 2011-2020, Sweden 2011-2020; Good Health Status: Iceland 2010-2018,United States 2010-2019; Fair Health Status: Iceland 2010-2018, United States 2010-2019; Bad Health Status: Iceland 2010-2018, United States 2010-2019.

Source: *Prepared by the author*

b. Results on the relationship between long-term care expenditures and variables

The correlation analysis results of the variables were given in Table 3.

Table 3: *Correlation Analysis Results*

	LTCE	LEAB	GOOD HEALTH	FAIR HEALTH	BAD HEALTH
LTCE	1	0.64	0.62	-0.51	-0.68
LEAB	0.64	1	0.47	-0.38	-0.56
GOOD HEALTH	0.62	0.47	1	-0.96	-0.90
FAIR HEALTH	-0.51	-0.38	-0.96	1	0.76
BAD HEALTH	-0.68	-0.56	-0.90	0.76	1

Source: Prepared by the author

According to Table 3, there was a positive correlation between long-term care expenditures and life expectancy at birth (r=0.64). It is understood from Figure 1 that long-term care expenditures between 0-500 PPP dollars were concentrated in the 73 years and over, while the expenditures of 500 dollars and above were mostly made in the 78-84 age group (Figure 1).

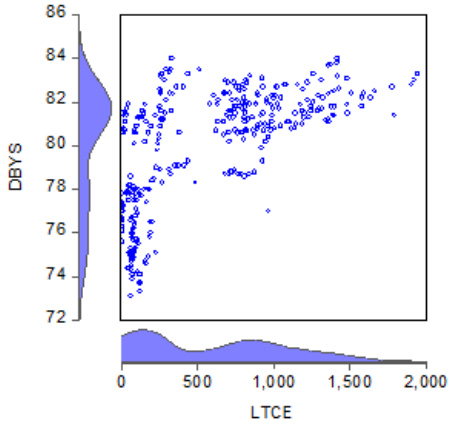


Figure 1. Long-term care expenditures and life expectancy at birth, 29 countries, 2010-2020

There was a positive correlation between long-term care expenditures and Good Health Status (r=0.62). According to Figure 2, while long-term care expenditures between 0-500 PPP dollars were pointed to the Good Health Status of 29.5-89 % population; spending 500 PPP dollars or more was pointed to the Good Health Status of 60-90 % population.

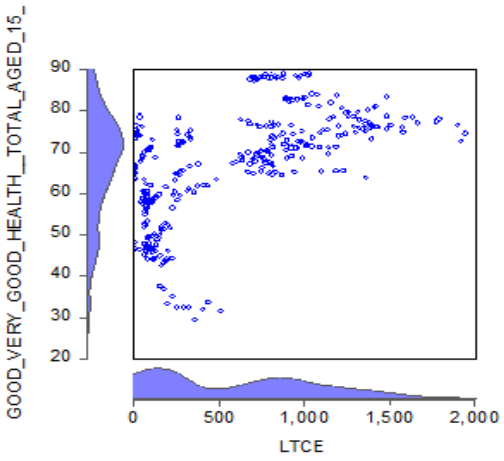


Figure 2. Long-term care expenditures and Good Health Total Aged 15+, 29 countries, 2010-2020

There was a negative correlation between long-term care expenditures and Fair Health Status (r=- 0.51). According to Figure 3, while long-term care expenditures between 0-500 PPP dollars were pointed to the Fair Health Status of 15-55 % population approximately; spending 500 PPP dollars or more was pointed to the Fair Health Status of 8-30 % population approximately.

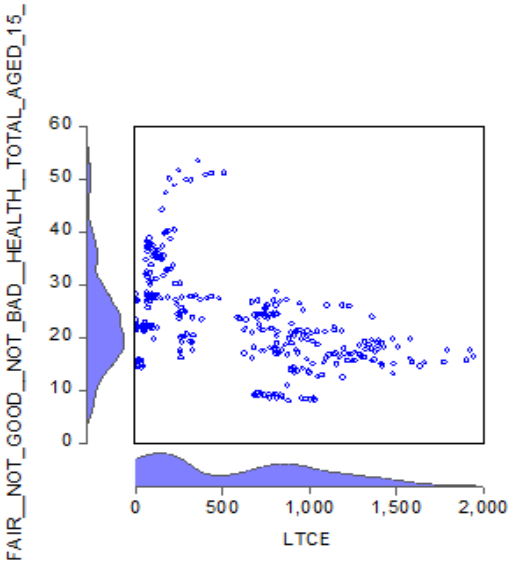


Figure 3. Long-term care expenditures and Fair Health Total Aged 15+, 29 countries, 2010-2020

There was a negative correlation between long-term care expenditures and Bad Health Status (r=- 0.68). According to Figure 4, while long-term care expenditures between 0-500 PPP dollars were pointed to the Bad Health Status of 5-27 % population approximately; spending 500 PPP dollars or more was pointed to the Bad Health Status of 2.9-12 % population approximately.

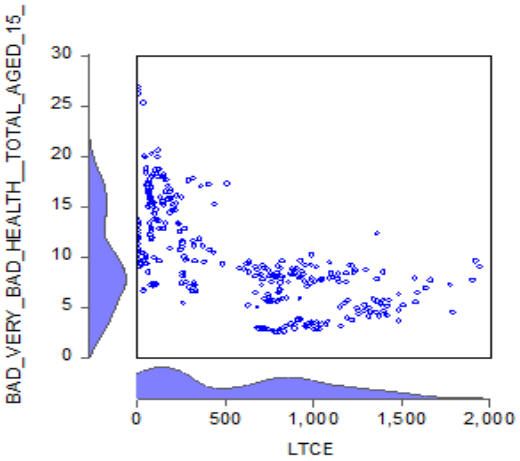


Figure 4. Long-term care expenditures and Bad Health Total Aged 15+, 29 countries, 2010-2020

c. Results on the relationship between life expentancy at birth and variables

There was a positive correlation between life expectancy at birth and Good Health Status (r=0.47). From Figure 5, it is understood that in the age range 80-84 around 60-90% of the population indicated Good Health Status.

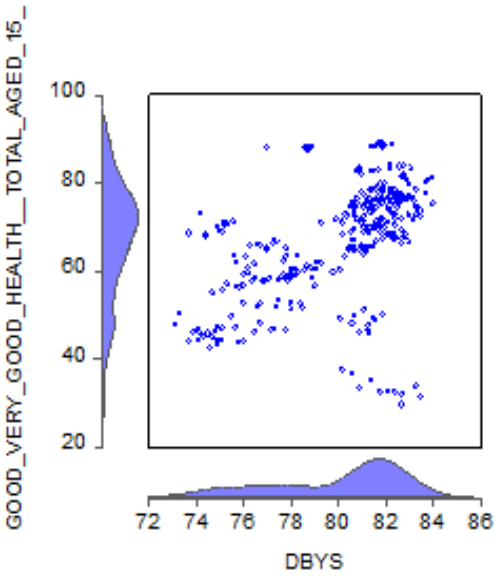


Figure 5. Life Expentancy At Birth and Good Health Total Aged 15+, 29 countries, 2010-2020

There was a negative correlation between life expectancy at birth and Fair Health Status (r=-0.38). From Figure 6, it is understood that in the age range 80-84 around 8-30 % of the population indicated Fair Health Status.

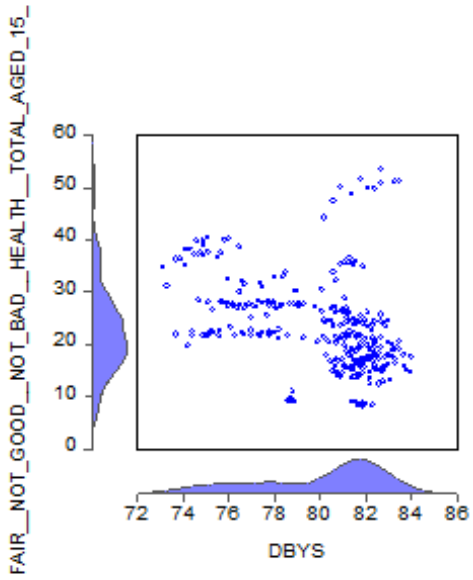


Figure 6. Life Expentancy At Birth and Fair Health Total Aged 15+, 29 countries, 2010-2020

There was a negative correlation between life expectancy at birth and Bad Health Status (r=-0.56). From Figure 7, it is understood that in the age range 80-84 around 2.5-22 % of the population indicated Bad Health Status.

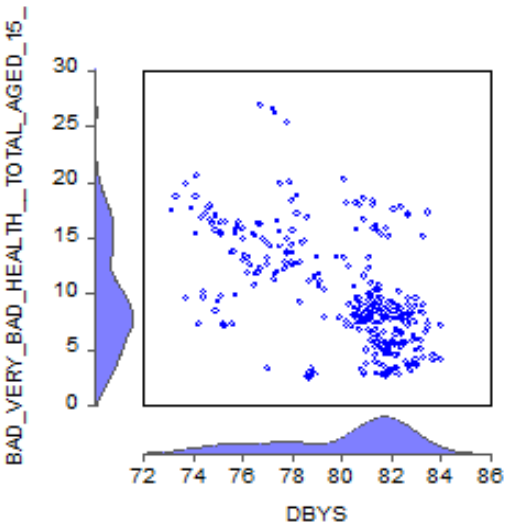


Figure 7. Life Expentancy At Birth and Bad Health Total Aged 15+, 29 countries, 2010-2020

Discussion and Recommendations

There are many influential factors on perceived health status such as age, gender, education, marital status, working conditions, genetics, diseases burdens etc.

In a study, when averages in health status perceptions are detailed according to rural-urban, gender, marital status, income, and education level, the most important factors affecting positive health perceptions are marital status, education level, and income level, respectively. In a study conducted in Turkey, the European Union, and OECD countries, approximately 30-32% of people reported that they had health problems, while 65-70% of the population stated that their health status was "good" (Aydın, 2019). In this study, our results showed that while 67% of the population perceived their health status as good in the countries subject to the study, 23.2% evaluated it as fair health status and 9.2% as bad health status.

Population satisfaction with regard to coverage of primary health care services at the level of 10 industrialized countries, 12 health indicators (eg infant mortality, life expectancy, and age-adjusted mortality rates) and overall costs of systems was studied. While the results reveal overall concordance for primary care, health indicators and satisfaction-expenditure ratio in nine out of 10 countries; USA and West Germany were found to be at low levels (Starfield, 1991).

In a study using health expenditure, maternal mortality, and neonatal mortality, it was concluded that health expenditures can improve the aforementioned indicators of low HDI countries by mediating the relationship between HDI and neonatal and maternal mortality rates (Nuhu et al, 2018).

In a study using disability rate, mortality rate, and quality of life, it was concluded that there is a strong relationship between Human Development Index and health indicators (Prashanthy, 2021).

In the evaluation of 29 countries between 2010-2020;

- The average life expectancy in the countries studied is 80 years old.
- Long-term care expenses average around 600 PPP dolars.
- Long-term care expenditures have increased by over 102% in 11 years.
- Average life expectancy increased by around 1.3% in 11 years.
- The percentage of the population with good health increased by around 2.7% in 11 years.
- The percentage of the population with a fair health status increased by 1.08% in 11 years.
- The percentage of the population with bad health status decreased by around 10.7% in 11 years.

A moderate and positive correlation was found between long-term care expenditures and average life expectancy. Results showed that long-term care expenditures between 0-500 PPP dollars were concentrated in the 73 years and over, while the expenditures of 500 PPP dollars and above were mostly made in the 78-84 age group (Figure 1).

A moderate and positive correlation was found between long-term care expenditures and the percentage of the population with good health status. According to Figure 2, while long-term care expenditures between 0-500 PPP dollars were pointed to the Good Health Status of 29.5-89 % population; spending 500 PPP dolars or more was pointed to the Good Health Status of 60-90 % population.

A low level and negative correlation was found between long-term care expenditures and the percentage of the population with fair and bad health status. In other words, while the increase in long-term care expenditures causes a decrease in the fair-bad health level; the decrease in long-term care expenditures causes an increase in the fair-bad health level.

A low level and positive correlation was found between average life expectancy and the percentage of the population with good health status. From Figure 5, it is understood that in the age range 80-84 around 60-90% of the population indicated Good Health Status.

A low level and negative correlation was found between average life expectancy and the percentage of the population with fair-bad health status. From Figure 6, it is understood that in the age range 80-84 around 10-30 % of the population indicated fair health status. From Figure 7, it is understood that in the age range 80-84 around 2.5-22 % of the population indicated bad health status.

The results of the evaluation of the countries within the framework of the variables;

- Countries where long-term care expenditures and life expectancy at birth increase together, with a decrease in the level of good and fair health status and an increase in bad health status; Germany.
- Countries where long-term care expenditures and life expectancy at birth increase together, with a decrease in the level of good health status and an increase in the level of fair and bad health status; Iceland, and Norway.
- Countries where a decrease in long-term care expenditures and an increase in life expectancy at birth, with a decrease in the level of good and fair health status and an increase in bad health status; Luxemburg.
- Countries where an increase in long-term care expenditures and a decrease in life expectancy at birth, with an increase in good-fair-bad health status together; United States.
- Countries where long-term care expenditures and in life expectancy at birth increase together, with an increase in the level of good health status and a decrease in fair-bad health status; Croatia, Hungary, Slovenia, Estonia, Austria, Belgium, Slovak Republic, and Canada.
- Countries where long-term care expenditures and life expectancy at birth increase together, with an increase in the level of good-fair health status and a decrease in bad health status; Portugal, Greece, Poland, Czech, Denmark, Netherlands, Spain, Latvia, Finland.
- Countries where long-term care expenditures and life expectancy at birth increase together, with an increase in the level of good-bad health status and a decrease in fair health status; France, Ireland, and Switzerland.
- Countries where long-term care expenditures and life expectancy at birth increase together, with an increase in fair health status and a decrease in good-bad health status; Lithuania, Korea and Sweden.
- Countries where where an decrease in long-term care expenditures and increase in life expectancy at birth decrease, with an increase in good health status and a decrease in fair-bad health status; Romania.

Generally the increase in long-term care expenditures with the increase in life expectancy points to the percentage of the population with a better good health status. Therefore, it is thought that it will guide the policies to be followed for long-term care expenditures.

REFERENCES

Aydın K. (2019). Yaşam Koşulları Ve Sağlık Hastalık Algıları. Sosyoloji Araştırmaları Dergisi, Cilt / Volume 22 Sayı / Number 1 : (32-68)

Aywater, R. M. (1949). Commission on Chronic Illness. Am J Public Health Nation's Health, 39(10), 1343–4. https://doi.org/ 10.2105/ajph.39.10.1343.

Bilir, N. (2006). Değişen Sağlık Örüntülerinde Halk Sağlığı Çalışanlarının Rolü: Kronik Hastalıklar ve Yaşlılık Sorunları. Toplum Hekimliği Bülteni, 25(3), 1-6.

Gerteis J, Izrael D, Deitz D, LeRoy L, Ricciardi R, Miller T, Basu J.(2014). Multiple Chronic Conditions Chartbook. AHRQ Publications No, Q14-0038. Rockville, MD: Agency for Healthcare Research and Quality. April.

Goodacre, S., C. Collins And C. Slattery (2010). Cambridge VCE Health & Human Development Units 3&4, Cambridge University Press.

Hajat C, Siegal Y and Adler-Waxman A (2021) Clustering and Healthcare Costs With Multiple Chronic Conditions in a US Study. Front. Public Health 8:607528. doi: 10.3389/fpubh.2020.607528

Nuhu KM, McDaniel JT, Alorbi GA, Ruiz JI. (2018). Effect of healthcare spending on the relationship between the Human Development Index and maternal and neonatal mortality. Int Health;10(1):33-9.

OECD, Eurostat and World Health Organization (2017), A System of Health Accounts 2011: Revised edition, OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264270985-en https://stats.oecd.org/.Accessed Time: 29.10.2022

Prashanthy MR. (2021). A comparative analysis of Misery Index and its impact on health indicators across the globe. Indian J of Forensic Medicine & Toxicology;15(4):35- 0.

Rudmik L, Drummond M. (2013). Health Economic Evaluation: Important Principles And Methodology. Laryngoscope, 123:1341-1347.

Starfield, B. (1991). Primary care and health a cross-national comparison. Journal of American Medical Association 266:16, 2268-2271. https://doi.org/10.1001/jama.1991.03470160100040

Ulutürk S. (2015).Sağlık Ekonomisi, Sağlık Statüsü, Sağlığın Ölçülmesinde Kullanılan Ölçütler ve Önemi: Türkiye Örneği. Finans Politik ve Ekonomik Yorumlar, Cilt: 52 Sayı: 603; 47-63.

Polycyclic Aromatic Hydrocarbons and Their Effects on Health

Hakan TOĞUÇ

1. Definition

Polycyclic aromatic hydrocarbons (PAHs) are structures formed by the combination of two, three or more aromatic rings. Under the influence of high temperature, pyrolysis of hydrocarbons occurs. PAHs with less than four benzene rings are defined as mild PAHs, and PAHs with four or more benzene rings are defined as severe PAHs (Wu, Tao & Liu, 2006). Coal, oil, cigarette or hookah smoke, smoke from industrial facilities, and foods cooked on barbecue are among the sources of PAHs (Li et al, 2006).

Polycyclic aromatic hydrocarbons are a semi-volatile group of organic pollutants that can be formed and spread through various agricultural activities and industrial areas (Wick et al, 2011). It has been found that it can easily contaminate the air, water, soil and nutrients (Srogi, 2007). PAHs are compounds with higher affinity for heavy metals and other organic pollutants due to their strong hydrophobic properties. Due to their low water solubility, they are difficult to detect, so accurate and appropriate analysis methods must be developed (Hu et al, 2015).PAHs can be formed in foods as a result of heat treatments such as barbecue, grilling, smoking and roasting, as well as the compounds found in polluted air conditions in industrial production areas, accumulating on plants and contaminating vegetables, fruits and cereals (Moazzen et al, 2015).

These groups of compounds have potentially harmful effects on ecosystems and human health, as many of them have been shown to be carcinogenic, teratogenic and mutagenic. (Sojinu, Sonibare & Zeng, 2011). Various approaches and strategies, including physical and chemical, have been developed, optimized and used to reduce the effects of these pollutants and rehabilitate polluted areas (Moazzen et al, 2015). Some of these traditional remediation techniques are not common due to their technological complexity, high cost, and significant limitations, such as lack of public acceptance. Most of the techniques are invasive and simply relocate the contamination problem, which often requires more waste management (Sojinu, Sonibare & Zeng, 2011).

2. History

Although the fact that chemicals such as PAH can cause cancer was determined as a result of a research conducted in London in 1775, there is not enough information about the damage of these compounds on the tissue. In 1920, it was determined that tumors formed on the skin of mice after soot extracts were applied to the skin of mice by Japanese workers. In 1953, it was found by Doll that smoking is the main cause of lung cancer. After the analysis, BaP in cigarette smoke and tar was found to have a carcinogenic effect (Simko, 2002).

3. Physical and Chemical Properties

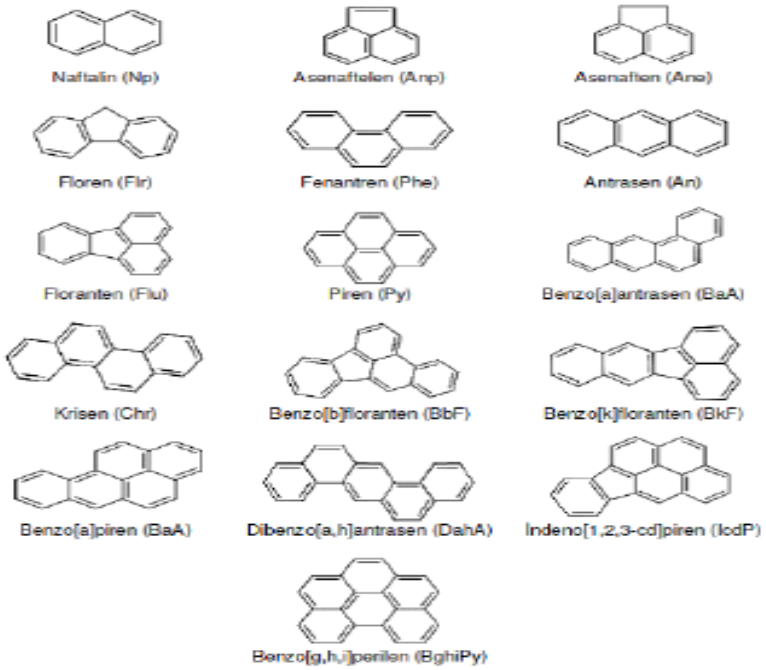
PAHs are compounds that occur as a result of incomplete combustion of hydrocarbons, and often occur as a complex mixture of combustion products instead of a single compound (ATSDR, 1995). But it can also be produced as a pure compound for research purposes. PAHs in pure compound form are white, green, light yellow, colorless, solid and have a slight pleasant odor. Most of these hydrocarbons do not have a specific area of use, except for PAHs produced for purposes

other than research. Some PAH compounds can be used in healthcare and in the manufacture of paints, pesticides or plastics (ATSDR, 1995). Figure 1 shows the molecular structures of some PAH groups. PAHs react with nitric acid and nitrous oxide to form sulfur dioxides, nitro derivatives, and sulphonic and sulfuric acid forms by reacting with sulfuric acid (WHO, 1998). In addition, they can react with ozone and hydroxyl radicals (Douben, 2003). Factors such as the solubility of PAHs in water, their diffusion in the environment, their vapor pressure, and logKow (WHO, 1998).

4. Classification

It is known that there are more than 100 PAH compounds in nature. However, 16 different PAH compounds in Figure 1, which are thought to have higher carcigenic and toxic effects, are recognized as priority pollutants by the United States Environmental Protection Agency (US-EPA) (Rubailo et al, 2008).

Figure 1. 16 different PAH compounds thought to be carcinogenic.



5. Presence in Foods

PAH compounds formed due to environmental pollution (Szterk, 2013) in industrial production areas pass into the soil, water and air and contaminate agricultural products such as vegetables, fruits and cereals (Shi, Zhang & Liu, 2016). In addition, these compounds have been found in processed and heat-treated foods such as roasted coffee, dairy products, vegetable oil, tea, and packaging materials (Londoño, Reynoso & Resnik, 2015). PAH compounds formed in coffee occur during roasting of coffee beans (Szterk, 2013). PAH compounds found in oilseeds; As a result of contamination with environmental factors such as soil, water and air, it can infect these seeds, and it can be transferred to vegetable oils in stages such as drying method and solvent extraction during the seed stage (Shi, Zhang & Liu, 2016). European Commission regulations (835/2011); propolis etc. reported that various herbal products such as green tea and bee products contain PAH compounds depending on the production techniques (EC, 2011).

It is thought that PAH compounds can be formed in foods by basically three different methods. First method; It is the formation of PAH compounds as a result of pyrolysis of organic substances such as carbohydrates, proteins, fats, etc. with high temperature (500-900 °C). Second method; The oil droplets dripping from the foods cooked with coal fire can turn into volatile PAHs as a result

of dripping on hot coal and burning with intense fire. These volatile PAH products are more likely to contaminate the surface of the food as the smoke point increases. The third method is; The formation of PAH products with the incomplete combustion of coal and the contamination of these products on the food surface (Esposito et al, 2015). In particular, fats melted in meat cooked on barbecue or charcoal fall on hot coal and become pyrolysis (Alomirah et al, 2011). Thus, the PAH compounds formed are carried towards the meat by the smoke and accumulate on the surface. Later, it is carried into the food and accumulates in lipid components (Santos, Gomes & Roseiro, 2011). Due to the lipophilic nature of PAH compounds in this transport, the fat and water content of the food has an important role in the transport rate (Santos, Gomes & Roseiro, 2011).

Polycyclic aromatic hydrocarbons are environmental pollutants formed as a result of the effects of solid fuels, fossil fuels and biological organisms (Huang and Penning, 2014). In addition, smoking, cooking, stove use, and incense are also shown to be among the main sources of PAH (21). For this reason, the PAH rate is high in kitchens, and it can also be taken into the body from air, water, soil, food and water consumption (WHO, 2014).

6. PAHs in the Food Chain

The sources of PAHs in foods by contact with foods are numerous. PAHs are industrially released into nature as pyrolysis from organic substrate compounds originating from human activities. PAHs emitted to the environment as a result of the combustion of wood or coal and the emission of exhaust fumes from motor vehicles become contaminated with food (WHO, 2014).

Many PAH compounds have low water solubility and low volatility. Therefore, they tend to remain in the form of small organic particles such as ash. As a result of the contamination of these compounds with soil, foods such as legumes, cereals, vegetables and fruits are grown in contaminated soil (WHO, 2014). In a study conducted in England, the amount of B[a]P in the peel of vegetables as a result of soil contamination was found to be between 590 and 2301 μg/kg. The high amount of PAH in the soil causes contamination by passing from the plants that animals consume while grazing to their milk and from there to dairy products (WHO, 2014).

7. PAH Formation During Food Preparation and Cooking

Foods may be contaminated with PAH before preparation, but the PAH content in foods can often increase during cooking (Wick et al, 2011). In the researches on the formation of PAH in the cooking stages of foods; While the method that produces the most PAH is seen as roasting and frying, the least PAH formation is observed in the boiling method (WHO, 2014). The contribution of these methods to the PAH content can be explained as follows.

7.1. Food Smoking

The smoking method, which is one of the oldest cooking methods, is often used in the storage and flavoring of foods such as meat, fish and cheese. In this method, it is applied at 650-700 ºC in wood fire. The smoke from the fire comes into contact with the food, and the food continues to be smoked as the temperature of the fire decreases (WHO, 2014). Liquid smoke flavorings (LSF) can also be used to reduce PAH formation in smoking. This method provides lower PAH formation compared to smoking (WHO, 2014).

7.2. Grilling

In this method, in which heat is transmitted to the food by using an oven or pan, high-temperature butter, margarine or vegetable oils can be used. During this process, PAH formation can occur mostly as a result of cooking fatty beef with barbecue (WHO, 2014).

7.3. Frying

The method of cooking foods with animal or vegetable oil at high temperature is called frying. In this cooking method, since the heat in the hot oil is applied directly to all surfaces of the food, it provides faster cooking (Singh, Varshney & Agarwal, 2016). In the frying method, the PAH content of the food differs according to the processing of the oil used. Repeated use of the used oil significantly increases the PAH content in the food. As a result, both the fried food and the air in the kitchen are contaminated with PAH with the gas released (Singh, Varshney & Agarwal, 2016).

7.4. Drying

Drying is one of the oldest methods applied in foods. The aim is to prolong the shelf life of the food by preventing bacterial growth in the food. This method can be applied to meat and meat products, vegetables, fruits, cereals and legumes. In this method, the air of the environment and the drying floor are among the factors that increase contamination during the drying phase (Singh, Varshney & Agarwal, 2016).

7.5. Steaming

Steam cooking method is the method of cooking food as a result of transferring heat from steam. Since pyrolysis is minimized in this method, PAH formation is also low. This method is the most important cooking method that supports low PAH formation. In this method, it was determined that PAH was formed 35 times less than the grill method, 24 times less than the smoking method, and 15 times less than the roasting method (Chen and Lin, 1997).

8. PAH – Impact on Health

The effects of PAHs on human health depend on the PAH concentration and exposure time of the contact contamination (Buha, 2011). The health of the individual is also related to other factors such as basic health status, genetic characteristics, environmental factors, age and gender before exposure (Buha, 2011). Epidemiological studies have shown that combinations of PAHs and duration of exposure affect basic health (ATSDR, 2011). Naphthalene, which is frequently found in potable water from PAHs that enter our lives in many ways, binds to molecules in kidney, liver and lung tissues with covalent bonds and increases the toxicity rate with this method (Sudip, Om & Rakesh, 2002). In addition, it is known to cause eye defects and hemolytic anemia (Srogi, 2007). In the short term, many symptoms such as vomiting, nausea, diarrhea, abdominal pain, headache, excessive sweating, and tachycardia can be seen with high exposure (IPCS, 2002). At the same time, mild irritation and dermatitis can be observed on the skin after high exposure in a short time (CDC, 2009). In addition to all these, urban air pollution and PAHs taken with food have negative effects on the immune system (Liu et al, 2013). In addition, both short-term and long-term effects of Acenapthene caused adverse effects on the immune system and skin diseases, while PAHs such as Benzo (a) anthracene caused tumors as a result of animal exposure (Kim et al, 2013).

9. Conclusion and Recommendations

- ✓ In cases where the smoking method is not applied consciously, it is inevitable that undesirable compounds will occur in the food to be consumed. These hydrocarbons can accumulate in the human body over time and cause important health problems, especially breast, pancreatic and stomach cancer, where they can have mutagenic and carcinogenic effects.
- ✓ Instead of the traditional smoking techniques that are frequently used in our country; Smoked technology should be used in systems where smoke aroma, oven temperature and humidity inside the oven can be controlled, and the cooking smoke can be prepared outside the oven and then filtered and delivered to the nutrients in the modern oven. In this way, consumption of richly flavored incense products will not pose a health problem, and it will be possible to produce standard smoked foods similar to the practices in European countries.

- ✓ In addition, it should be preferred to bake and boil foods of animal origin (fatty meat) instead of grilling them by placing them on coal or direct fire. Pure charcoal should be used as fuel type. When grilling, the cooking process should be done on embers after the flames are extinguished, not on the burning fire.
- ✓ The distance between meat and fire should be at least 7 cm, ideally 10-15 cm.
- ✓ Measures should be taken to prevent air, soil and water pollution and inspections should be carried out effectively.

Bibliography

Agency for Toxic Substances and Disease Registry (ATSDR). (1995). Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Atlanta (GA): *Department of Health and Human Services*, Public Health Service, USA.

Agency for Toxic Substances and Disease Registry (ATSDR). (2011). Toxic Substances-Polycyclic Aromatic Hydrocarbons (PAHs). *Department of Health and Human Services*, Public Health Service, USA.

Alomirah, H., Al-Zenki, S., Al-Hooti, S., Zaghloul, S., Sawaya, W., Ahmed, N., Kannan, K. (2011). Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (PAHs)from grilled and smoked foods. *Food Control*, 22, 2028-2035.

Buha, A. (2011). Polycyclic aromatic hydrocarbons. *Toxipedia*, 1:12–16.

Centers for Disease Control and Prevention (CDC) (2009) Fourth National Report on Human Exposure to Environmental Chemicals.

Chen, B.H., Lin, Y.S. (1997). Formation of polycyclic aromatic hydrocarbons during processing of duck meat. *Journal of Agricultural and Food Chemistry*. 45(4), 1394-1403.

Douben, P.E.T. (2003). PAHs: An Ecotoxicological Perspective. *Introduction*. 1–6. Ed: P.E.T Douben Wiley.

Esposito, M., Citro, A., Marigliano, L., Urbani, V., Seccia, G., Maotta, M.P., De Nicola, C. (2015). Inşuence of different smoking techniques on contamination by polycyclic aromatic hydrocarbons in traditional smoked *Mozzarella di Bufala Campana. Int J Dairy Technol,* 68(2).

European Commission (EC). (2011). Commission Regulation (EC) No 835/2011, of 19 August 2011, amending regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, L215, 4-8.

Hu, X., Kang, F., Gao, Y., Zhou, Q. (2015). Bacterial diversity losses: A potential extracellular driving mechanism involving the molecular ecological function of hydrophobic polycyclic aromatic hydrocarbons, *Biotechnology Reports*. 5, 27–30.

Huang, M., Penning, T.M. (2014). Processing contaminants: Polycyclic aromatic hydrocarbons (PAHs). *Encyclopedia of Food Safety*, 416-423.

International Programme on Chemical Safety (IPCS). (2000). Human exposure assessment; Environmental Health Criteria, 214. www. inchem.org/documents/ehc/214. Accessed 13 Nov 2022.

Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C. (2013). A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ Int,* 60:71–80

Li, J., Zhang, G., Li, X. D., Qi, S. H., Liu, G. Q., Peng, X. Z. (2006). Source seasonality of polycyclic aromatic hydrocarbons (PAHs) in a subtropical city, Guangzhou, South China, *Science of the Total Environment*. 355, 145– 155.

Liu, J., Zhang, L., Winteroth, L., Garcia, M., Weiman, S., Wong, J., Sunwoo, J., Nadeou, K. (2013). Epigenetically mediated pathogenic effects of phenanthrene on regulatory T cells. *J Toxicol,* 12:104–116

Londoño, V.A.G., Reynoso, C.M., Resnik, S.L. (2015). Polycyclic aromatic hydrocarbons (PAHs) survey on tea (*Camellia sinensis*) commercialized in Argentina. *Food Control*, 50, 31-37.

Moazzen, M., Ahmadkhaniha, R., Gorji, M. E., Yunesian, M., Rastkari, N. (2013). Magnetic solid-phase extraction based on magnetic multi-walled carbon nanotubes for the determination of polycyclic aromatic hydrocarbons in grilled meat samples, *Talanta*. 115, 957–965.

Rubailo, A.I., Oberenko, A.V. (2008). Polycyclic Aromatic Hydrocarbons as Priority Pollutants. Journal of Siberi-an Federal University, *Chemistry*, 4, 344-354.

Santos, C., Gomes, A., Roseiro, L.C. (2011). Polycyclic aromatic hydrocarbons incidence in

Shi LK, Zhang DD, Liu YL. 2016. Incidence and survey of polycyclic aromatic hydrocarbons in edible vegetable oils in China. *Food Control*, 62, 165-170.

Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke şavouring food additives, *J Chromatogr B Analyt Technol Biomed Life Sci.* 25;770(1-2):3-18.

Singh, L., Varshney, J.G., Agarwal, T. (2016). Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. *Food Chemistry*, 199:768-781.

Sojinu, O.S., Sonibare, O.O., Zeng, E.Y. (2011). Concentrations of polycyclic aromatic hydrocarbons in soils of a mangrove forest affected by a forest fire. *Toxicol Environ Chem* 93(3), 450–461

Srogi, K. (2007). Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review, *Environ Chem Lett,* 5 (4), 169–195

Sudip, K.S., Om, V.S., Rakesh, K.J. (2002.) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. *Trends Biotechnol* 20:243–248

Szterk, A. 2015. Acridine derivatives (PANHs, azaarenes) in raw, fried or grilled pork from different origins, and PANH formation during pork thermal processing. *J Food Compos Anal*, 43, 18-24.

World Health Organisation (WHO). (1998). Environmental Health Criteria 202, Selected non-heterocyclic polycyclic aromatic hydrocarbons. IPCS, International Programme on Chemical Safety. *World Health Organisation*, Geneva.

World Health Organization (WHO). (2014). WHO Guidelines for indoor air quality: selected pollutants. ISBN, 97892, 89002134.

Wick, A.F., Haus, N.W., Sukkariyah, B.F., Haering, K.C., Daniels, W.L. (2011). Remediation of PAH-contaminated soils and sediments: A literatüre review. CSES Department, *Internal Research Document*. 1–102

Wu, S. P., Tao, S., Liu, W. X. (2006). Particle size distributions of polycyclic aromatic hydrocarbons in rural and urban atmosphere of Tianjin. *China, Chemosphere,* 62, 357–367.

Anal Fissure

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Definition and Etiology

An anal fissure is a tear in the anoderm (Fig. 1). Being distal to the dentate line, it is a painful and distressing condition. Patients often describe a sharp pain, as if their skin is being cut by a knife or as if they "pass glass", during stool passage. In some cases, pain continues for hours.

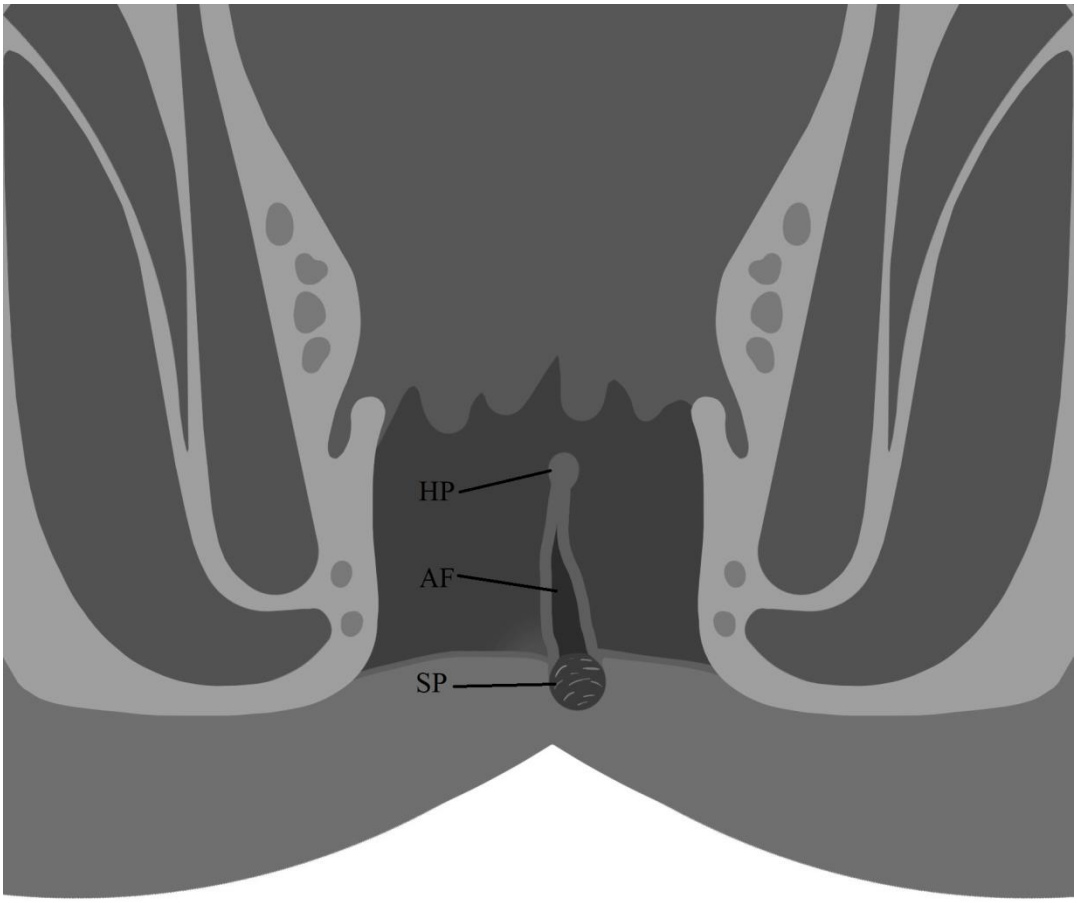

FIGURE 1. The Illustration of an Anal Fissure. HP: Hypertrophied Papillae, AF: Anal Fissure, SP: Sentinel Pile

The true prevelance is hard to presume, since most cases heal spontaneously, such as anal fissures occurring during pregnancy, and thus patients do not seek immediate medical care. However, it is a common disease, which comprise an important part of the outpatient general surgery practices. In a contemporary study, the life time risk of this clinical picture was found to be 7.8% (1). Both genders are equally affected and middle aged individuals are at increased risk (2).

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There are two main theories, which explain the source of this condition; the increased internal anal sphincter (IAS) pressure and decreased epithelial blood supply (3). Frankly, when IAS pressure increases chronically, the blood flow to anoderm, especially to its posterior part, decreases. So these two theories actually support each other, as most of the tearings occur at the posterior line. Nevertheless, it is logical to assume, that some patients are prone to this condition. When a triggering event, such as constipation, chronic diarrhea or anal intercourse, damages the anoderm, a vicious cycle begins. Patients will feel uncomfortable while passing stools. This will result in more hardened stools and thus more damage to the anoderm. Actually, any alterations of bowel movements caused by pain eventually contribute to the cycle. Consequently, treatments aim to break this cycle by decreasing the IAS tone and thus increasing the blood supply to the sensitive anoderm.

Classification and Diagnosis

90% of tears occur at the posterior line and almost 10% occur at the anterior line. These fissures are called typical anal fissures. Tears occurring elsewhere are classified as atypical or secondary anal fissures and associated with an underlying disease. The underlying condition can be an anorectal cancer, leukemia, Crohn's Disease, HIV/AIDS, tuberculosis, syphilis, HSV, or psoriasis (4). The key to treat an atypical fissure, is to treat the underlying disease.

Fissures, which last less than 6 weeks, are classifed as acute fissures, whereas fissures, which last more, are referred as chronic. Acute fissures are superficial tears and associated with bright red bleeding during and after stool passage (Fig. 2). Chronic fissures on the other hand, have three distinct features; skin tags at external apex, hypertrophied papilla at the internal apex and exposed IAS fibers in between (Fig. 3) (5). Considering both bleeding and palpable skin tags together, it does not come as a surprise, that patients mistake an anal fissure for hemorrhoids.

Gentle traction of buttocks often exposes an anal fissure. If it can not be visualized or an atypical fissure is found, examination under anesthesia is prompted. A physician is advised to avoid digital rectal examination or proctoscopy not to aggravate the pain. If a secondary cause is suspected or there is a suspicion of malignancy, the symptoms must be relieved prior to an endoscopic evaluation. Anal manometry should also be postponed as passing the mash is also a painful process and therefore patients often do not comply adequately.

Most fissures heal within 6 weeks with conservative measures. Chronic fissures require medical treatment and if the conservative and medical treatments fail, surgery becomes a necessity.

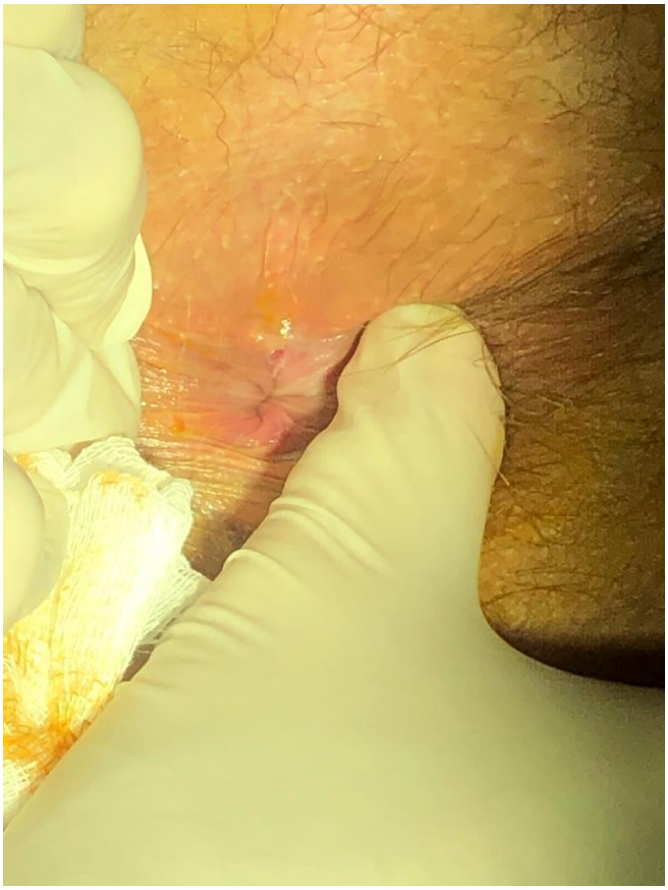

FIGURE 2. An acute anal fissure. The sphincter is not exposed and a skin tag is absent.

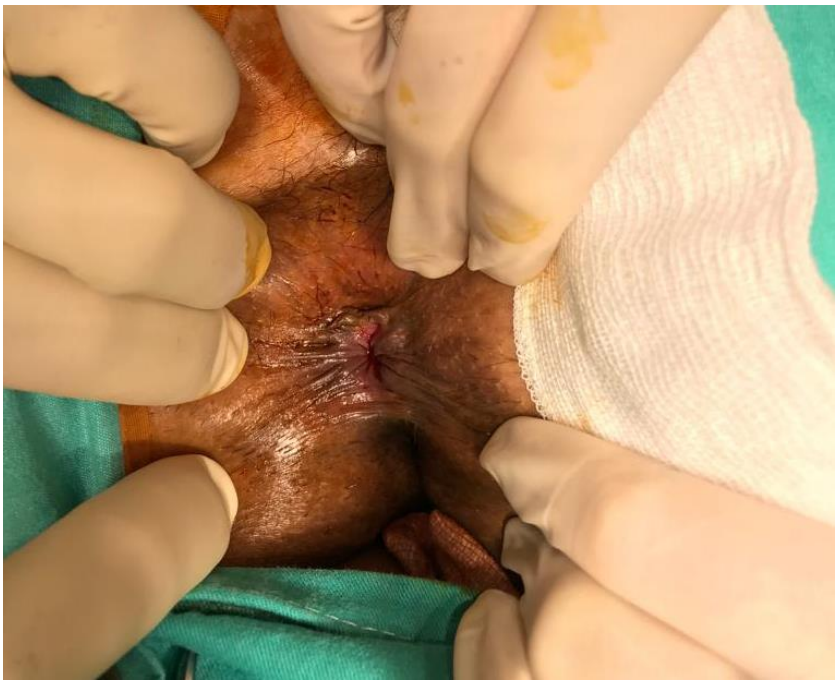

FIGURE 3. Chronic Anal Fissure. An exposed IAS segment and a small sentinel pile are visible.

Conservative Management

Following the exclusion of secondary causes, if it is deemed necessary, the treatment begins with conservative measures and is further tailored based on whether the condition is acute or chronic. Up to 90% of acute anal fissures heal spontaneously just with dietary modifications and warm sitz baths (6). However, chronic anal fissures (CAF) have more than 50% failure rates, when they are treated with conservative methods alone (7). The risk of failure is even more than that in recurrent cases.

The aim is to ensure an adequate stool consistency with sufficient fibre intake and relax the IAS with warm application. Our conservative approach is as follows;

1. 25g daily fibre consumption for women and 38g daily fibre consumption for men (8).
2. Warm sitz baths for 15 minutes at least twice daily or after each stool passage.
3. Analgesics (topical or peroral), in case of severe pain.

The source of fibre can be numerous and whether it is relevant is beyond the scope of this topic. Nevertheless, two studies showed improved healing rates with 20g daily bran divided in two doses and 15g daily bran divided in 3 doses (9, 10).

Patients with chronic or recurrent anal fissures are commenced on medical treatment. In our clinic, patients are assessed according to their pain relief and fissure healing before they are deemed as candidates for surgery. Therefore we do not have a strict time line for surgical intervention for patients under medical treatment, as different medical treatment options require different periods of time to take effect.

Medical Treatment

If conservative management fails or the patient presents with chronic anal fissure, considering medical treatment options such as topical agents, botilinum toxin (BT), etc., along with conservative management becomes appropriate.

Nitric Oxide

As stated previously, one of the main underlying causes of anal fissure is the increased anal resting pressure. To help facilitate relaxation of the IAS and thus reduce this pressure, topical Nitric Oxide (NO) application was proposed in the early 90's (11). Although various NO donors with different concentrations were used historically, the contemporary treatment utilizes glyceryl trinitrate (GTN) at a concentration of either 0.2% or preferably 0.4% applied twice daily (12).

Initially this agent promised good and smooth healing rates in CAF. However, a cochrane analysis demonstrated a healing rate of approximately 50% with GTN, with rates of recurrence reaching up to 70% (13). Additionally, NO in general causes severe headaches in 50% of patients, which results in discontinuation of the drug and thus the desired long term medical treatment (>6-8 weeks) can not be achieved in 20% of patients (14). And since NO also causes vasodilation, patients who are prone to hypotension or patients who are under phosphodiesterase 5 inhibiton therapy due to hypertension are advised to avoid this agent.

Calcium Channel Blockers

Oral and topical calcium channel blockers (CCB) are proposed as an alternative to relax IAS. Nifedipine and diltiazem are the most utilized agents in this regard. Topical forms are favored, since they are associated with fewer side effects, although there is no difference in recurrence rates (15).

The commercially available drugs usulually contain either 2% diltiazem or 0.2-0.5% nifedipine. However, to our knowledge, both in Europe and the USA there is no available

commercial preparation and these drugs are custom-made. Given the fact, that both dosage and substance might vary, more scientific effort is required to optimize CCBs in anal fissure treatment. Nevertheless, the available data suggest that they are associated with fewer side effects, such as flushing and headache, compared with GTN (16).

Botulinum Toxin

Clostridum botulinum is a bacteria, which produces the famous botulinum toxin (BT). It is a protein which more or less paralyzes the muscle activity. There are 7 types of BT and BT-A is utilized in CAF treatment. Also, there are 3 subtypes of BT-A. We prefer abobotulinumtoxinA (Dysport) in our routine practice, however our preference is based on drug's availability rather than scientific facts.

BT has an overall healing rate of 60-80% in CAFs with a significantly less incontinence risk compared to surgery (17). As one can appreciate, this rate is desirable due to the fact that every recovered individual is a patient, who is saved from the complications of a surgical approach. However, debates are ongoing about its dosage and site of injection.

Generally, 50 IU of BT-A is injected to the IAS in a single session (Fig. 4). In a contemporary study, increasing the dosage was associated with better healing rates (18). However, a recent meta-analysis demonstrated that increasing the dosage only increases the complications, such as temporary incontinence, local hypersensitivity reactions, hematoma formation, etc (19). This study also states that injection site is also important, as injecting the BT outside of the fissure is associated with both increased healing and complication rates. Consequently, combining both the dosage and site of injection facts and lack of data, it is quite early to come to a conclusion about an optimal BT administration. Well structured prospective studies are necessary, in this regard.

In our daily practice, we prefer BT-A in patients with CAF and high fecal incontinence (FI) risk in conjunction with medical therapy, when medical therapy alone fails to relieve the symptoms.

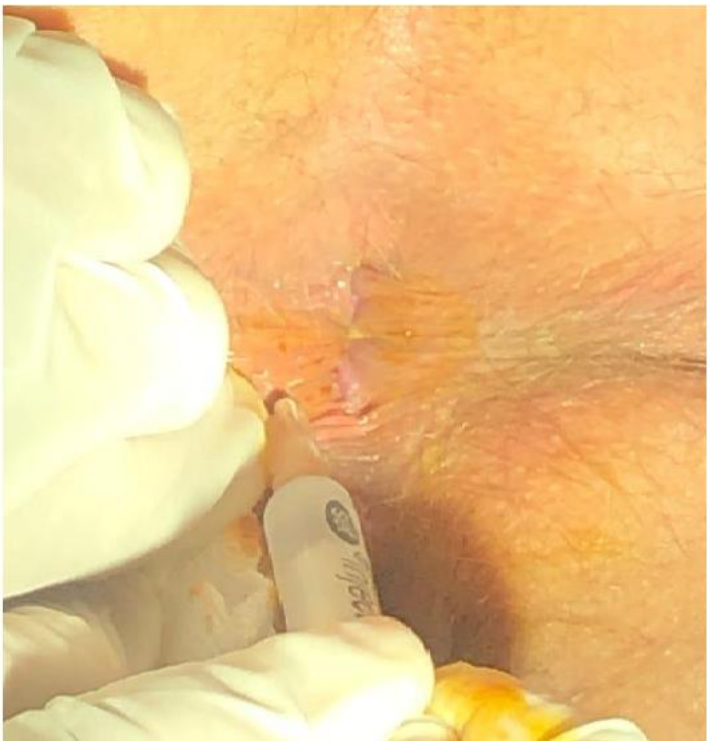

FIGURE 4. BT injection.

Miscellaneous Medical and Minimal Invasive Approaches

Neuromodulation, pelvic floor physical therapy (PFPT) and moist exposed burn ointment (MEBO) therapies have been proposed as alternatives.

PFPT utilizes approximately 8 weeks of biofeedback therapy in conjunction with conservative management. In a recent randomized controlled trial (RCT) PFPT showed 55% healing rate in patients with CAF who did not respond to conservative and medical therapy (20). A drawback of this study is that the results are short to mid-term.

MEBO is an ointment which is applied to patients with burns to promote wound healing. It is also utilized for pressure ulcer treatment. The mechanism of healing is believed to be the enhanced neovascularization and fibroblast proliferation (21). For this reason an ongoing RCT hypothesise that MEBO in conjunction with a CCB is superior to MEBO or CCB alone, in CAF treatment (22). Its results are yet to be published.

A subgroup of patients suffer from CAF due to pelvic floor disorders which result in obstructive defecation syndrome. These patients often describe prolonged straining and chronic constipation. An IAS hypertonicity might not even accompany the symptoms. Therefore, the treatment is directed towards the underlying cause rather than the CAF by utilizing neuromodulation.

Neuromodulation refers to either Sacral Nerve Stimulation (SNS) or posterior tibial nerve stimulation (PTNS) in pelvic floor disorders. While the latter is performed mostly transcutaneously, the former requires sedation and surgery. A recent systematic review evaluated PTNS in CAF treatment and suggested up to 75% mid-term healing rates (23). In another systematic review, SNS was also evlauated along with PTNS (24). The authors favored PTNS over SNS. However, both studies clearly stated that the data are sparse and methodological variations render a healthy conclusion impossible at the moment. Additionally, neuromodulation is an expensive procedure and to our knowledge there is little to no reference to the cost-effectivity in the available studies.

In summary, more RCTs with standardized methodology are required to assess the true value of above mentioned approaches.

Operative Management

IAS is the extention of circular muscle layer of rectum and relaxing it is the fundemantal of any CAF therapy. Where conservative and medical managements fail, surgery comes in handy.

Historically, anal dilation was the treatment of choice. However, this approach resulted in undesirable FI rates and therefore has been abondoned (25). As for the contemporary surgical approaches, assessing the patient's FI risk and evaluating any evident FI prior to surgery are mandatory. The most common FI risk factors are as follows (26);

1. Vaginal Delivery (even uncomplicated deliveries pose a significant FI risk)

2. Anal or Pelvic Surgery History (Including transabdominal gynecological interventions, such as hysterectomy)

3. Pelvic Radiotherapy

4. Anal Intercourse

5. History of Sexual Assaults

Choice of surgical intervention must be tailored according to the above-mentioned risk factors. In a nutshell, a surgical approach must avoid the risk of FI as much as possible, while maintaining adequate IAS relaxation and tissue healing.

Sphincterotomy

The golden standard of CAF treatment is sphincterotomy. IAS is dissected, so that anal resting pressure is diminished. However, some questions and debates about this procedure should be addressed;

1. Where to dissect the sphincter? Should a surgeon dissect the already exposed sphincter muscle or rather do a lateral sphincterotomy ?

- Lateral (3 or 9 o'clock) internal sphincterotomy (LIS) (Fig. 5) is the desired technique, since posterior sphincterotomy can lead to a keyhole deformity and thus fecal soiling (27). In a recent RCT, posterolateral internal sphincterotomy was evaluated and found to be superior to LIS (28). This is an interesting finding and we believe more effort should be focused on this matter in the future.

2. To what extent should the IAS be dissected ?

- The extent of a sphincterotomy should not exceed 1/2 of the IAS or the apex of the fissure. However, women patients should be given special care when dissecting the sphincter. Murad-Regadas et al. suggested 1/4 of the IAS or <1 cm should be dissected (29). In a more recent study, an even more conservative approach (dissection of 1/5 of the total IAS length) is proposed and deemed favorable (30). It is a fact that women are under greater risk of FI and more prospective studies are required to establish an optimized dissection extent in female patients.

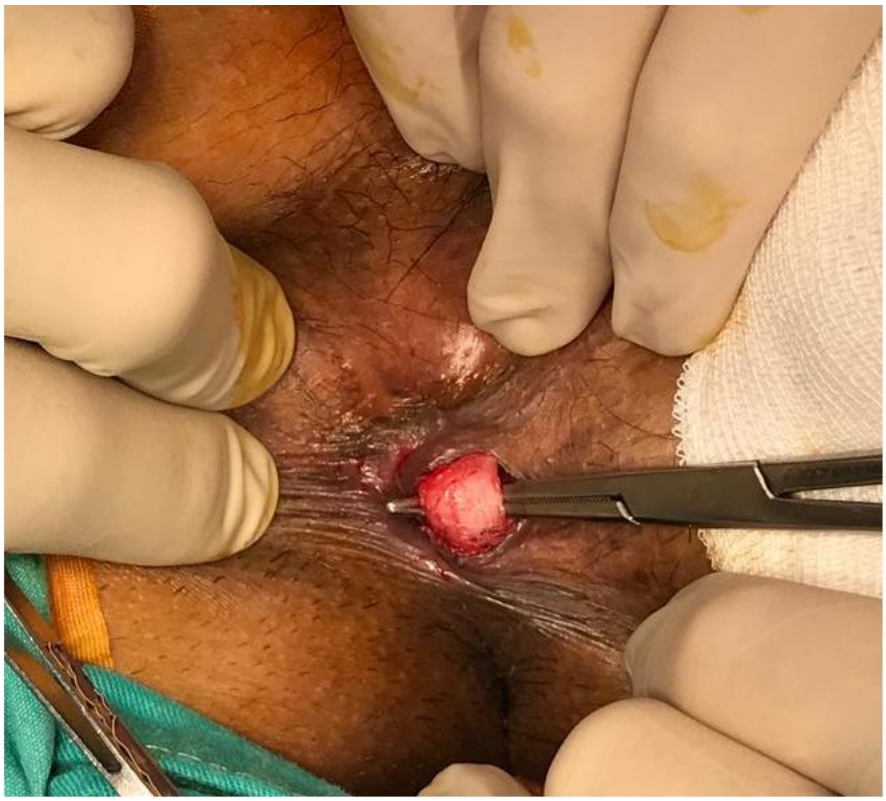

FIGURE 5. Surgically exposed IAS during LIS procedure

3. Open or closed technique ?

- Debates are still ongoing. According to a cochrane review, there is no difference in terms of healing or FI (31). A study evaluated postoperative endosonographic findings and concluded that open technique is superior in terms of completion of sphincterotomy (32). In a contemporary trial, a cataract knife assisted closed technique was compared to open LIS and found

to be superior in terms of pain, bleeding, incontinence and recurrence (33). With the available data, we suggest that every surgeon should prefer the method, with which he/she feels comfortable.

4. Does FI occur frequently after LIS?

-Historically, LIS was associated with almost 50% FI rate (flatus, liquid stool, etc.) (34). However, with the new sphincter preserving limited dissections, contemporary studies report less than 10% FI incidence (35).

Consequently, apart from some technical debates, LIS is a standard and successive surgical procedure in CAF treatment. In our clinic, we perform LIS procedure in patients with no FI risk factors, when conservative treatments fail and a hypertrophied IAS is present.

Fissurectomy and Fissurotomy

Some fissures have fibrotic edges, which may hinder healing. In such cases, curettage of the fissure base, excision of fibrotic edges, sentinel pile (skin tag) and hypertrophied papillae, namely fissurectomy was proposed. This method showed good results (>90% healing rate) especially when it is combined with BT-A (36). However, more recent studies revealed a worse postoperative FI profile following fissurectomy, which rendered its practice limited (37).

Another approach is unroofing the fissure, namely fissurotomy. With this technique a sphincterotomy can be avoided and the inventors of this method reported up to 98% healing rates (38). However, to our knowledge, the data have not been expanded since then in this regard and therefore this method lacks the scientific evidence to be performed in daily practice.

Special Considerations

Fissures without IAS Hypertonicity

A CAF without an apparent increased anal resting pressure, clearly complicates the treatment strategy, as it renders topical treatments, BT-A and sphincterotomy useless. In such cases flap techniques (Flap Anoplasty) must be the treatment of choice. Although most of these procedures were proposed as an alternative to LIS in patients with anal hypertonicity to reduce the risk of FI, their results are satisfying with more than 80% overall healing rates and less than 2% de novo FI events (39). A recent study even suggested the utilization of the skin tag as flap in patients with anal hypotonicity and reported nearly 100% healing rates with no de novo FI (40). However, this study has a limited number of patients and therefore can not be recommended as a standard treatment option yet.

Consequently, anal advancement flaps are the only surgical treatment options for the patients with low anal tone and they should follow conservative treatments, if deemed necessary.

Crohn's Disease

Fissures are common findings in patients with Crohn's Disease. The percentage of the affected individuals can reach up to 50% (41). However, this condition is mostly due to chronic inflammation rather than IAS hypertonicity. Therefore, efforts should be directed towards Crohn's remission before initiating an interventional therapy for the anal fissure. Conservative and medical therapy can be started even before Crohn's remission. The above-mentioned interventional therapies (fissurectomy, BT-A, LIS, flap anoplasty) can be performed only when the Crohn's Disease Activity Index is less than 150 (42).

Child Patients

Conservative and medical treatments are deemed appropriate in children with either acute or chronic anal fissures (43). Surgery should be avoided as much as possible and must be reserved only for patients, whose symptoms do not improve with other methods. However, CAF

is not a common condition in children and the data regarding this issue are sparse. Therefore, a threshold time can not be established for timing of the surgery.

Concomitant Anal Lesions

Both sentinel pile and the hypertrophied papillae can be excised during a fissure surgery. However, excisional hemorrhoidectomy should be avoided when performing LIS, as it increases the risk of FI (44).

Human Immunodeficiency Virus (HIV)

Fissures can present in atypical locations. However, most of the HIV+ patients present with typical fissures (either posterior or anterior) (45). Since infectious complications and impaired wound healing are common problems in HIV+ patients, antiviral therapy should precede fissure treatment. Conservative and medical treatments are favored in this patient population. In male patients, anal hypotonicity is more prevalent in this group and the surgery, in case it becomes necessary, must be tailored accordingly.

Recurrent Anal Fissure

The surgeon should query the treatment before the recurrence. For example; did the patient undergo surgery, such as sphincterotomy, or did the fissure heal with only conservative measures and then recur? When an anal fissure recurs, conservative measures and medical treatments should be the first line treatments. Frankly, any treatment options are viable. However, anal tone must be checked first and if a sphincterotomy was performed and the disease recurred, a sphincter-saving procedure must be chosen.

References

1. Mapel, D. W., Schum, M., & Von Worley, A. (2014). The epidemiology and treatment of anal fissures in a population-based cohort. *BMC gastroenterology*, *14*, 129. https://doi.org/10.1186/1471-230X-14-129

2. Jahnny B, Ashurst JV. Anal Fissures. [Updated 2021 Nov 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK526063/

3. Wehrli H. (1996). Atiologie, Pathogenese und Klassifikation der Analfissur [Etiology, pathogenesis and classification of anal fissure]. *Swiss surgery = Schweizer Chirurgie = Chirurgie suisse = Chirurgia svizzera*, (1), 14–17.

4. Zaghiyan, K. N., & Fleshner, P. (2011). Anal fissure. *Clinics in colon and rectal surgery*, *24*(1), 22–30. https://doi.org/10.1055/s-0031-1272820

5. Nelson R. (2010). Anal fissure (chronic). *BMJ clinical evidence*, *2010*, 0407.

6. Zaghiyan, K. N., & Fleshner, P. (2011). Anal fissure. *Clinics in colon and rectal surgery*, *24*(1), 22–30. https://doi.org/10.1055/s-0031-1272820

7. Nelson R. L. (2014). Anal fissure (chronic). *BMJ clinical evidence*, *2014*, 0407.

8. Turner, N. D., & Lupton, J. R. (2011). Dietary fiber. *Advances in nutrition (Bethesda, Md.)*, *2*(2), 151–152. https://doi.org/10.3945/an.110.000281

9. Jensen S. L. (1986). Treatment of first episodes of acute anal fissure: prospective randomised study of lignocaine ointment versus hydrocortisone ointment or warm sitz baths plus bran. *British medical journal (Clinical research ed.)*, *292*(6529), 1167–1169. https://doi.org/10.1136/bmj.292.6529.1167

10. Jensen S. L. (1987). Maintenance therapy with unprocessed bran in the prevention of acute anal fissure recurrence. *Journal of the Royal Society of Medicine*, *80*(5), 296–298.

11. Lund, J. N., & Scholefield, J. H. (1997). Glyceryl trinitrate is an effective treatment for anal fissure. *Diseases of the colon and rectum*, *40*(4), 468–470. https://doi.org/10.1007/BF02258394

12. Scholefield, J. H., Bock, J. U., Marla, B., et al. (2003). A dose finding study with 0.1%, 0.2%, and 0.4% glyceryl trinitrate ointment in patients with chronic anal fissures. *Gut*, *52*(2), 264–269. https://doi.org/10.1136/gut.52.2.264

13. Nelson, R. L., Thomas, K., Morgan, J., & Jones, A. (2012). Non surgical therapy for anal fissure. *The Cochrane database of systematic reviews*, *2012*(2), CD003431. https://doi.org/10.1002/14651858.CD003431.pub3

14. Watson, S. J., Kamm, M. A., Nicholls, R. J., et al. (1996). Topical glyceryl trinitrate in the treatment of chronic anal fissure. *The British journal of surgery*, *83*(6), 771–775. https://doi.org/10.1002/bjs.1800830614

15. Sahebally, S. M., Ahmed, K., Cerneveciute, Ret al. (2017). Oral versus topical calcium channel blockers for chronic anal fissure-a systematic review and meta-analysis of randomized controlled trials. *International journal of surgery (London, England)*, *44*, 87–93. https://doi.org/10.1016/j.ijsu.2017.06.039

16. Sanei, B., Mahmoodieh, M., & Masoudpour, H. (2009). Comparison of topical glyceryl trinitrate with diltiazem ointment for the treatment of chronic anal fissure: a randomized clinical trial. *Acta chirurgica Belgica*, *109*(6), 727–730. https://doi.org/10.1080/00015458.2009.11680524

17. Perry, W. B., Dykes, S. L., Buie, W. D., Rafferty, J. F., & Standards Practice Task Force of the American Society of Colon and Rectal Surgeons (2010). Practice parameters for the management of anal fissures (3rd revision). *Diseases of the colon and rectum*, *53*(8), 1110–1115. https://doi.org/10.1007/DCR.0b013e3181e23dfe

18. Brisinda, G., Chiarello, M. M., Crocco, A., et al. (2022). Botulinum toxin injection for the treatment of chronic anal fissure: uni- and multivariate analysis of the factors that promote healing. *International journal of colorectal disease*, *37*(3), 693–700. https://doi.org/10.1007/s00384-022-04110-0

19. Vitoopinyoparb, K., Insin, P., Thadanipon, K., et al. (2022). Comparison of doses and injection sites of botulinum toxin for chronic anal fissure: A systematic review and network meta-analysis of randomized controlled trials. *International journal of surgery (London, England)*, *104*, 106798. https://doi.org/10.1016/j.ijsu.2022.106798

20. van Reijn-Baggen, D. A., Elzevier, H. W., Putter, H., et al. (2022). Pelvic floor physical therapy in patients with chronic anal fissure: a randomized controlled trial. *Techniques in coloproctology*, *26*(7), 571–582. https://doi.org/10.1007/s10151-022-02618-9

21. Li, W., Ma, Y., Yang, Qet al. (2017). Moist exposed burn ointment for treating pressure ulcers: A multicenter randomized controlled trial. *Medicine*, *96*(29), e7582. https://doi.org/10.1097/MD.0000000000007582

22. El Charif, M. H., Doughan, S., Kredly, R., et al. (2021). MEBO versus topical Diltiazem versus a combination of both ointments in the treatment of acute anal fissure: a randomized clinical trial protocol. *BMC complementary medicine and therapies*, *21*(1), 75. https://doi.org/10.1186/s12906-021-03227-z

23. Perivoliotis, K., Baloyiannis, I., Ragias, Det al. (2021). The role of percutaneous tibial nerve stimulation (PTNS) in the treatment of chronic anal fissure: a systematic review. *International journal of colorectal disease*, *36*(11), 2337–2346. https://doi.org/10.1007/s00384-021-03976-w

24. Bananzadeh, A., Sohooli, M., Shamsi, T., et al. (2022). Effects of neuromodulation on treatment of recurrent anal fissure: A systematic review. *International journal of surgery (London, England)*, *102*, 106661. https://doi.org/10.1016/j.ijsu.2022.106661

25. Nielsen, M. B., Rasmussen, O. O., Pedersen, J. F., et al. (1993). Risk of sphincter damage and anal incontinence after anal dilatation for fissure-in-ano. An endosonographic study. *Diseases of the colon and rectum*, *36*(7), 677–680. https://doi.org/10.1007/BF02238595

26. Lunniss, P. J., Gladman, M. A., Hetzer, F. Het al. (2004). Risk factors in acquired faecal incontinence. *Journal of the Royal Society of Medicine*, *97*(3), 111–116. https://doi.org/10.1258/jrsm.97.3.111

27. Yüksel, O., Bostanci, H., Leventoğlu, S., et al. (2008). Keyhole deformity: a case series. *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract*, *12*(6), 1110–1114. https://doi.org/10.1007/s11605-008-0471-5

28. Alawady, M., Emile, S. H., Abdelnaby, M., et al. (2018). Posterolateral versus lateral internal anal sphincterotomy in the treatment of chronic anal fissure: a randomized controlled trial. *International journal of colorectal disease*, *33*(10), 1461–1467. https://doi.org/10.1007/s00384-018-3087-6

29. Murad-Regadas, S. M., Fernandes, G. O., Regadas, F. S., et al. How much of the internal sphincter may be divided during lateral sphincterotomy for chronic anal fissure in women? Morphologic and functional evaluation after sphincterotomy. *Diseases of the colon and rectum*, *56*(5), 645–651. https://doi.org/10.1097/DCR.0b013e31827a7416

30. Brillantino, A., Izzo, D., Iacobellis, F., et al. (2021). Safety and effectiveness of minimal sphincterotomy in the treatment of female patients with chronic anal fissure. *Updates in surgery*, *73*(5), 1829–1836. https://doi.org/10.1007/s13304-020-00874-8

31. Nelson, R. L., Chattopadhyay, A., Brooks, W., et al. (2011). Operative procedures for fissure in ano. *The Cochrane database of systematic reviews*, *2011*(11), CD002199. https://doi.org/10.1002/14651858.CD002199.pub4

32. García-Granero, E., Sanahuja, A., García-Botello, S. A., et al. (2009). The ideal lateral internal sphincterotomy: clinical and endosonographic evaluation following open and closed internal anal sphincterotomy. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland*, *11*(5), 502–507. https://doi.org/10.1111/j.1463-1318.2008.01645.x

33. Nasir, G. J. A., & Sadat, G. (2022). Comparison of Open and Closed Lateral Sphincterotomy in Patients with Chronic Anal Fissure. *Journal of Pharmaceutical Research International*, *34*(30B), 1-6. https://doi.org/10.9734/jpri/2022/v34i30B36071

34. Nyam, D. C., & Pemberton, J. H. (1999). Long-term results of lateral internal sphincterotomy for chronic anal fissure with particular reference to incidence of fecal incontinence. *Diseases of the colon and rectum*, *42*(10), 1306–1310. https://doi.org/10.1007/BF02234220

35. De Robles, M. S., & Young, C. J. (2022). Real world outcomes of lateral internal sphincterotomy vs botulinum toxin for the management of chronic anal fissures. *Asian journal of surgery*, *45*(1), 184–188. https://doi.org/10.1016/j.asjsur.2021.04.027

36. Lindsey, I., Cunningham, C., Jones, O. M., et al. (2004). Fissurectomy-botulinum toxin: a novel sphincter-sparing procedure for medically resistant chronic anal fissure. *Diseases of the colon and rectum*, *47*(11), 1947–1952. https://doi.org/10.1007/s10350-004-0693-x

37. Bara, B. K., Mohanty, S. K., Behera, S. N., et al. (2021). Fissurectomy Versus Lateral Internal Sphincterotomy in the Treatment of Chronic Anal Fissure: A Randomized Control Trial. *Cureus*, *13*(9), e18363. https://doi.org/10.7759/cureus.18363

38. Pelta, A. E., Davis, K. G., & Armstrong, D. N. (2007). Subcutaneous fissurotomy: a novel procedure for chronic fissure-in-ano. a review of 109 cases. *Diseases of the colon and rectum*, *50*(10), 1662–1667. https://doi.org/10.1007/s10350-007-9022-5

39. Sahebally, S. M., Walsh, S. R., Mahmood, W., et al. (2018). Anal advancement flap versus lateral internal sphincterotomy for chronic anal fissure- a systematic review and meta-analysis. *International journal of surgery (London, England)*, *49*, 16–21. https://doi.org/10.1016/j.ijsu.2017.12.002

40. Sobrado Júnior, C. W., Hora, J., Sobrado, L. Fet al (2019). Anoplasty with skin tag flap for the treatment of chronic anal fissure. Anoplastia com plicoma sentinela para o tratamento de fissura anal crônica. *Revista do Colegio Brasileiro de Cirurgioes*, *46*(3), e20192181. https://doi.org/10.1590/0100-6991e-20192181

41. Gibson, M. S., & Singh. (2020, July 29). Anal fissure epidemiology and demographics - wikidoc. Retrieved September 22, 2022, from https://www.wikidoc.org/index.php/Anal_fissure_epidemiology_and_demographics#:~:text=The%20incidence%20of%20anal%20fissure%20is%20approximately%201100%20(700%2D1700,is%20approximately%2030%2D50%25.

42. D'Ugo, S., Franceschilli, L., Cadeddu, F., et al. (2013). Medical and surgical treatment of haemorrhoids and anal fissure in Crohn's disease: a critical appraisal. *BMC gastroenterology*, *13*, 47. https://doi.org/10.1186/1471-230X-13-47

43. Patkova, B., & Wester, T. (2020). Anal Fissure in Children. *European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery ... [et al] = Zeitschrift fur Kinderchirurgie*, *30*(5), 391–394. https://doi.org/10.1055/s-0040-1716723

44. Wang, W. G., Lu, W. Z., Yang, C. M., et al. (2018). Effect of lateral internal sphincterotomy in patients undergoing excisional hemorrhoidectomy. *Medicine*, *97*(32), e11820. https://doi.org/10.1097/MD.0000000000011820

45. Mallari, A. O., Schwartz, T. M., Luque, A. E., et al. (2012). Anal cancer screening in HIV-infected patients: is it time to screen them all?. *Diseases of the colon and rectum*, *55*(12), 1244–1250. https://doi.org/10.1097/DCR.0b013e31826ab4fb

Changes In Jaw Surgery After The Pandemic And Their Effects On Oral Health

İlhami Sancar ŞİMŞEK[1]

Introduction

The Covid-19 pandemic, which broke out in China at the end of 2019 and affected the whole world in a short time, deeply affected the production and delivery of health services, as well as all areas of life. During the pandemic process, while there were uncertainties about the exact effects of the disease, its way of spreading, which risk factors it has, and which groups it is risky for, on the other hand, there were disruptions in all areas from social life to public order, from production to education (da Silva et al, 2022; Buchy et al, 2021; Carbone et al, 2021; Sharma et al, 2021). One of the areas most affected by this situation is the areas of oral and maxillofacial surgery.

Oral health is actually an important issue that is related to many health areas and closely concerns public health and the quality of life of individuals. However, since the problems in general and non-elective health services have not been resolved, especially in developing or underdeveloped countries, oral health and related units gain importance only in the presence of a situation that affects high pain or other health conditions. (Poirier et al, 2022; Gill et al, 2022; Poudel et al, 2021; Petersen et al, 2020; Tynan et al, 2018). For this reason, it can be stated that many factors such as physicians, health workers, institutions or equipment working in the field of oral health are insufficient both compared to other health services and compared to the population.

One of the biggest challenges faced by those working in the field of oral health has been the cessation of health tourism, which has increased its orientation due to the already limited opportunities for dental health during the pandemic and especially due to very low profit margins against very high costs in the domestic market. Although health is a constitutional right of individuals and a social service, the provision of health services in reality brings a certain economic cost. In dental and oral health, this situation has a much greater effect than other health services, and external dependence is at the highest level in terms of materials (Chavda et al, 2022; Emrani et al, 2021; Imai and Tanaka, 2021; Mallah et al, 2021; Murugan et al, 2021). Moreover, this situation is valid not only for Turkey, but also for many countries in the world. Despite the dependency on foreign materials in Turkey, highly qualified workforce has made our country one of the most attractive centers in oral health and global health tourism. However, the pandemic process made this situation very difficult and closed the foreign markets where dentists and surgeons met the material-currency problems they had experienced in the domestic market.

Internally, the most important problems experienced in health services are the general perspective on oral health and the low importance given to oral health in social or cultural terms. Oral health, which was postponed or neglected even in normal times, was postponed more for many different reasons during the pandemic process and many oral health services, especially jaw surgery, were evaluated electively. Examining and revealing all these factors and the effects of the process on oral health is more important than many studies, research or clinical findings in oral

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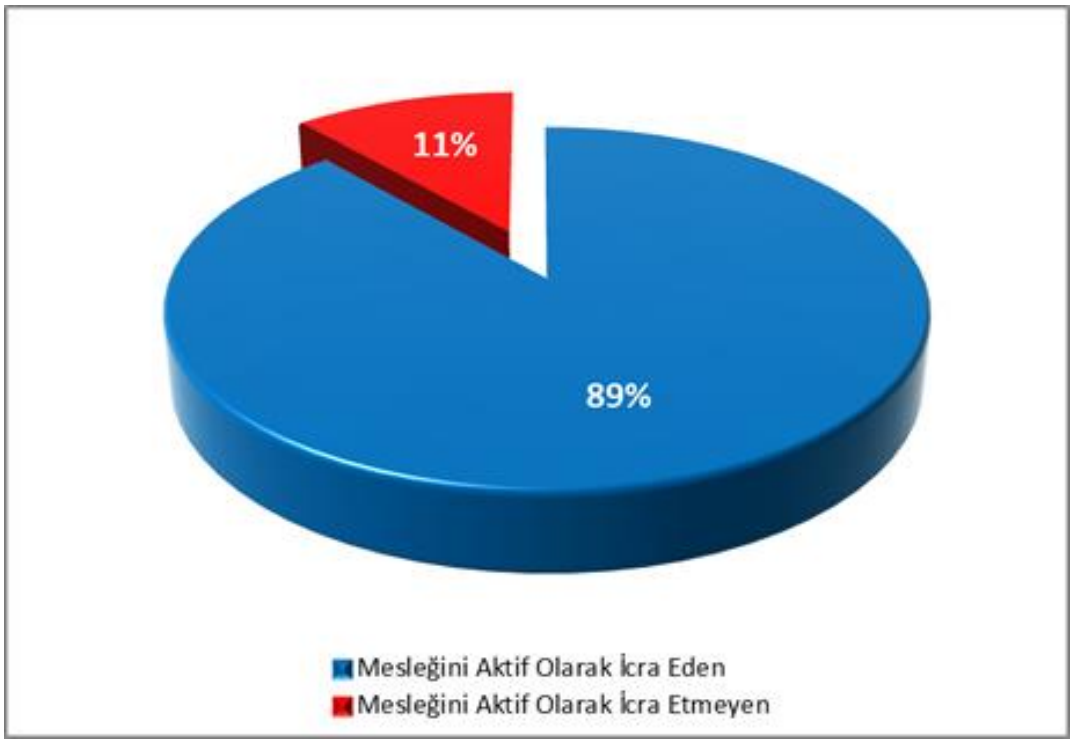
health and maxillofacial surgery, and there are a series of factors that will provide a direct economic input to the field. makes a recommendation.

General Situation of Oral Health and Maxillofacial Surgery in Turkey

In Turkey, the situation of oral and maxillofacial surgery, or more generally, oral health services, should be examined under two headings as material-material and workforce. In terms of materials and materials, although production has been made for new domestic implants, consumables or coating types in recent years, these products have not yet been sufficiently accepted in the market and brands of flammable origin are dominant in the market. In this respect, it is possible to state that foreign dependency as a material in oral health is high in Turkey, therefore it is closely affected by exchange rate movements, and price stability is a disadvantage to either the dentist or the patients. In particular, the products of countries such as Switzerland and Germany, which dominate the market, almost hold the market superiority and monopoly in this field (Peker et al, 2021; Kuzu, 2019). In this respect, it can be stated that oral health in Turkey does not have a positive impression in terms of materials and materials. The increase in foreign exchange and commodity prices along with the deteriorated economies during the pandemic process, on the other hand, revealed how important domestic consumables are and how necessary a self-sufficient system is despite all globalization and transportation-communication possibilities.

The second issue is the workforce or human resources. Although there are other important workforce elements in oral and dental health, such as dental practitioners and technicians trained in associate degree departments, dentists are predominantly important in dental health services and are seen as an important indicator of the level of oral and dental health of the country. The distribution of those who became dentists in Turkey in 2021 according to their ability to practice their profession is shown in Figure 1.

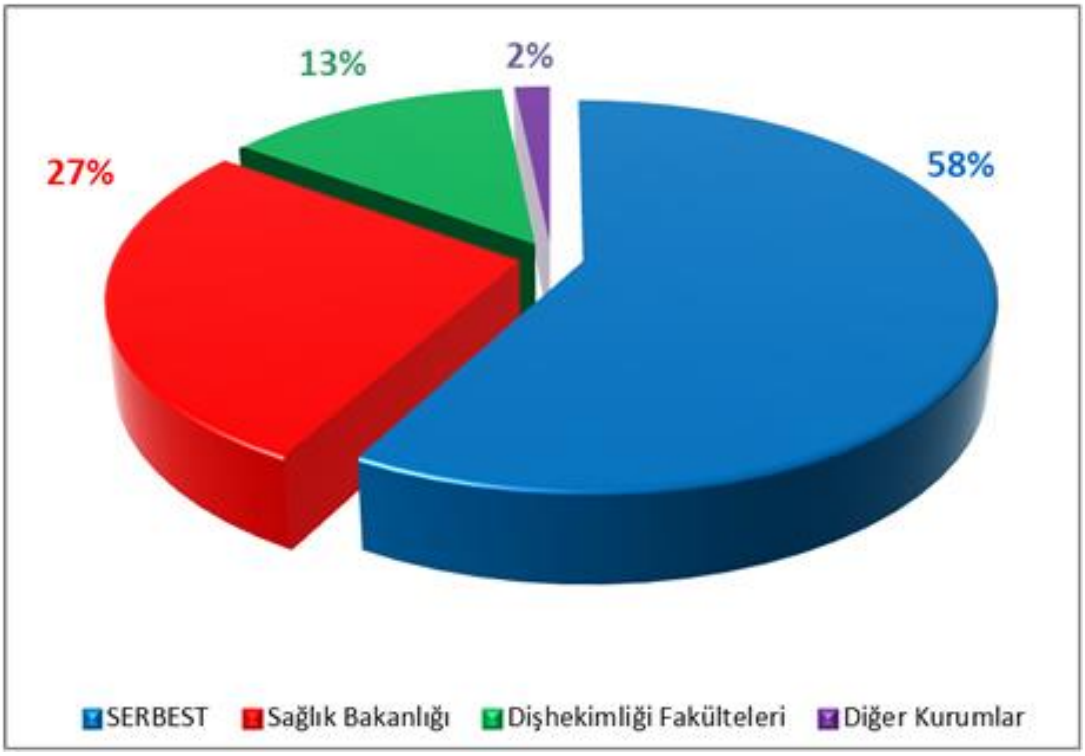
Figure 1. Distribution of those who became dentists in Turkey in 2021 according to their ability to practice their profession



Kaynak: Türkiye Diş Hekimleri Birliği, https://www.tdb.org.tr/sag_menu_goster.php?Id=92, Erişim: 22.09.2022

According to Figure 1, 89% of those who became dentists in our country in 2021 are practicing their profession, while 11% cannot. In fact, although 89% is seen as a very good figure for a profession, the rate of 11% is quite high in an area whose training constitutes a very serious economic cost according to the country's economy and the financial strength of individuals. Moreover, while the population's access to dentists in general is quite low, a significant rate of 11% cannot practice their profession either for economic or other reasons. Places where dentists work in Turkey in 2021 is shown in Figure 2.

Figure 2. Places where dentists work in Turkey in 2021



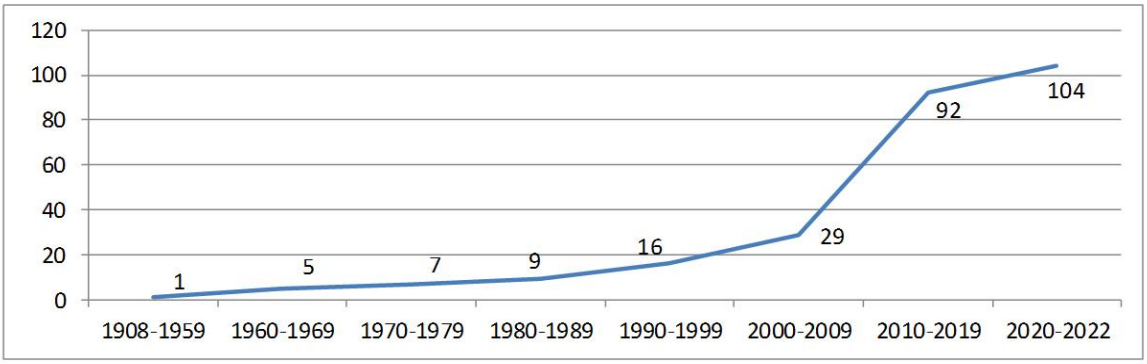
Kaynak: Türkiye Diş Hekimleri Birliği, https://www.tdb.org.tr/sag_menu_goster.php?Id=92, Erişim: 22.09.2022

According to the 2021 data of the Turkish Dental Association, 58% of the dentists in the country are self-employed, 27% work in the ministry of health and 13% in dentistry faculties. The ratio of those working in institutions other than these is around 2%.

In general, according to the distribution by place of work, self-employment is the most preferred type of employment in the country. Although this is followed by the ministry of health and universities, the sum of the two is not as much as the rate of self-employed. Therefore, it is possible to state that dentistry services and oral and dental health services are mostly provided in a private sector in Turkey, and this situation also brings with it costs and workload on dentists such as marketing, advertising and operation.

The change in the number of dentists in Turkey by years is given in Figure 3.

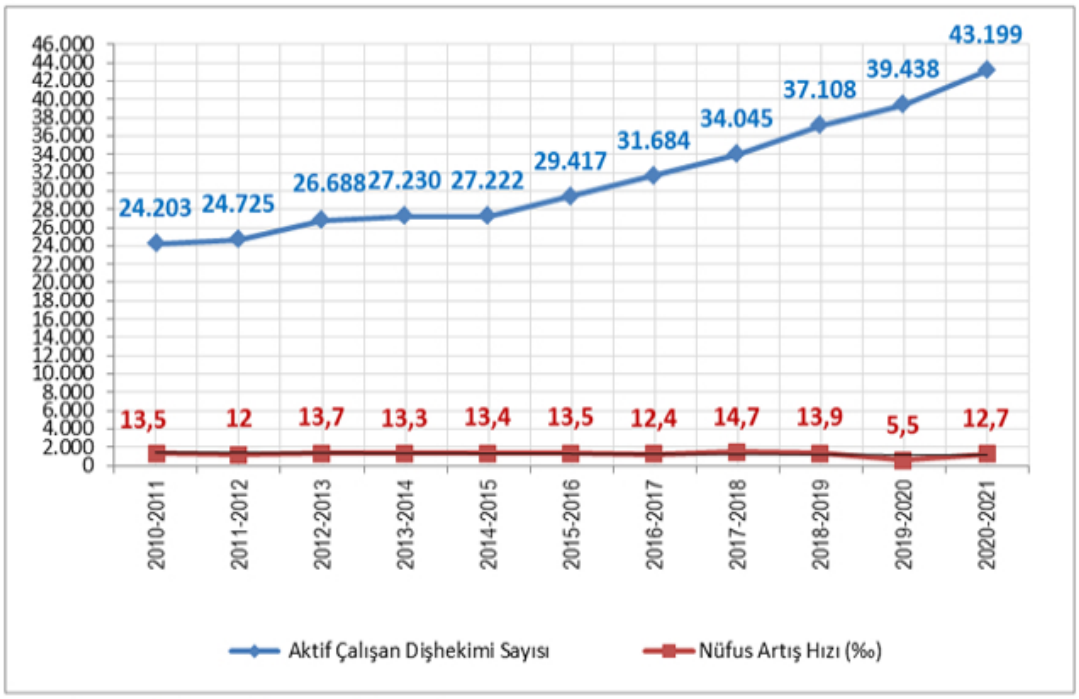
Figure 3. Change in the number of dentists in Turkey by years



Kaynak: Türkiye Diş Hekimleri Birliği, https://www.tdb.org.tr/sag_menu_goster.php?Id=84, Erişim: 22.09.2022

According to the change in the number of dentists over the years, there was a first acceleration in the 1990s and a second acceleration in the mid-2000s. Since the pandemic process in 2019, the sharp increase momentum that started in 2009 has experienced a serious decrease in the rate of increase. However, when evaluating this change, it is necessary to take into account the change in the population. The distribution of the change in the number of dentists by years according to the population is as in Figure 4.

Figure 4. Distribution of the change in the number of dentists over the years by population



Kaynak: Türkiye Diş Hekimleri Birliği, https://www.tdb.org.tr/sag_menu_goster.php?Id=387, Erişim: 22.09.2022

When the population growth rate and the number of actively working dentists are evaluated together, it is seen that the rate of dentists and the number of dentists per capita have increased significantly. It can be stated that health tourism, which has increased significantly towards the end of the 2000s, and the fact that one of the sectors that have the highest share in global health services, is oral and dental health, has a significant impact on this change.

Changes in Oral Health and Maxillofacial Surgery after the Pandemic

One of the most important changes experienced after the pandemic is the decrease in universities providing dental education. The distribution of the number of newly opened dental faculties by years, according to state and foundation universities, is given in Table 1.

Table 1. Distribution of the number of newly opened dental faculties by years, according to state and foundation universities

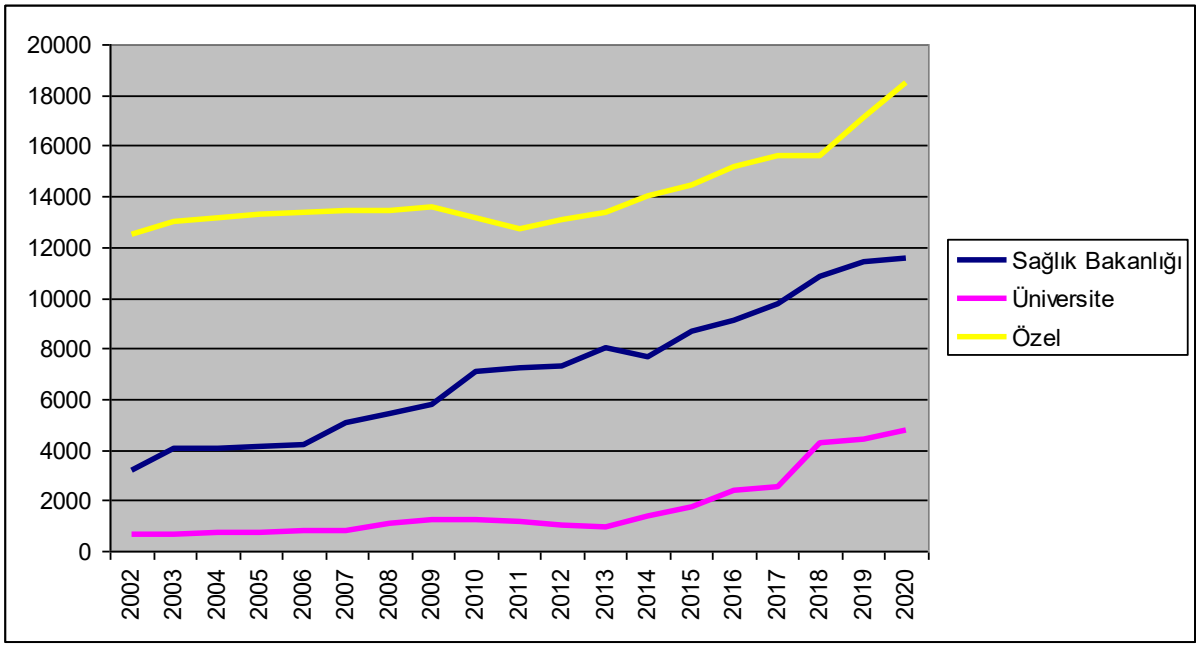
YIL	DEVLET ÜNİVERSİTESİ	VAKIF ÜNİVERSİTESİ
1908-1959	1	-
1960-1969	4	-
1970-1979	2	-
1980-1989	2	-
1990-1999	5	2
2000-2009	10	3
2010-2019	42	21
2020-2022	9	3
TOPLAM	**75**	**29**

Kaynak: Türkiye Diş Hekimleri Birliği, https://www.tdb.org.tr/sag_menu_goster.php?Id=84, Erişim: 22.09.2022

According to the data in Table 1, it is seen that the rate of increase in dentistry faculties, whose number increased rapidly between 2010 and 2019 with the effect of global health tourism, decreased after the pandemic. Although the faculties of dentistry and the process of training dentists have high economic costs and returns, due to the serious decrease in the number of international students after the pandemic, as well as the serious declines in dental health, like all other fields in global health tourism, new It is observed that the increase in the number of dental faculties has decreased significantly.

In addition to the training given at universities, there have been some changes in the employment characteristics in the sector after the pandemic. According to the 2022 research conducted by the Turkish Statistical Institute (TUIK), the distribution of the institutions where dentists work over the years is given in Figure 5.

Figure 5. According to the 2022 research conducted by the Turkish Statistical Institute (TUIK), the distribution of the institutions where dentists work depending on the years



Kaynak: TÜİK hane halkı araştırması, https://biruni.tuik.gov.tr/medas/?kn=149&locale=tr, Erişim: 26.09.2022

According to the data in the figure, after 2019, employment rates in both state and university decreased, while private employment increased. In fact, while the main reason for the increase in the number of people employed in the private sector after the pandemic is the decrease in the employment of dentists in public and universities, it is possible to state that there has been a serious decrease in the number of physicians going abroad due to the effect of the pandemic.

In general, the most important changes in oral health and maxillofacial surgery after the pandemic are the economic dimension of oral and dental health and the changes in the number of patients. Although health services are a constitutional and human right that individuals have in the global sense, private health services are provided in parallel with public or social health services due to insufficient or insufficient quality of public health services. Oral and dental health, on the other hand, is the area in which the private sector tends the most in terms of both the treatment and the time to reach the doctor, and the quality of the service and materials provided in public institutions. Therefore, the number of patients is of vital importance in oral and dental health services. It can be stated that the most important disruption experienced in the pandemic process and economic reasons come first in the field of oral and dental health, as in all other areas.

The situation is even worse in terms of maxillofacial surgery, and it is seen that foreign dependency in terms of materials is more effective here. In addition, serious problems have been experienced in jaw surgery due to the unlimited or insufficient interest and public support in the country, which is directed to the fields related to the pandemic. In addition to the problems of finding patients, important economic problems have been experienced due to the fact that the materials used in maxillofacial surgery are dependent on the exchange rate, the exchange rate has a sharp fluctuation, and the production has decreased all over the world due to the pandemic.

Conclusion

Although oral health is a health field that is important in terms of nutrition, good looks and acceptance in the society, it is considered within the scope of secondary health services due to the deficiencies in other health areas with high mortality rates. The company does not guarantee oral health. In fact, although the reasons for this seem to be the ones listed above, basically the most important reason for this is the cost and financial return calculations. In particular, the fact that insurance companies do not offer packages to secure dental health services within the scope of complementary health expresses the economic aspect of the situation more clearly.

At the beginning of the economic reasons, the high level of foreign dependency in terms of equipment and vehicles in the health services provided in the field of dentistry and oral health, the absence of an economic exchange rate in the country, and therefore, the foreign dependency and low predictability of each service in oral health come to the fore. Due to the unbalanced increase in exchange rates, sectors are faced with the tables that may cause harm, let alone providing income, in the ongoing oral health services. For this reason, in countries with a high level of external dependence in terms of materials and equipment, oral health services are consistently seen as elective or secondary health care services.

Turkey has overcome this negative situation or disadvantage with its qualified workforce and has become a country that provides the highest quality service at the most affordable prices in terms of oral and dental health in global markets. In this process, the low profit margin of the patients in the domestic market or the quality of health services that may be of relatively lower quality have increased as a result of the services provided by dentists and surgeons from outside. However, with the pandemic, the situation regarding oral and dental health in health tourism and global markets has been reversed. In this process, dentists and surgeons had serious economic problems, the costs, especially materials and rents, were at very high levels, therefore, these gains of the dentists, who had a very important place in the world and showed serious individual efforts, almost melted away after the pandemic. Although oral health services have increased significantly in the domestic market, it is possible to state that the main and hard blow comes from global health tourism, which has a high economic impact.

In fact, while the operations in terms of oral and dental health and especially jaw surgery continue locally in developed countries, the whole process from operations performed in jaw surgery to minor procedures has been seriously disrupted in Turkey. After the Covid-19 pandemic, even weddings, mass celebrations or ceremonies were considered obligatory and not considered elective, while even very serious procedures in jaw surgery that adversely affected health were seen as elective.

While the expected effect of the pandemic in other health fields is the formation of a health awareness and individuals to pay more attention to their health, it has brought serious economic difficulties within the framework of those who provide these services in oral and maxillofacial surgery. National action plans are needed for the continuation of the achievements of all parties working in the field of oral health, especially dentists and surgeons, with their individual efforts. The pandemic process is actually important in oral and dental health, as in any other field, in terms of revealing how negative results can be caused by external dependence in terms of materials and equipment. Again, the post-pandemic events show how fragile and in need of encouragement oral and maxillofacial surgery is economically. Oral and especially maxillofacial surgery should be strengthened and necessary support should be given, both in terms of directly affecting the health and quality of life of individuals and in terms of bringing foreign currency directly to the country from foreign markets in economic terms.

References

Buchy P, Buisson Y, Cintra O, et al. (2021). COVID-19 pandemic: lessons learned from more than a century of pandemics and current vaccine development for pandemic control. *Int J Infect Dis*. 112:300-317. doi:10.1016/j.ijid.2021.09.045

Carbone M, Lednicky J, Xiao SY, Venditti M, Bucci E. (2021). Coronavirus 2019 Infectious Disease Epidemic: Where We Are, What Can Be Done and Hope For. *J Thorac Oncol*. 16(4):546-571. doi:10.1016/j.jtho.2020.12.014

Chavda VP, Kapadia C, Soni S, et al. (2022). A global picture: therapeutic perspectives for COVID-19. *Immunotherapy*, 14(5):351-371. doi:10.2217/imt-2021-0168

da Silva SJR, do Nascimento JCF, Germano Mendes RP, et al. (2022). Two Years into the COVID-19 Pandemic: Lessons Learned. *ACS Infect Dis*. 8(9):1758-1814. doi:10.1021/acsinfecdis.2c00204

Emrani J, Ahmed M, Jeffers-Francis L, et al. (2021). SARS-COV-2, infection, transmission, transcription, translation, proteins, and treatment: A review. *Int J Biol Macromol*. 193(Pt B):1249-1273. doi:10.1016/j.ijbiomac.2021.10.172

Gill SA, Quinonez RB, Deutchman M, et al. Integrating Oral Health into Health Professions School Curricula. *Med Educ Online*. 2022;27(1):2090308. doi:10.1080/10872981.2022.2090308

Imai K, Tanaka H. (2021). SARS-CoV-2 Infection and Significance of Oral Health Management in the Era of "the New Normal with COVID-19". *Int J Mol Sci*. 22(12):6527. Published 2021 Jun 18. doi:10.3390/ijms22126527

Kuzu, Ö. F. (2019). Türkiye'deki Ağız ve Diş Sağlığı Politikalarının İncelenmesi ve Yeni Model Önerisi: Bir Kamu Hastanesi Örneği, Sivas Cumhuriyet Üniversitesi Sosyal Bilimler Enstitüsü Sağlık Kuruluşları Yöneticiliği Ana Bilim Dalı, Yüksek Lisans Tezi.

Mallah SI, Ghorab OK, Al-Salmi S, et al. (2021). COVID-19: breaking down a global health crisis. *Ann Clin Microbiol Antimicrob*. 20(1):35. Published 2021 May 18. doi:10.1186/s12941-021-00438-7

Murugan C, Ramamoorthy S, Kuppuswamy G, Murugan RK, Sivalingam Y, Sundaramurthy A. (2021). COVID-19: A review of newly formed viral clades, pathophysiology, therapeutic strategies and current vaccination tasks. *Int J Biol Macromol*. 193(Pt B):1165-1200. doi:10.1016/j.ijbiomac.2021.10.144

Peker K, Ak G, Onur OD, Isler S, Acikgoz MM. (2021). The impacts of the Covid-19 pandemic on dental public health and ethical issues. *Sağlık Bilimlerinde İleri Araştırmalar Dergisi,* 4(Suppl.1): S83-S95. https://doi.org/10.26650/JARHS2021-945653

Petersen PE, Baez RJ, Ogawa H. (2020). Global application of oral disease prevention and health promotion as measured 10 years after the 2007 World Health Assembly statement on oral health. *Community Dent Oral Epidemiol*. 48(4):338-348. doi:10.1111/cdoe.12538

Poirier BF, Hedges J, Smithers LG, Moskos M, Jamieson LM. (2022). Child-, Family-, and Community-Level Facilitators for Promoting Oral Health Practices among Indigenous Children. *Int J Environ Res Public Health*. 19(3):1150. Published 2022 Jan 20. doi:10.3390/ijerph19031150

Poudel P, Griffiths R, Arora A, et al. (2021). Oral Health Status, Knowledge, and Behaviours of People with Diabetes in Sydney, Australia. *Int J Environ Res Public Health*. 18(7):3464. Published 2021 Mar 26. doi:10.3390/ijerph18073464

Sharma A, Ahmad Farouk I, Lal SK. (2021). COVID-19: A Review on the Novel Coronavirus Disease Evolution, Transmission, Detection, Control and Prevention. *Viruses*. 13(2):202. Published 2021 Jan 29. doi:10.3390/v13020202

Tynan A, Deeth L, McKenzie D. (2018). An integrated oral health program for rural residential aged care facilities: a mixed methods comparative study. *BMC Health Serv Res*. 18(1):515. Published 2018 Jul 3. doi:10.1186/s12913-018-3321-5

Investigation Of Growth Differentiation Factor-15 (Gdf-15), Paraoxonase And Arylesterase Levels In The Pericardial Fluid Of Patients Undergoing Cardiac Surgery

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Introduction

The pericardium is a double-layered fibro-serous layer that covers the surface of the heart and contains the pericardial fluid. Pericardium and pericardial fluid form a closed homeostatic field that allows the heart to function easily. Interventions and illnesses may cause deterioration of the homeostatic environment and accompanying mechanical stress (1). Pericardial fluid contains growth factors, cardiac hormones and cytokines(2). Growth differentiation factor-15 (GDF-15), known as macrophage inhibitory peptide, is in the transforming growth factor β (TGF-β) superfamily. It is synthesized in response to conditions such as oxidative stress, inflammation, injury, and tissue hypoxia (3). It has been reported that it is seriously elevated in diabetes, kidney dysfunction etc.cases (4). Despite the high level of GDF-15 in patients with heart failure, the synthesis site of GDF-15 could not be fully explained because it is not seen in cardiomyocytes (5). Deaths not related to cardiovascular diseases (CVD) and CVD are associated with elevated GDF-15 levels(3). Therefore, the function of GDF-15 has not been fully elucidated (6).

Paraoxonase 1 (PON1) is a glycoprotein grouped as the aryldialkylphosphatase enzyme (7). It has anti-oxidant and anti-atherogenic effects (8). In addition, it is an enzyme that has arylesterase and lactonase functions and has the ability to hydrolyze many substrates such as glucuronides, organophosphate compounds, arylesters, and cyclic thiolactone carbonates (9). With this function, it hydrolyzes oxidized lipids from low-concentration lipoproteins (LDL) (10). Also, PON1 is associated with its anti-inflammatory and anti-oxidative properties among the subgroups of high-density lipoprotein (HDL) (8).

Examining the levels of GDF-15and antioxidant enzymes paraoxonase and arylesterase which were previously studied only at serum level in many different disease prognoses such as Pulmonary Embolism, Heart Failure, Thalassemia Major, Acute Pulmonary Proembolism, Ovarian Surface Epithelial Malignant Tumor, Left Ventricular Systolic Function Disorder, will shed light on the investigation of cardiovascular diseases.

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Material and Method

The study was initiated with the approval of the Ethics Committee numbered 16/03/38. Pericardial fluid samples were taken from the patients who underwent surgery during the opening of the pericardium after sternotomy.

Patient Population and Procedures

In this study, 40 (28 Male + 12 Female) patients who underwent open heart surgery due to various cardiovascular diseases in the Cardiovascular Surgery operating rooms of Harran University Faculty of Medicine and Mehmet Akif Inan Training and Research Hospital were included. The age of these patients was a minimum of 38 and a maximum of 85, and the mean age was 60.97±8.79 years.

Average weight and height of the patients, respectively; while it is 86.23±9.77 kg, 173.5±4.79 cm in men, it is 73.50±5.61 kg, 158.5±2.16 cm in women. 97.5% of all patients had coronary bypass surgery. All of the patients reported that they smoked cigarettes. In addition, 21 of all patients had diabetes mellitus, 26 had hypertension and did not have any other diseases.

Patients with left ventricular ejection fraction <30%, active infection, systemic inflammatory disease, and chronic lung and kidney disease were excluded from the study.

Obtaining Pericardial Fluid

In patients undergoing open heart surgery, after mediansternotomy with standard cardiopulmonary bypass procedures, the pericardium was opened and pericardial fluid was aspirated (at least 2 ml) with a sterile syringe. Bloody pericardial fluid samples were excluded from the study. Pericardial fluid, taken with a sterile syringe, was transferred to a sterile gel-free tube and transported to the laboratory in a cold environment. Then, the pericardial fluid in the sterile tube was centrifuged at 1000 g at + 4 °C for 20 minutes and the supernatant was taken into a sterile eppendorf tube and stored at -80 ^{0}C to be studied.

Study of Pericardial Fluid Samples

Before the study, 40 samples belonging to the patients, which were removed from –80 °C, were brought to room temperature and expected to dissolve. It was again passed through a second centrifuge (1000 g + 4 °C 20 min) in order to avoid the particles in it. The study was started by transferring the supernatant part on the eppendorf tube to another eppendorf tube.

Measuring the Amount of GDF 15

Elabscience Human GDF-15 (GrowthDifferentiationFactor 15) Eliza Kit was used for this study. This eliza kit is suitable for measuring serum, plasma and other biological fluids, and its minimum detectable dose is 14.06 pg/mL.

Paraoxonase Enzyme Activity Measurement

Paraoxonase activity, a lipophilic, hydrophobic, antioxidant enzyme linked to HDL-Cholesterol, was measured using a commercial RelAssay kit. In the method, the paraoxonasenzyme paraoxon (O,O-diethyl-O-pnitrophenylphosphate) hydrolyzes its substrate, resulting in the formation of a colored pnitrophenol product. The absorbance of the formed product was monitored in the kinetic mode at 412 nm, and the enzyme activity was expressed as U/L (11).

Arylesterase Activity Measurement

Arylesterase activity of paraoxonase enzyme, an antioxidant enzyme, was also measured using a commercial RelAssay kit. This test is based on the colorimetric measurement of phenol

released from the phenylacetate substrate by enzymatic activity. The results were expressed as kU/L because the enzyme activity was at very high levels(12).

Total Oxidant Status Level Measurement

The total oxidant status (TOS) level of the samples was measured using commercial kits. In the measurement, a colorimetric method based on the cumulative oxidation of ferrous ion to ferric ion by the oxidant molecules contained in the samples was used. Results were expressed as μmol H2O2 Equivalent/L (13).

Measurement of Total Antioxidant Status

Total antioxidant level (TAS) level of the samples was measured using commercial kits. The measurement method is based on the reduction of the colored ABTS* cationic radical by all antioxidant molecules in the sample, resulting in decolorization of the colored radical in proportion to the total concentrations of the antioxidant molecules. Trolox, a water-soluble analogue of vitamin E, is used as the calibrator. Results were expressed as mmol TroloxEquivalent/L (14).

Oxidative Stress Index Measurement

Oxidative Stress Index (OSI), which is shown as an indicator of Oxidative Stress, is expressed as a percentage of the ratio of Total Oxidative Status/Level (TOS) to Total Antioxidant Status/Level (TAS). When calculating the Oxidative Stress Index (OSI) of the samples, the TAS levels are multiplied by 10 to equalize the TOS levels and the units. The results were expressed as ArbutraryUnits (AU) (15).

$$OSİ = \frac{\text{TOS, μmol H2O2 Equiv. / L.}}{\text{TAS, μmol TroloxEquiv. / L.}} \text{ X } 100$$

Statistical analysis

Statistical analyzes were performed using the SPSS Version 17 (SPSS Inc. Chicago USA) computer program. The significance of the difference between the means of the groups was compared with the One-WayANOVA test. The relationship between the parameters was investigated by Pearson Correlation Analysis. Values less than ($p< 0.05$) were considered statistically significant.

RESULTS

GDF-15, Paraoxonase, Arelisterase, TAS, TOS and OSI values are shown in Table-I.

The lowest value of GDF-15 in the pericardial fluid of the patients was 13.57 pq/mL, the highest value was 27.45 pq/mL, and the mean was 18.21 pq/mL. Considering the paraoxonase values, the lowest value was 21 U/L, the highest value was 100 U/L, and the mean was 67.91 U/L. Arelisterase values were measured as a minimum of 321 U/L, a maximum of 399 U/L, and an average of 368 U/L.

GDF-15, Paraoxonase, Arelisterase, TAS, TOS and OSI correlation data are shown in Table-II.

When correlation analyzes were examined, a positive correlation was observed between GDF-15 and paraoxonase values ($r=0.101$), but no statistical significance was observed ($p=0.673$) (Figure-1. Correlation between GDF-15 and PON1 Levels). A negative correlation was observed

between GDF 15 and arelisterase (r = -0.017), no significant statistical finding was obtained (p=0.945) (Figure-2. Correlation between GDF-15 and Arylesterase Levels). A positive correlation was observed between paraoxonase and arelisterase (r=0.039), no statistically significant finding was obtained (p=0.871).

When the correlations of GDF-15, Arylesterase, Paraoxonase values and TAS, TOS and OSI values were evaluated, no significant statistical data could be obtained for all parameters (P>0.05)

DISCUSSION AND CONCLUSION

In recent studies, pericardial fluid concentrations for different cardiovascular diseases make important contributions to the detection of many pathophysiological mechanisms. Pericardial fluid contains growth factors, cardiac hormones and cytokines (2). Known as a macrophage inhibitor peptide, GDF-15 is synthesized in response to conditions such as oxidative stress, inflammation, injury, and tissue hypoxia (3).

Meloux et al. performed cerebral embolization in wistar male rats by microcircular intervention of the left or right internal carotid artery. They evaluated stroke lesions and cardiac function. They found that there was an increase in the levels of GDF-15 and catecholamines immediately after the stroke. They showed that GDF-15 levels were significantly increased in rats with ischemic stroke (16). In addition, they thought that GDF-15 gene polymorphism was one of the strong factors in the progression of ischemic stroke, as the stroke affected large arteries and the level of GDF-15 increased further (17)

Serum samples were obtained at each endomyocardial biopsy before and after heart transplantation by Tokavanich et al. As a result of the examinations, they observed that the level of GDF-15 increased significantly in patients with heart failure, and the level of GDF-15 decreased after transplantation. It has been stated that GDF-15 follow-up may be useful in distinguishing patients with a high risk after transplantation and may contribute to risk stratification in terms of both primary graft dysfunction and the possibility of death in these patients (18). Rothenbacher et al. investigated the relevance of baseline GDF-15 levels to overall mortality in an eight-year review of 1,470 older adults. According to the results; They explained that there is a correlation between GDF-15 levels and all factors that can cause death(19). Wollert et al. explained that, GDF-15 is associated with mortality in heart failure with atrial fibrillation. As a result of the study, it has been proven that GDF-15 levels in serum have a strong correlation with the occurrence, progression and risk prediction of CVD in the general population and patient groups(3).

HDL are high-density lipoproteins. Density,. consists of subgroups that differ in terms of lipid diversity, protein content, etc .It has many functions such as prevention of inflammation, controlling the production of adhesion molecules that cause great vascular integrity, and apoptosis. It also shows anti-atherosclerotic properties by maintaining endothelial barrier activations (20). PON-1(8), which has anti-oxidant and anti-atherogenic effects, is strongly dependent on HDL (8). Sun et al. found that those without coronary artery disease (CAD) had higher arylesterase functions of PON-1 than those with CAD (21). Zuin et al. proved that PON-1 arylesterase level is decreased in patients with CAD (22). Murillo-González et al. examined serum PON-1 activity and the relationship of its polymorphisms with CVD using four different substrates and explained that it can be a guiding biomarker in the progression of CVDs(23)., Didas et al. explained that attenuation of arylesterase ,an enzyme in the esterase family(24), activity may increase the likelihood of developing chronic kidney disease (CKD) in type 2 diabetes patients (T2DM) and that it can be used as a biomarker to predict the development of CKD in T2DM (25).

In our study, the level of GDF-15, paraoxonase and arylesterase, filtered into the pericardial fluid in patients undergoing open heart surgery, was determined in cardiovascular diseases and the relationship between these parameters and the pathophysiology of the disease was investigated. For this purpose, it is important that we use the pericardial fluid of patients during open heart surgery. Because, according to many studies, the concentrations of some substances in the pericardial fluid are of great importance for the diagnosis or prediction of pericardial or non-pericardial disorders (2).

Although there was no statistical difference between GDF-15 levels and arelisterase levels, a negative correlation was observed. When paraoxonase and arelisterase are examined, a positive correlation relationship is observed even though it is not statistically significant. This situation stands out as an expected situation. GDF-15 increases in conditions due to oxidative stress, on the contrary, PON-1 and arylesterase activity are observed. However, when the correlation analyzes of OSI values were evaluated in the study, the opposite results were obtained. There have been studies on GDF-15, paraoxonase and arylesterase levels in many different disease prognoses such as pulmonary embolism, heart failure, thalassemia major, acute pulmonary proembolism, surface epithelial malignant tumor of the ovary, left ventricular systolic dysfunction, only at serum level. Although there are no contradictory results and significant statistical results, the level of GDF-15, antioxidant enzymes paraoxonase and arylesterase, in the pericardial fluid, is one of the pioneering studies in the literature as far as we know, and it will be a pioneer for future studies.

Limitations of the Study

These limitations are the lack of standard methodology due to the fact that the study was conducted for the first time, the small number of patients participating in the study, the sensitive pericardial fluid taken, being adversely affected by environmental conditions, and the absence of a control group.

CONCLUSION

In our study, GDF-15, paraoxonase and arylesterase in pericardial fluid and the correlation between them were measured. As it is the first in the literature, we believe that it will shed light on future studies. For this reason, there is a need for studies with larger series, multicenter, prospective, controlled and standardized methodology regarding the parameters to be investigated in pericardial fluid.

REFERENCES:

1- Fatehi Hassanabad A, Zarzycki A, Deniset JF, et al. An overview of human pericardial space and pericardial fluid. CardiovascPathol. 2021 Jul-Aug;53:107346.

2- Trindade F, Vitorino R, Leite-Moreira A, et al. Pericardial fluid: an underrated molecular library of heart conditions and a potential vehicle for cardiac therapy. Basic ResCardiol. 2019;114:10.

3- Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. ClinChem. 2017 Jan;63(1):140-151.

4- Buendgens L, Yagmur E, Bruensing J, et al. Growth differentiation factor-15 is a predictor of mortality in critically illpatients with sepsis. DisMarkers 2017; 2017: 5271203.

5- Ahmad T, Wang T, O'Brien EC, et al. Effects of left ventricular assist device support on biomarkers of cardiovascularstress, fibrosis, fluid homeostasis, inflammation, and renal injury. JACC Heart Fail 2015; 3: 30–39.

6- Tuegel C, Katz R, Alam M, et al. GDF-15, Galectin 3, Soluble ST2, and Risk of Mortality and Cardiovascular Events in CKD. Am J KidneyDis. 2018 Oct;72(4):519-528.

7- Mahrooz A, Mackness M, Bagheri A, et al. The epigenetic regulation of paraoxonase 1 (PON1) as an important enzyme in HDL function: the missing link between environmental and genetic regulation. Clin. Biochem. 2019;73:1–10.

8- Furlong CE, Marsillach J, Jarvik GP, et al. Paraoxonases-1, -2 and -3: what are their functions? Chem. Biol. Interact. 2016;259:51–62.

9- Tajbakhsh A, Rezaee M, Rivandi M, et al. Paraoxonase 1 (PON1) andstroke; the dilemma of genetic variation. Clin. Biochem. 2017;50(18):1298–1305.

10- Kuppan K, Mohanlal J, Mohammad AM, et al. Elevated serum OxLDL is associated with progression of type 2 Diabetes Mellitus to diabetic retinopathy. Exp. EyeRes. 2019;186:107668.

11- Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet. 1983. 35, 1126- 1138.

12- Haagen L, Brock A. A new automated method for phenotyping arylesterase (EC 3.1.1.2) based upon inhibition of enzymatic hydrolysis of 4-nitro-phenyl acetatebyphenylacetate. Eur J ClinChemClinBiochem. 1992 Jul;30(7):391-5.

13- Erel O. A new automated colorimetric method for measuring total oxidant status. ClinBiochem, 38 (12), 2005, 1103-1111.

14- Erel O. A novel automated direct measurement method for total antioxidant capacit yusing a new generation, morestable ABTS radicalcation. ClinicalBiochemistry. 37 (2004) 277–285.

15- Aycicek A, Varma M, Ahmet K, et al. Maternal active or passive smoking causes oxidative stress in placental tissue. Eur J Pediatr. 2011 May;170(5):645-51.

16- Meloux A, Rigal E, Rochette L, et al Ischemic Stroke Increases Heart Vulnerability to Ischemia-Reperfusion and Alters Myocardial Cardioprotective Pathways. Stroke. 2018 Nov;49(11):2752-2760.

17- Xiang Y, Zhang T, Guo J, et al.. The association of growth differentiation factor-15 gene polymorphisms with growth differentiation factor-15 serum levels and risk of ischemic stroke.JStrokeCerebrovascDis. 2017; 26:2111–2119.

18- Tokavanich N, Sinphurmsukskul S, Kongruttanachok N, et al. Circulating growth differentiation factor-15 as a novel biomarker in heart transplant. ESC Heart Fail. 2021 Aug;8(4):3279-3285.

19- Rothenbacher D, Dallmeier D, Christow H, et al. Association of growth differentiation factor 15 withotherkey biomarkers, functional parameters and mortality in community-dwellingolderadults. Age Ageing. 2019 Jul 1;48(4):541-546.

20- Linton MF, Yancey PG, Davies SS, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. Endotext. MDText.com, 2019, Inc.; South Dartmouth, MA, USA.

21- Sun T, Hu J, Yin Z, et al. Low serum paraoxonase1 activity levels predict coronary artery disease severity. Oncotarget. 2017;8(12):19443–19454.

22- Zuin M, Trentini A, Marsillach J, et al. Paraoxonase-1 (PON-1) Arylesterase Activity Levels in Patients with Coronary Artery Disease: A Meta-Analysis. DisMarkers. 2022 Mar 10;2022:4264314.

23- Murillo-González FE, Ponce-Ruiz N, Rojas-García AE, - et al. PON1 lactonase activity and its association with cardiovascular disease. ClinChimActa. 2020 Jan;500:47-53.

24- Özgün GS, Özgün E, Eskiocak S, et al. Diyabetik Sıçanlarda Taurinin Paraoksonaz, Arilesteraz ve Laktonaz Aktivitelerine Etkileri. Türk Klinik Biyokimya Derg 2016;14(3):157-165.

25- Didas N, Thitisopee W, Porntadavity S, et al.Arylesterase activity but not PCSK9 levels is associated with chronic kidney disease in type 2 diabetes. IntUrolNephrol. 2020 Sep;52(9):1725-1732.

Table I. GDF-15, Paraoxonase, Arelisterase, TAS, TOS and OSI values ,

	Min	Max	Mean	Std. Deviation
Pericard_Gdf15	13,57	27,45	18,2170	4,17715
Paraoxonase	21,00	100,00	67,9100	19,72111
Arelisterase	321,00	399,00	368,5200	17,78363
TAS	,90	1,68	1,3340	,17901
TOS	5,70	19,00	14,5875	2,96044
OSİ	,38	1,67	1,1112	,25381

Table II. GDF 15, Paraoxonase, Arylesterase, TAS, TOS and OSI Correlation Table

	TAS	TOS	OSİ	PON1	ARE	GDF_15
TAS r	1	,11-5	-,544*	-,032	-,282	-,025
p		,628	,013	,893	,228	,918
TOS r	,115	1	,755**	-,178	-,242	-,143
p	,628		,000	,453	,303	,549
OSİ r	-,544*	,755**	1	-,104	,009	-,071
p	,013	,000		,663	,969	,767
PON1r	-,032	-,178	-,104	1	,039	,101
p	,893	,453	,663		,871	,673
AREr	-,282	-,242	,009	,039	1	-,017
p	,228	,303	,969	,871		,945
GDF_15r	,025	-,143	-,071	,101	-,017	1
p	,918	,549	,767	,673	,945	

PON1: Paraoxonase, ARE: Arelisterase, * The correlation is significant at the 0.05 level, ** The correlation is significant at the 0.01 level.

The Relationship Of Nitric Oxide With Cardiovascular Diseases And Pulmonary Hypertension

Mehmet ÖZDİN[1]

Introduction

Cardiovascular diseases (CVD) are among the most important health problems affecting public health in the world. Atherosclerosis forms the basis of CVDs. Nutrition, environmental and genetic factors play an significant role in the formation. It is formed by the accumulation of fat and tissue residues under the cells lining the vein, which is the inner most layer of the vein. In this way, it progresses with stenosis or obstruction in the lumen of the vessel and causes disruption of tissue blood supply. Atherosclerosis is a multifactorial disease characterized by thickening of the vessel wall and loss of vessel elasticity due to this thickening (İlikay & et al., 2016) (Özdin, 2021a, 2021b). Atherosclerosis is the leading cause of death worldwide. One third of deaths in the world are due to these atherosclerotic diseases (Çoban, Erginel & Tuna, 2014). More than 600,000 people die annually from CVD in the United States (Mangge & et al., 2014). Various metabolic, inflammatory, infectious or hemodynamic factors are involved in the development of atherosclerosis, plaque formation and activation. Vascular endothelial dysfunction is significant in the pathogenesis of atherosclerosis causing coronary heart disease (CHD). Vascular endothelial dysfunction, which develops when endothelial cells cannot fulfill their basic functions such as vascular homeostasis and nitric oxide (NO) production, causes atherosclerotic plaque formation. Despite the complexity of CHD in its etiological, pathophysiological, clinical and epidemiological developments, it is of great importance to understand its formation mechanisms, since atherosclerosis is a preventable condition. Atherosclerotic lesions begin at an early age, aging poses a risk for CVD. Middle-aged men under 60 years of age have a 2 to 5 times greater risk of CHD than women, while the gender-dependent risk decreases with age. Family history affects 40% of the total risk profile. Other major risk factors for atherosclerosis include hypertension, type II diabetes mellitus (type II DM), elevated blood cholesterol levels, sedentary life and obesity (Özdin, 2003). (Güray, 1997).

NO was discovered in the 1970s. Robert F. Aorta drawing attention to acetylcholine, which plays a role in contraction, indicate that it is guanylate cyclase in the cytoplasm that causes relaxation. Endotel stressed that the relaxation factor is NO. It was determined to be a NO producing compound (Furchgott & Zawadski, 1990). NO is a very important molecule produced in the body by the nitric oxide synthetase (NOS) enzyme from L-arginine, a semi-essential amino acid. It must also be taken from outside. In this context, for a healthy life, attention should be paid to foods containing L-arginine and L-citrulline amino acids. The most important point to be considered here is to express the importance of natural NO production (Sarı, 2020). In this study,

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it was aimed to research the relationship of nitric oxide with CVD and pulmonary hypertension (PH).

Nitric Oxide Biosynthesis

Physiological stimuli such as acetylcholine, glutamate, bradykinin, adenosine diphosphate (ADP) activate nitric oxide synthetase (NOS) in endothelial cells. When this enzyme, which is dependent on Calcium-Calmodulin, is activated, it provides the conversion of L-Arginine and oxygen to L-Citrulline and NO by reacting. Thanks to the discovery and use of L-Arginine analogs that competitively inhibit the NOS enzyme, it has been possible to investigate the wide biological role of NO (Moncada & Higgs, 1993). NO formed as a result of this reaction diffuses into the vascular smooth muscle cells. NO actuates the enzyme by fixating to the heme group of the guanylate cyclase enzyme. The activated guanylate cyclase enzyme converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Causes muscle relaxation in cGMP. NO, reacts with heme-containing proteins, especially hemoglobin, and is converted to nitrate (NO_3-) and excreted in the urine (Edwards, 1995). Nitric oxide is a colorless gas and is very stable in the absence of oxygen. However, in contact with air, it quickly reacts with oxygen and turns into nitrogen dioxide (NO_2). Nitrogen Dioxide is a toxic gas that can cause tissue damage (Borland & Higenbottam, 1989).

Nitric Oxide In Cardiovascular And Pulmonary System

The main task of the vascular endothelium is to prevent the adhesion and aggregation of platelets and other blood cells. At the same time, it is to ensure the continuity of circulation by providing adequate blood flow to the blood vessels. Vascular ebdotel synthesizes and secretes NO in order to exert this effect. Systemic use of NO synthesis inhibitors enhancements blood pressure in small arteries and arterioles (Anggard, 1994). This indicates the importance of nitric oxide made in the vascular endothelium in regulating blood pressure and blood flow. As a matter of fact, any functional disorder in the endothelium (diabetes mellitus, homocystinemia, familial hyperlipidemia) or acquired (smoking, sedentary life, atherogenic diet) causes hypertension (Anggard, 1994, Vane, Anggard & Botting 1990). Atherosclerosis, familial hypercholesterolemia, and reduction in coronary blood flow in smokers cannot be increased by administration of acetylcholine. This is because the dilator response of endothelium to acetylcholine is decreased (Cox & et al., 1989, Celermajer & et al., 1992). Endothelium-dependent dilatation is reduced in essential hypertension and experimentally induced hypertension in animals, with clear evidence that this is due to an aberrance in the L-Arginine-NO system (Anggard, 1994). Persistent pulmonary hypertension (PPH) occurs either primary or secondary. Secondarily, it may occur due to respiratory system disorders such as perinatal asphyxia, meconium aspiration and severe respiratory distress syndrome. It may also occur due to sepsis, diaphragmatic hernia, congenital heart disease and polycythemia (Greenough, Morley & Roberton 1992). Although its exact occurrence is not known, it has been shown that increased endogenous NO activity at birth contributes to the normally observed decrease in pulmonary vascular resistance (Abman & et al., 1990). In PPH, the lack of resistance normally seen in the postnatal pulmonary vessels may be related to low endogenous NO production. In addition, the amino acid L-Arginine, which is the precursor of NO synthesis, may be insufficient in children with PPH (Vosatka, Kashyap & Botting 1994). Intravenous vasodilators, hyperventilation, high frequency oscillatory ventilation and extracorporeal membrane oxygenation are used as specific treatment in PPH. Intravenous vasodilators, prostacyclin and magnesium sulfate treatments cannot respond to the desired level and may cause systemic hypotension

((Vosatka, Kashyap & Botting 1994, Kaapa & et al., 1985, Abu-Osba & et al., 1992). In PH, NO selectively exerts a vasodilator effect on the pulmonary vessels. Inhaled NO reduces pulmonary vascular resistance without reducing systemic vascular resistance and cannot have a vasodilatory effect on the systemic vascular bed since it is rapidly inactivated by hemoglobin as soon as it enters the bloodstream (Abman & ark., 1992, Pepke-Zabe & et al., 1991). At the same time, methemoglobin concentration rarely rises above 1-2% during inhaled NO (INO) therapy (Kinsella & Abman, 1993a). Ventilation increases perfusion compatibility in PH. In addition, it increases cardiac output, pulmonary blood flow and oxygenation. Has all the features of an ideal pulmonary vasodilator (Rossaint & et al., 1993). They can also widen the vessels in non-ventilated lung areas, disrupting the ventilation-perfusion harmony. Because of these properties, INO is used in newborns with pulmonary hypertension, in children with congenital heart disease and respiratory distress syndrome in infants. In addition, INO has been used in adults with PH, adult respiratory distress syndrome (ARDS), and bronchopulmonary dysplasia (Kinsella & Abman, 1993b).). The most popular use of NO today is inhalation in pulmonary hypertension. Pulmonary vascular diseases are a serious complication of congenital heart diseases. Hypertensive crisis in congenital heart diseases is seen particularly after open heart surgeries. PH can overrun systemic arterial pressure. As a result, the return of blood to the left atrium is reduced. Cardiac output drops rapidly (Jones, Shore & Rigloy, 1981). Reversal of PH is very difficult and hypertensive crisis has an important place in postoperative mortality and morbidity (Hopkins, & et al., 1991). Hyperoxic hyperventilation and intravenous vasodilators are widely used in the treatment of PH (Schranz, & et al., 1992). Nitric oxide inhalation is seen as an effective alternative therapy. The use of 1 ppm (parts per million) in the treatment of pulmonary hypertensive crisis gives successful results (Sellden, & et al., 1993). The use of 20-80 ppm of INO in infants with congenital heart disease who develops PH is effective in reducing pulmonary arterial pressure (Roberts, & et al., 1993). Patients with cardiorespiratory problems need to take NO at a high oxygen concentration in order to benefit from INO sufficiently. However, since toxic NO production increases when INO is given at high oxygen concentration, NO administration and monitoring should be done carefully (Foubert, & et al., 1992, Bauchet & Renaudin, 1993).

Conclusion

Although NO, which is produced in almost all healthy tissues in the organism, is structurally simple, its activity is a very complicated mediator. NO; It provides blood pressure and vascular tone balance by vasodilation by stimulating guanylate cyclase in vascular smooth muscles. It is used by inhalation in the treatment of PH in the clinic, and it is a broad-spectrum chemical that is effective on other organs.

REFERENCES

Abman SH, Griebel JL, Parken DK, Schmidt JM, Swanton D, Kinsella JP. (1994). Effects of Inhaled Nitric Oxide in Children with Severe Hypoxemic Respiratory Failure. J Pediatr. 124:881-888.

Abman SH, Chatfied BA, Hall SL, MC Murty IF. (1990). Role of Endothelium-Derived Relaxing Factor During Transition of Pulmonary Circulation at Birth. Am J Physiol. 259:H1921-1927.

Abu-Osba YK, Galal O, Manasra K, Rejial A. (1992). Treatment of Severe Pulmonary Hypertension of the Newborn with Magnesium Sulphate. Arch Dis Child. 67:31-35.

Anggard E. (1994). Nitric Oxide: Mediator, Murderer and Medicine. The Lancet. 343:1199-1206.

Bauchet M, Renaudin V. (1993). Safety Requirements for Use of Inhaled Nitric Oxide in Neonates. Lancet 341:968

Borland CDR, Higenbottam TW. (1989). A Simultaneous Single Breath Measurement of Pulmonary Diffusing Capacity with Nitric Oxide and Carbonmonoxide. Eur Respir J. 2:56-63.

Celermajer DS, Sorensen KE, Gooch VM, et al. (1992). Noninvasive Detection of Endothelial Function in Children an Adults at Risk of Atherosclerosis. Lancet. 340:1111-1115.

Cox DA, Vita JA, Tresure CB, et al. (1989). Atherosclerosis Impairs Flow Mediated Dilation of Coronary Arteries in Human. Circulation. 80:458-465.

Çoban N, Erginel N, Tuna Ü. (2014). Ateroskleroz Gelişiminde Genetik Faktörlerin Rolü. Deneysel Tıp Araştırma Enstitüsü Dergisi. 4(7):3-12.

Edwards AD. (1995). The pharmacology of inhaled Nitric oxide. Arch Dis Child. 72:F127-130.

Foubert L, Fleming B, Latimer R, et al. (1992). Safety Guidelines for Use of Nitric Oxide. Lancet 339:1615-1616.

Furchgott RF, Zawadski JV. (1990). The Obligatory Role of Endothelial Cells in The Relaxation of Arterial Smooth Muscle by Acetylcholine. Nature, 228, 373.

Greenough A, Morley CJ, Roberton NRC. (1992). Acute respiratory disorders in the newborn. In: Roberton NRC, ed. Textbook of Neonatology. Edinburgh: Churchill Livingstone. 432-451.

Güray A, Samancı N, Ovalı F, Dağoğlu T. (1997). Nitrik Oksit: Fizyolojisi ve Klinik Önemi. T Klin J Med Sci. 17:115-119.

Hopkins RA, Bull C, Hawonth SG, De Laval MR, Stark J. (1991). Pulmonary Hypertensive Crises Following Surgery for Congenital Heart Defects in Young Children. Eur J Cardiothorac Surg 5:628-634.

İlikay S, Buğra Z, Öztürk O, Aydoğan HY. (2016). Koroner Kalp Hastalığında Endotelyal Nitrik Oksit Sentaz RS1799983 (GLU298ASP) Varyasyonunun Metabolik Etkilerinin İncelenmesi. Deneysel Tıp Dergisi. 6:12.21-31.

Jones ODH, Shore DF, Rigloy ML. (1981). The use of Tolazoline Hydrochloride as a Pulmonary Vasodilation in Potentially Fatal Episodes of Pulmonary Vasoconstriction after Cardiac Surgery in Children. Circulation 64(Suppl II):134-139.

Kaapa P, Kopp M, Ylikorkala O, Kouvalainen K. (1985). Prostacyclin in the Treatment of Neonatal Pulmonary Hlpertension. J Pediatr. 107:951-953.

Kinsella JP, Abman SH. (1993). Methaemoglobin During Nitric Oxide Therapy With High Frequency Ventilation. Lancet 342:615

Kinsella JP, Abman SH. (1993). Methaemoglobin During Nitric Oxide Therapy With High Frequency Ventilation. Lancet 342:615.

Mangge H, Becker K, Fuchs D, Gostner JM. (2014). Antioxidants, inflammation and cardiovascular disease. World J Cardiol. 26;6(6):462-77. doi: 10.4330/wjc.v6.i6.462.

Moncada S, Higgs A. (1993). The L-Arginin Nitric Oxide Pathway. N Engl J Med. 329:2002-2012.

Özdin M. (2021a). Homocysteine as a Risk Factor for Cardiovascular Diseases. Updates In Internal Sciences For. Chapter 12, 2021:251-264.

Özdin M. (2021b). Relationship of Paraoxonase With Cardiovascular Diseases. Trends In Interprofessional Care Management In Healthcare. Chapter 5:79-87.

Özdin M. (2003). Koroner Kalp Hastaları Ve Çeşitli Risk Faktörlerini Taşıyan Bireylerde Paraoksonaz 1 Aktiviteleri Ve Fenotiplerinin Araştırılması. Uzmanlık Tezi. Elazığ.

Pepke-Zabe J, Higenbottam TW, Dinhzuan AT, Stone D, Walwork J. (1991). Inhaled Nitric Oxide Causes Selective Pulmonary Vasodilation in Patients With Pulmonary Hypertension. Lancet 338:1173-1174.

Roberts JDJ, Lang P, Bigertello LM, Clahakes GJ, Zapol WM. (1993). Inhaled Nitric Oxide in Congenital Heart Disease. Circulation 87:447-453.

Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. (1993). Inhaled Nitric Oxide for the Adult Respiratory Distress Syndrome. N Engl J Med 328:399-405.

Sarı A. (2020). Nitrik Oksitin İnsan Sağlığındaki Önemi ve Üretimini Arttırıcı Moleküller. Physical Sciences (NWSAPS). 15(2):29-39.

Schranz D, Zepp F, Iversen J, Wippenman C, Huth R, Zimmen B, et al. (1992). Effects of Tolazoline and Prostacyclin on Pulmonary Hypertension in Infants after Cardiac Surgery. Crit Care Med 1420:1243-9.

Sellden H, Winbery P, Gustafsson LE, Lundell B, Book K, Fnestell CG. (1993). Inhalation of Nitric Oxide Reduced Pulmonary Hypertension after Cardiac Surgery in 3.2 kg Infant. Anesthesiology 78:577-80

Vane JR, Anggard E, Botting RM. (1990). Regulatory Functions of the Vascular Endothelium. N Engl J Med. 323:27-36.

Vosatka RJ, Kashyap S, Trifiletti RR. (1994). Arginine Deficiency Accompanies Persistent Pulmonary Hypertension of the Newborn. Biol Neonate. 66:65-70.

Phthalates and Their Toxic Effects on the Immune System

Senanur AKDEMİR[1]
Mehtap KARA[2]

Introduction

Plasticizers are added to substance and materials to increase their flexibility, workability and extensibility. Phthalates are a type of plasticizer and are also known as endocrine disrupting chemicals (EBC). Phthalates are commonly used in many products in the environment. Cosmetics, personal care products, medical devices and food packaging materials are just a few of the usage areas. The widespread and increasing use of phthalates is very worrying.

The immune system is a defense system that protects the body against external damage. It is divided into two as innate immunity (Hereditary) and acquired (Adaptive). Innate immunity gives non-specific responses while acquired immunity gives specific but slower responses. Damage to the immune system leaves the body vulnerable to pathogens.

With the increase in the use of phthalates, their toxicity on the body has come to the fore again. Studies show the toxicity of phthalates on many body systems. In addition to its endocrine disrupting properties, it has been observed that it has effects such as shortening the gestation period, increasing systolic pressure, focal testicular dysgenesis, neuronal degenerations and causing changes in immunological responses. It is thought that permanent damage remains on the baby, especially after exposure in the womb. It has been found that children are more exposed to phthalates than adults. While phthalates were used in children's toys in the past, it was noticed that children were exposed to phthalates in toys by sucking, and the use of phthalates in children playthings was banned in the following years.

When the toxicity of phthalates on the immune system was investigated, it was observed that they had positive effects on interleukin, CRP and TNFα levels. It is thought that autoimmune diseases may occur as a result of these exposures.

Exposure to phthalates is a current and important issue. Although it is not possible to completely prevent exposure due to their widespread use, its level can be reduced. Attention should be paid to the contents of the materials used, food packaging, drugs, medical devices, cosmetics and many other products. Reducing phthalate exposure is important for our health. With this compilation, it is aimed to raise people's awareness about the toxicity of phthalates to the human body and the ways of protection from phthalates.

Plasticizers

Additives that, when added to another substance, make the substance more flexible and softer are called 'Plasticizer'. This expression is mostly used for additives added to plastics (Godwin, 2017). The Council of the International Union of Pure and Applied Chemistry (IUPAC) defines plasticizer as "*A plasticizer is added to a substance and material (usually plastic or elastomer) to increase its flexibility, workability or extensibility*" (Altenhofen da Silva et al., 2011). Plasticizing technology initially

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focused on nitrocellulose type materials. In the 19th century, Alexander Parkes make a design of a new nitrocellulose plastic, using various oils to make the brittle polymer flexible. Parkes and his colleague Daniel Spill wanted to establish a plasticizer technology with this new nitrocellulose they had developed. However, they could not obtain the desired quality product with the castor oil they chose as a plasticizer (Hyatt, J.W. and Hyatt, I.S., 1870). After that, John Wesley Hyatt commercialized celluloids for the plasticizing process. In the early 1900s, phthalates and phosphates began to be used as plasticizers. Increasing interest and usage area of two important plasticizers, tricresyl phosphate and dibutyl phthalate (DBP) (Semon, W.L., 1933).

There are many classifications of plasticizers. Some of these are classifications based on chemical structures and performance. Plasticizers are divided into five according to their performance; general use, strong solvent, low temperature, low volatile and other plasticizers. Chemically, they are divided into six: phthalates, aliphatic dicarboxylic acid esters, phosphates, hydrocarbon extenders, epoxies, and others. Plasticizers can also be grouped into primary plasticizers and secondary plasticizers. Primary plasticizers are substances that are present in the plasticization system as the sole plasticizer or a high level of plasticizer. Secondary plasticizers are used in combination with primary plasticizers to improve the plasticization process, improve certain performances such as reduced flammability, or reduce costs (Godwin and Krauskopf, 2007).

There are different theories explaining the plasticization mechanism. Some of these are lubricant, gel, and free volume theories. According to the lubricant theory, the plasticizer acts as a molecular lubricant that allows the free move of polymer chains over each other if a force is applied to the polymer. According to the gel theory, the polymer molecules are loosely attached to each other at different points. The plasticizer added to the polymer increases the movement of the polymer chain (Daniels, 2009). According to the free volume theory, the free volume represents the internal volume in the polymer. As the free volume increases, the movement of the polymer chains will be easier, and thus the flexibility will increase (Cadogan, D.F. and Howick, C.J., 2000).

Plasticizer Types

Aside from having a lot of areas of use, plasticizers also have a lot of types. Some of their name and consumption rates in 2014 are given in Table 1. (Godwin, A., 2017).

Table 1. : Plasticizer types and their consumption rates in 2014 (Godwin, A., 2017).

Plasticizer Type	Consumption Rate (%)
Phthalate Esters	75
Terephthalate	7
Dibasic acid esters	3
Epoxy plasticizers	3
Trimellitate esters	2
Benzoates	2
Cyclohexanoates	<2
Polymeric Plasticizers	<2
Phosphates	1
Citrates	<1
Others	3

Annotation: The classification of plasticizers according to their chemical types and their consumption percentages in 2014 are given in Table 1. Compared to today, the biggest change in this table was seen in terephthalate plasticizers. With the prohibition of the use of some phthalate esters in children's toys, an

increase in the use of terephthalate has been observed and it is predicted that this increase will continue in the future (Bolton, D., 2013).

Immune System

The Immune System is body defense system that protects the body against diseases, foreign substances that may come from outside, and their pathogenic effects. The main function of the immune system is to recognize foreign substances and develop the necessary defense mechanisms against them (Songu and Katmış, 2012).

The immune system can be classified as innate and the adaptive immune system. Innate immunity is a non-specific defense system that is formed according to the innate characteristics of the person. It cannot distinguish the difference of pathogens. Adaptive immunity, on the other hand, is in a state of change. It responds specifically by distinguishing foreign substances and pathogens from its own cells (Songu and Katmış, 2012). The differences between the adaptive immunity and the innate immunity are given in Table 2. (Tomar, N and Rajat, K.D., 2014).

Table 2.: Plasticizer types and their consumption rates in 2014 (Godwin, A., 2017).

The Innate Immune System	**Adaptive Immune System**
Non-specific response	Specific response
Immediate response	Delay between antigen exposure and response
No immunological memory	Retains immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Annotation: The innate immune system cannot distinguish between pathogens. In contrast, the adaptive immune system specifically distinguishes pathogens but responds more slowly than innate immunity. While there is immunological memory originating from T memory cells in adaptive immunity, these cells are not found in innate immunity.

Lymphocytes, an important member of the adaptive immunity, contribute to cellular and humoral immunity. In cellular immunity, the effect of T lymphocytes and macrophages is dominant. Macrophage, one of the most effective immune cells, is activated after encountering T cell cytokines (Songu and Katmış, 2012). The innate immune system is called by this name because it provides protection against pathogens without being dependent on an external factor. The innate immune system reacts immediately when it encounters pathogens (McComb, S. et al., 2019). The main four defense barrier types of innate immunity are; anatomical (skin, mucosa), physiological (temperature changes, pH degree), phagocytic cells as monocytes, neutrophils, tissue macrophages and inflammatory (serum proteins). Cells of the innate immunity are; phagocytes, neutrophils, macrophages, natural killer (NK) cells, mast cells, basophils, dendritic cells and eosinophils. The classified version of the immune system is given in Figure 1. (Tomar, N and Rajat, K.D., 2014). Antibodies are an element of humoral immunity together with cytokines and complement system. Immune system cells can be found in the body and also have a network of organs. It is distributed throughout the body in a way that can quickly stop the spreading infection. The innate immune cells are usually formed in the bone marrow and reside in the blood and tissues. The cells of the adaptive immune system; T and B lymphocytes, mature in the thymus and bone marrow, also known as primary immune organs, and then remain in the lymphatic tissue. The accommodation sites of these lymphocytes are known as secondary immune organs. Some of these organs are; lymph nodes, spleen, appendix, tonsils, mucosa-associated lymphoid tissues, and adenoids (McComb, S. et al., 2019).

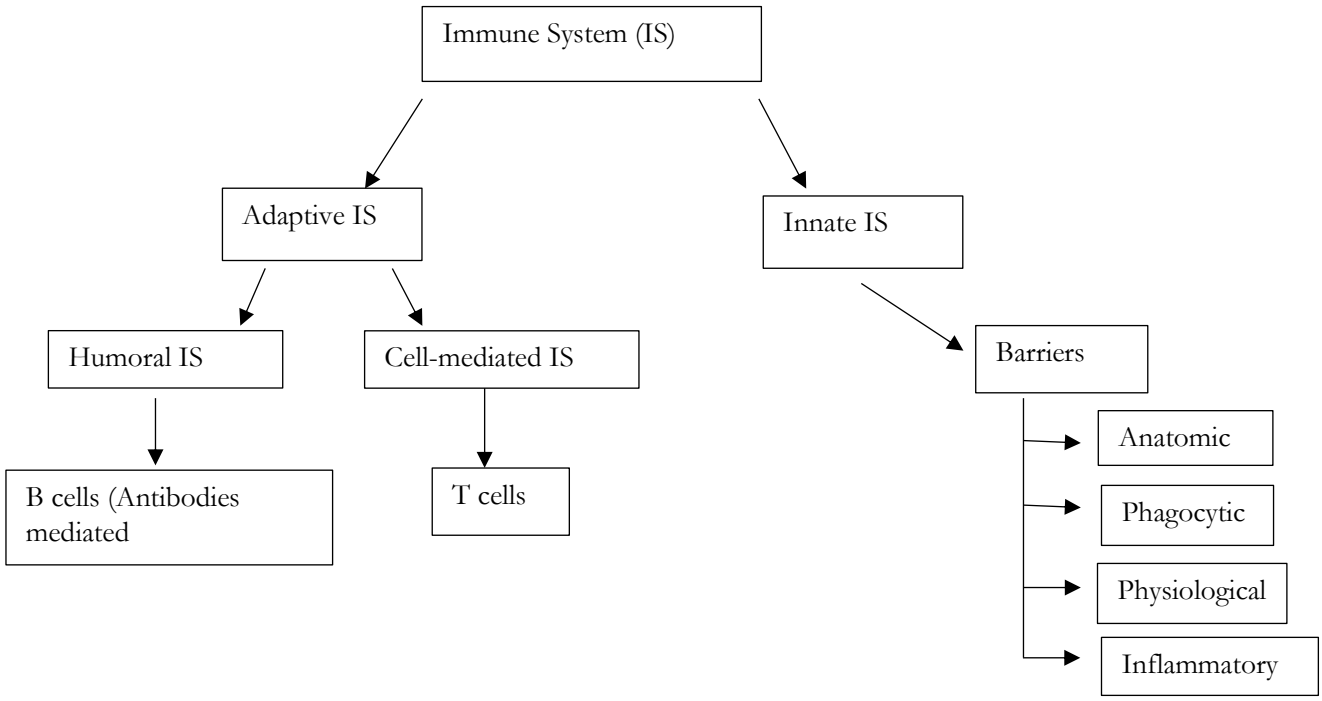

Figure 1.: Types of the Immune System (Tomar, N. and Rajat, K.D., 2014)

Phthalates

Plastics have brought enormous benefits to society since they were invented, but they have also had negative effects on the environment and human health. People are constantly exposed to plastics. Of these, the most worrying are; Phthalates are known as endocrine disrupting chemicals (EBK). Phthalates are mainly used for their softening effects in polyvinyl chloride (PVC) plastics (Wang, Y. and Qian, H., 2021). Phthalates also act as lubricants, solvents and impart positive effects to the products they are added to. There are potential routes of exposure by ingestion, inhalation, intravenous injection and skin contact (Schettler, T., 2006). While phthalate consumption is increasing, more than 3000000 metric tons of phthalates are used worldwide every year (Lyche, J.L., et al., 2009).

Phthalates are diester structured containing a benzene ring with two ester functional groups. They have low water solubility, with increasing chain length and high molecular weight. Therefore, while low molecular weight phthalates such as DMP (Dimethyl phthalate) and DBP are more soluble in water, phthalates with high molecular weight and long alkyl chains have low water solubility (Lyche, J.L., et al., 2009). Phthalates are generally lipophilic and this property affects their spreading and environmental degradation properties (Schettler, T., 2006). The general chemical structures of phthalates are shown in Figure 2 (EPA, 2012).

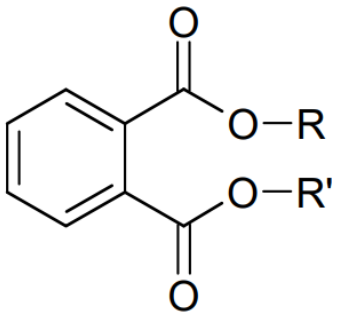

Figure 2.: General chemical structures of phthalates (EPA, 2012).

Annotation: Dialkyl ortho-phthalates (or phthalate esters) have the chemical structure shown above. R groups can be linear, branched, linear-branched or circular (EPA, 2012).

Phthalate Types

Phthalates are phthalic acid diesters and they are classified depends on molecular weight as high and low. High molecular weight phthalates include various compounds that are widely used to more flexible and durable plastics. Among them, the most widely used chemical is diethylhexyl phthalate (DEHP) (Filardi, T. et al., 2020). Phthalates such as DEHP, DBP, diethyl phthalate (DEP), di-isononyl phthalate (DiNP) and di-iso-decyl phthalate (DiDP) are mainly used to produce PVC (Wang, Y. and Qian, H. , 2021). Low molecular weight phthalates are common mainly in personal care products, cosmetics, pesticides and food packaging plastics (Filardi, T. et al., 2020). With low molecular weight short-branched phthalates such as DMP and DEP are widely produced and used in medical products and medical devices. Phthalate types and where they can be found are shown in Table 3. (Wang, Y. and Qian, H., 2021).

Table 2.: Phthalate Types and where they can be found (Wang, Y. and Qian, H., 2021)

Compounds	**Abbreviation**	**Where It Can Be Found**
Diethylhexyl phthalate	DEHP	Plasticizer
Dibutyl phthalate	DBP	Nail polishers; plasticizer; an additive to adhesives or printing inks
Diethyl phthalate	DEP	Toothbrushes; automobile parts; tools; toys; food packaging; cosmetics; insecticides; aspirin
Mono-(2- ethylhexyl) phthalate	MEHP	Vinyl tiles; food conveyor belts; carpet tile; artificial leather

Annotation: It is almost impossible to avoid exposure to phthalates, as they are ubiquitous in the environment. In addition, exposure to phthalates is increasing due to the use of plastic. Some of the phthalates we encounter in our daily lives and they can be found are given in the table above (Wang, Y. and Qian, H., 2021).

Phthalate firstly metabolized with hydrolysis after absorption into the cell, then conjugated with hydrophilic glucuronide conjugate and catalyzed via uridine 5'-diphosphoglucuronyl transferase. The type of phthalates exert their toxicological effect in the body with different mechanisms. Short-branched phthalates hydrolyzed into monoester phthalates and then excreted via urine, and long-branched phthalates metabolized as hydroxylation and oxidation, which are then excreted in urine and feces (Wang, Y. and Qian, H., 2021).

Toxic effects of Phthalates

In 2006, the National Toxicology Program (NTP) classified the effect of DEHP on the reproductive system development of one-year-old boys as 'concerning'. At the same time, the effects on the reproductive system of boys older than one year of age and exposed in the womb have been classified as 'somewhat alarming'. Some studies show that exposure to DEHP increases the risks of asthma, bronchial obstruction, ADHD and its symptoms and altered pregnancy durations. The National Research Council concluded that multiple phthalates can cause cumulative adverse effects on male reproductive system development (EPA (United States Environmental Protection Agency), 2017).

Scientific Committee on Toxicity, Ecotoxicity and the Environment has reached tolerable daily intake values (TDI) for phthalates based on NOAEL (No Observable Adverse Effect Level) levels from animal studies. They concluded that predominantly used phthalates such as DEHP and DiNP had the lowest calculated TDI values (Heudorf, U. et al., 2007). The NOAEL and TDI values of phthalates in toys are shown in Table 3. (CSTEE, 1998)

Table 3.: The NOAEL and TDI values of phthalates in toys (CSTEE, 1998).

Phthalate	**NOAEL value mg/kg/day**	**Tolerable daily intake µ g/kg/day**
DiNP	15	150
DEHP	3,7	37
DNOP	37	370
DiDP	25	250

Phthalate esters and their metabolites residues commonly detected in different consumer products, urine, breast milk, and amniotic fluid. Due to phthalates can also cross the placenta, they could be teratogenic. Babies and young children consume more calories and relatively higher levels of fatty foods than adults. Children's estimated total dietary DEHP intake is highest, compared to younger adolescents. In addition to dietary exposure, oral phthalate ingestion also occurs via mouth, suck, or chew toys. The passage of DEHP from toys with saliva solubilization. With the release from some toys being higher than the maximum pass-through level determined by the Scientific Committee on Toxicity, Ecotoxicity and Environment, the European Union (EU) banned the use of phatalate types in children's toys and childcare products in 1999. In 2005 the use of DEHP banned (Lyche, J.L., et al., 2009). In the United States (USA), the use of DiNP is still allowed in toys, and it has been shown that children take up significant levels of DiNP as a result of chewing activities (Kavlock et al., 2002). Human exposure can either be assessed based on concentrations found in environmental samples and food, or indirectly reconstructed from urinary metabolite levels that reflect internal exposure. There is substantial evidence to suggest that phtatalate types negatively effects the male reproductive system functions, and it has therefore been suggested that they may have a similar mechanism of action. High doses phthalates inhibit testosterone production in both fetal and adult Leydig cells. In contrast, low doses of phthalates

increase the number of adult Leydig cells and increase testosterone production. Side effects of phthalate exposure related with dose and exposure duration. Serum thyroid hormone (TH) and thyroid stimulating hormone (TSH) levels disruptions were related with MEHP concentrationsthat can be indirectly affects the male and female reproductive system. Estimated concentrations of DEHP in the general population were found to be highest in children and decrease with age. The critical period for effects on male reproductive development appears to be late pregnancy and the postpartum period. Recent data have shown that phthalates with a similar mechanism of action, when administered as a mixture, cause cumulative, dose-added effects on fetal testosterone production and testicular histopathology (Lyche, J.L., et al., 2009).

There are many studies showing that phthalates have serious toxic effects on many systems such as cardiovascular system, reproductive system, nervous system and endocrine system. (Mariana, M. & Cairrao, E., 2020) (Holahan, M.R., & Smith, C.A., 2015) (Hannon, P.R. & Flaws, J.A., 2015) (Wang, Y. et al., 2019) (Holahan, M.R. and Smith, C.A., 2015) (Philippat, C. et al., 2012).

According to a study, deodorants, perfumes, shampoos, creams, make-up materials, soaps and lotions are hygiene and beauty products that are used by pregnant women and contain high levels of phthalates. DEHP is the most abundant phthalate in make-up products. However, this rate may vary according to factors such as geographical conditions, diet, plastic use (Bustamante-Montes, L.P., 2021). Exposure to phthalates during a sensitive period such as pregnancy causes metabolic dysfunction in the mother and disrupts fetal development. (Filardi, T. et al., 2020).

Phthalates and Their Toxic Effects on the Immune System

For a functioning immune system, organs, cells and secretions provide body homeostasis. The first line of defense is innate immune reactions. Stimulation of this system depends on previous encounter with the pathogen; therefore, some time is required for it to react (Toskala, E., 2014). Epidemiological studies have investigated the possible relationship between exposure to PVC or phthalates and respiratory tract diseases in environments such as the home and workplace (Jaakkola, J.J. and Knight, T.L., 2008). It has been suggested in both in vivo and in vitro experimental studies that phthalates have adjuvant effects on immunoglobulin production, enable differentiation from T helper cells, affect cytokine secretion, increase enzyme and histamine secretion, and increase the phagocytic ability of innate immune cells (Bornehag, C.G. and Nanberg). , E., 2010). Monocytes and macrophages are cells of the innate immune system. Monocytes are found between the bone marrow and the bloodstream. They can migrate between tissues and differentiate into macrophages in the tissues they pass through (Verschoor, C.P. and Puchta, A., 2012).

In studies investigating the relationship between inflammatory responses and phthalate esters; while there correlation between high molecular weight phthalate esters and CRP(C-reactive protein), IL-6(Interleukin-6) and IL-10(Interleukin-10), no relationship was found with low molecular weight phthalate esters. According to studies, phthalate ester metabolites MBzP (monobenzyl phthalate), MiBP (monoisobutyl phthalate) and MnBP (mono-n-butyl phthalate) are directly associated with high levels of CRP, while other phthalate ester metabolites MCNP (monocarboxynonyl phthalate) and MCPP (mono- 3-carboxypropyl phthalate) is related with high levels of IL-6 (Franken, C., et al., 2017). Studies with DEHP also show that phthalates may cause dysregulation in inflammatory responses and increase TNFα (Tumor necrosis factor) production from monocytes and macrophages Hansen, J.F., et al., 2015a). In another review, the effects of two phthalate ester metabolites MEP and MiBP on inflammation elements (TNFα, CRP, alkaline phosphatase (ALP)) and absolute neutrophil count, ferritin, fibrinogen, IL-1β, IL-6 and IL-10 were investigated. The results found were not in line with other studies (Sweeney, M.R. et al., 2019). In addition, phthalate esters were found to be positively associated with the anti-inflammatory cytokine IL-10. The effect of phthalates on both pro-inflammatory and anti-inflammatory responses has also been demonstrated by two different in vitro studies (Hansen, J.F., et al., 2015b).

Both studies show an increase in IL-10 levels after exposure to phthalate esters. IL-10, produced by natural killer (NK) cells during a systemic infection, acts as regulators that stabilize inflammation and limit immune-mediated damage to the host (Martinez-Espinosa, I., et al., 2021). IL-10 secreted by NK can also inhibit the function of effector T cells and reduce the efficacy of anti-tumor immunity (Sun., H., et al., 2021). Increasing IL-10 levels reduces inflammation-mediated damage but can also lead to weak immune responses against the tumor. As mentioned before, phthalates are divided into two according to their molecular weight. High molecular weight phthalates and low molecular weight phthalates differ in terms of industrial use and exposure routes (North, M.L., et al., 2014). While low molecular weight phthalates are used in personal care products, medicines and other consumer products; high molecular weight phthalates are mostly found in PVC food packaging, home furniture and other construction materials (Beszterda, M. and Franski, R., 2018).

It is thought that DEHP and monoethylhexyl phthalate reduce the permeability of the mitochondrial membrane in the human lymphoblast cell line (TK6), thus leading to Reactive oxygen species (ROS) production and caspase activation (Rosado-Berrios, C.A. et al., 2011) (Nowak, K. et al., 2019).

Discussion

Phthalates are a group of plasticizers added to plastics to give them flexibility and reduce their brittleness. They are also called endocrine disrupting chemicals. According to their molecular weights; they are divided into two groups as high molecular weight phthalates and low molecular weight phthalates. They are found in many diverse and different areas such as food packaging, cosmetics, medical devices, drugs, construction materials, personal care products. Exposure is inevitable as these chemicals are ubiquitous in the environment.

The immune system is a system that protects the body against pathogens that may come from outside and develops a defense system. There are two forms of immunity, innate and adaptive. Innate immunity produces non-specific responses and is the first line of defense. Elements of the innate immune system are natural killer cells, macrophages, dendritic cells, and skin. Adaptive immunity, unlike innate immunity, responds specifically and is in constant change. Adaptive immunity elements are T and B lymphocytes. It is divided into humoral and cellular immunity. Antibodies, which are an element of the humoral system, form an important part of the immune system. Antibodies recognize the part of the pathogen called the antigen. The system called the complement system is involved in the formation of the response in both the acquired immunity and the innate immunity. There are three types of pathways that activate the complement system; the classical pathway, the alternative pathway, and the mannose-binding lectin pathway.

The toxic effects of phthalates on humans are classified as worrisome. Many studies have investigated the toxic effects of phthalates on the systems of the human body. It has been observed that changes occur in the time of birth as a result of exposure during pregnancy. It is known to reduce testosterone production and delay puberty when exposed to high doses. Exposure is very important especially in the womb and in the pediatric period.

Studies show that phthalates have positive effects on interleukin levels, CRP and TNFα. It is also thought that phthalate exposure may lead to autoimmune diseases.

Exposure to phthalates is an important risk factor today. In order to protect ourselves from phthalates, we should pay attention to the packaging materials of the materials we use and the food we eat. Since there is a possibility that phthalates can be transmitted through the air, we should ventilate the environments we are in regularly. Even if it is not possible to be completely protected from phthalates, which are ubiquitous in the environment, it is important for our health to minimize exposure.

REFERENCES

Godwin, A.D., 2017, Plasticizers, Applied Plastics Engineering Handbook, 2 (24), 533-553.

Altenhofen da Silva, M. et al., 2011, Polyvinylchloride (PVC) and natural rubber films plasticized with a natural polymeric plasticizer obtained through polyesterification of rice fatty acid, Polymer Testing, 30 (5), 478-484.

Hyatt, J.W. and Hyatt, I.S., 1870, Improvement in treating and molding pyroxyline, US Patent 105,338.

Semon, W.L., 1933, Synthetic rubber-like composition and method of making same, US Patent 1,929,453.

Godwin, A.D. and Krauskopf, L.G., 2007, Monomeric Plasticizers, Handbook of Vinyl Formulating, 2 (7), 173-238.

Daniels, P.H., 2009, A brief overwiev of theories of PVC plasticization and methods used to evaluate PVC-plasticizer interaction, Journal of Vinyl and Additive Technology, 219-223.

Cadogan, D.F. and Howick, C.J., 2000, Plasticizers, Ullman's Encyclopedia of Industrial Chemistry, 27, 599-618.

Bolton, D., 2013, Eastman Chemical Webinar Presentation. Suppliers going DEHP-free, it's easier than you think.

Songu, M. and Katılmış, H., 2012, Enfeksiyonlardan korunma ve immün sistem, Journal of medical updates, 2(1), 31-42.

Tomar, N. and Rajat, K.D., 2014, A brief outline of the immune system, Immunoinformatics, 3-12.

McComb, S. et al., 2019, Introduction to the Immune System, Immunoproteomics, 2 (1), 1-24.

Schettler, T., 2006, Human exposure to phthalates via consumer products, International Journal of Andrology, 1 (29), 134-139.

Lyche, J.L. et al., 2009, Reproductive and Developmental Toxicity of Phthalates, Journal of Toxicology and Environmental Health, 12 (4), 225-249.

Kavlock, R. et al., 2002, NTP Center of Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate, Reproductive Toxicology, 16, 489-527.

Holahan, M.R., Smith, C.A., 2015, Phthalates and neurotoxic effects on hippocampal network plasticity,NeuroToxicology, 48, 21-34.

Hannon, P.R. and Flaws, J.A., 2015, The effects of phthalates on the ovary, Frontiers in Endocrinology, 6 (8), 1-19.

Wang, Y. et al., 2019, Phthalate-Induced Fetal Leydig Cell Dysfunction Mediates Male Reproductive Tract Anomalies, Frontiers in Pharmacology, 10 (1309), 1-11.

Philippat, C. et al., 2012, Exposure to Phthalates and Phenols during Pregnancy and Offspring Size at Birth, Children's Health, 120 (3),464-470.

Bustamante-Montes, L.P., 2021, Phthalates exposure during pregnancy a study in a Mexican cohort, Toxicology Reports, 8, 1040-1045.

Filardi, T. et al., 2020, Bisphenol A and Phthalates in Diet: An Emerging Link with Pregnancy Complications, Nutrients, 12(2), 1-15.

Wang, Y., Qian, H., 2021, Phthalates and Their Impacts on Human Health, Healthcare, 9(5), 1-9.

Mariana, M. and Cairrao, E., 2020, Phthalates Implications in the Cardiovascular System, Journal of Cardiovascular Development and Disease, 26(7), 1-19.

EPA, 2017, Phthalates Biomonitoring, America's Children and the Environment, 3, 1-19.

Heudorf, U. et al., 2007, Phthalates: Toxicology and exposure, International Journal of Hygiene and Environmental Health, 623-634.

CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment), 1998a. Phthalate migration from soft PVC toys and child-care articles. Opinion expressed at the CSTEE third plenary meeting, Brussels, April 24, 1998

EPA, 2012, Phthalates, U.S. Environmental Protection Agency, 1-16.

Calder, P.C. et al., 2017, Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition, Ageing Research Reviews, 40, 95-119.

Toskala, E., 2014, Immunology, International Forum of Allergy&Rhinology, 4(2), 21-27.

Jaakkola, J.J. and Knight, T.L., 2008, The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis, Environmental Health Perspectives, 116(7), 845-853.

Bornehag, C.G. and Nanberg, E., 2010, Phthalate exposure and asthma in children, International Journal of Andrology, 333-345.

Franken, C. et al., 2017, Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents, International Journal of Hygiene and Environmental Health, 220(2), 468-477.

Sweeney, M. R., O'Leary, K. G., Jeney, Z., Braunlin, M. C., & Gibb, H. J. (2019). Systematic review and quality ranking of studies of two phthalate metabolites and anogenital distance, bone health, inflammation, and oxidative stress. Critical Reviews in Toxicology, 49(4), 281-301.

Hansen, J.F. et al., 2015a, Influence of phthalates on cytokine production in monocytes and macrophages: a systematic review of experimental trials, PLoS One, 10(3), 1-18.

Hansen, J.F. et al., 2015b, Influence of phthalates on in vitro innate and adaptive immune responses, PLoS One, 10(6), 1-13.

Martinez-Espinosa, I. et al., 2021. Role of IL-10-producing natural killer cells in the regulatory mechanisms of inflammation during systemic infection, Biomolecules, 12(1), 1-21.

Sun, H. et al., 2021. IL-10-producing ILCs: molecular mechanisms and disease relevance, Frontiers in Immunology, 12, 1-10.

North, M.L. et al., 2014. Effects of phthalates on the development and expression of allergic disease and asthma, Annals of Allergy, Asthma&Immunology, 112(6), 496–502.

Beszterda, M. and Franski, R., 2018, Endocrine disruptor compounds in environment: as a danger for children health, Pediatric Endocrinology, Diabetes&Metabolism, 24(2), 88–95.

Nowak, K. et al., 2019, Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells, Environment international, 125, 350–364.

Rosado-Berrios, C.A. et al., 2011, 2011Mitochondrial permeability and toxicity of diethylhexyl and monoethylhexyl phthalates on TK6 human lymphoblasts cells, Toxicology in Vitro, 25(8), 2010-2016.

Ashman, R.B., 2012, Leucocytes: methods and protocols, Methods in Molecular Biology, 844.

Galligan, T., 2021, Six tips to avoid phthalates after study highlights health harms, billion-dollar costs, Environmental working group, https://www.ewg.org/news-insights/news/2021/10/six-tips-avoid-phthalates-after-study-highlights-health-harms-billion#:~:text=Stay%20away%20from%20plastic%20wrap,their%20release%20into%20the%20food., [Ziyaret Tarihi: 16.05.2022].

James, M., 2013, How to avoid phthalates (Even though you can't avoid phthalates), Huffpost, https://www.huffpost.com/entry/phthalates-health_b_2464248, [Ziyaret Tarihi: 16.05.2022].

Matsko, C.M., 2020, How to avoid phthalates, Plastic Reuse, https://www.wikihow.com/Avoid-Phthalates#aiinfo, [Ziyaret Tarihi: 16.05.202].

Can Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio Be Used to Predict Viral Etiology in Patients with Lower Respiratory Tract Infection in the Pediatric Intensive Care Unit?

Merve MISIRLIOGLU[1]

Introduction

Viral infections are the most common causes of lower respiratory tract infections (LRTIs) in childhood. Failure to determine the etiology in patients with LRTIs in the pediatric intensive care unit (PICU) may lead to inappropriate treatment, unnecessary antibiotic use, and development of antibiotic resistance, and prolonged hospitalization and intensive care unit stays (1-2).

The neutrophil/lymphocyte ratio (NLR) is calculated using the neutrophil and lymphocyte values in the complete blood count and is an easily accessible, new biomarker that has been gaining in popularity recently and can predict the progression and mortality in many rheumatologic, cardiac and endocrinologic inflammatory diseases. It is thought to indicate the severity of inflammation. Platelet/lymphocyte ratio (PLR) is used to predict the inflammatory process, disease activity, response to treatment and prognosis in inflammatory diseases and malignancies (2).

Identification of the cause of the disease; It ensures that unnecessary treatment agents are not used, the clinical course is predicted, and necessary treatment manipulations and precautions are taken. In this study, it was aimed to investigate NLR and PLR values in predicting the etiology of viral respiratory pathogens in patients with LRTIs admitted to the PICU due to respiratory failure.

Materials and Methods

Samsun Training and Research Hospital pediatric intensive care unit provides service with 6 beds as a secondary level. Between October 2021 and April 2022, 126 patients were hospitalized in the PICU. 26 patients who were admitted to the PICU due to respiratory failure and whose viral panel was studied from nasopharyngeal swab were included in the study. Patient data were obtained retrospectively from patient files in electronic media. Patients' age, gender, month of illness, length of stay in hospital and PICU, NLR, PLR values with leukocyte, lymphocyte, neutrophil and platelet measurements in complete blood count, respiratory failure treatment approaches (high flow nasal cannula oxygen therapy, noninvasive and invasive mechanical ventilation) was recorded.

Patients in the 1 month-18 age group who were hospitalized due to respiratory failure due to lower respiratory tract infection in the PICU and whose respiratory tract viral panel was studied from nasopharyngeal swab were included. Patients with respiratory failure other than lower

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respiratory tract infection, patients under the age of 1 month and over 18 years of age, those hospitalized outside of the PICU, and patients whose respiratory tract viral panel was not studied from nasopharyngeal swab were excluded from the study. In addition, cases with conditions that may affect the hematological parameters of the patients (chemotherapy, hematological malignancy, immunosuppression) were not included in the study. Approval was obtained from Samsun University Clinical Research Ethics Committee for the study (SUKAEK-2022/7/6).

Statistical analysis

SPSS version 21.0 (IBM SPSS) program was used for statistical analysis. Risk factors affecting respiratory viral panel positivity were analyzed by Binary Logistic Regression Analysis and Enter method was used to include parameters in the model. ROC Analysis was used to examine the parameters determining respiratory tract infection positivity, and DeLong Test was used to determine which of the parameters was better in determining positivity. Analysis results were presented as mean ± standard deviation. Significance level was accepted as $p<0.05$.

Results

In the pediatric intensive care unit. 126 patients were hospitalized for a period of 7 months. 26 patients who were admitted to the PICU due to respiratory failure due to lower respiratory tract infection and whose viral panel was studied from nasopharyngeal swab were included in the study. 61.50% (n=16) of the patients were male and their mean age was 20.77±39.50 (1-204) months; respiratory viral pathogen was positive in 80.76% (n=21). In 5 patients, dual pathogens were detected and co-infection was present. There was no mortality in any of our cases. The length of stay in the PICU was 4.88±2.97 (3-14) days and the length of stay in the hospital was 9.15±3.25 (4-19) days. The characteristics of the patients are given in Table-1.

The results of whole blood parameters checked during the patients' admission to the intensive care unit; It is shown in Table-2. NLR was 3.18±3.65 (0.19-12.14) and PLR was 142.42 ±108.62 (12.48-350.90). To determine the parameters that can be used to predict respiratory viral panel positivity; the effects on positivity were examined by Binary Logistic Regression Analysis. univariate and multivariate models were established. Since the PLR and NLR parameters cannot enter the model at the same time (multi-connection problem), two multivariate models have been established in which both are included separately. Other parameters were also excluded from the model due to the multicollinearity problem. As a result of the analyzes performed, complete blood count, biochemistry and blood gas parameters were not found to be an effective risk factor for respiratory tract viral panel positivity ($p>0.05$) (Table-3).

Discussion

Lower respiratory tract infections; pneumonia and bronchiolitis consist of bronchitis. However, since it is difficult to distinguish from each other, especially in pediatric patients in the younger age group, this is the definition that covers these tables (3). Although the agents of infection vary according to age, viruses appear as the most common pathogens under the age of five (4). Respiratory Syncytial Virus (RSV) is the most common cause of bronchiolitis and pneumonia, and is a virus that causes epidemics especially in winter and spring (5). In our study, the most common agents were RSV and Rhinovirus. In another study conducted in critically ill children in our country, RSV was found to be the most common, and Rhinovirus was the second most common (1).

As a result of the differentiation of stem cells in the bone marrow, leukocytes, erythrocytes and platelets are produced. Although the density of the leukocyte series varies according to age, the majority of them are neutrophils. In the case of inflammation, the lymphocyte count decreases, while the neutrophil and platelet count increase. It is thought that NLR indicates the severity of inflammation (6). Numerous studies conducted in pediatric patient groups have reported an association between increased NLR and PLR and inflammation. NLR and PLR can be easily used in the diagnosis of diseases with systemic inflammation such as Henoch-Schoenlein Purpura, asthma, acute pyelonephritis, and acute appendicitis and in monitoring their progression. However, NLR was found to be a factor affecting mortality and morbidity in intensive care patients (7-9). In an adult study, it was reported that NLR could be a useful biomarker for mortality and acute respiratory distress syndrome (ARDS) development in patients with miliary tuberculosis and ARDS (10). Another hematological parameter whose number changes in inflammations is platelets. The platelet count increases not only in case of acute inflammations, but also in chronic inflammatory diseases. There are data showing that PLR, which can be used to predict disease activity, response to treatment, and prognosis in the inflammatory process, affects mortality and morbidity (11,12).

In a PubMed-based study we conducted, we could not find any publication in the literature examining the relationship between lower respiratory tract infection etiology and NLR and PLR in the pediatric age group. To the best of our knowledge, our study is the first study on the relationship between NLR and PLR in patients hospitalized in the pediatric intensive care unit due to respiratory failure due to LRTIs. In a study conducted in adult COVID-19 patients, they stated that there was a statistically significant difference between NLR healthy and covid patients, who also received and did not receive intensive care support (13). In a study conducted with 216 adult pneumonia patients in the intensive care unit, NLR and PLR were studied in the differentiation of bacterial and viral pneumonia, but no difference was found; stated that it is not an ideal biomarker for viral / bacterial differentiation (14). In our study, no statistically significant difference was found in NLR and PLR values in predicting respiratory viral positivity.

As a result; In respiratory tract infections, the correct diagnosis and the application of the right treatment can reduce the use of unnecessary antibiotic or antiviral treatment, prevent drug resistance and prevent morbidities that may develop. PLR and NLR are easily accessible and inexpensive parameters that can be used to detect the inflammatory state and do not require complex calculations. According to the results of our study, we determined that PLR and NLR could not be used to predict viral etiology in critically ill children with LRTIs; however, prospective studies with larger patient groups are needed.

References

1- Sık G, Demirbuga A, Annayev A, Cabiri A, Deliceo E, Citak A. The frequency of viral pathogens and clinical characteristics of patients hospitalized in the pediatric intensive care unit with the diagnosis of lower respiratory tract infection. J Pediatr Inf 2020;14(1):27-32. doi: 10.5578/ced.202011.

2- Karakoyun M, Ataoglu EA, Büyükkayhan D, Elevli M. The frequency and clinical features of respiratory syncytial virus infections in children under the age of 2 who presented with respiratory tract infection findings. Online Turkish Journal of Health Sciences 2018; 3:56-69. doi: 10.26453/otjhs.391181.

3- McIntosh K. Community-acquired pneumonia in children. N Eng J Med 2002; 346:429-437. doi: 10.1056/NEJMra011994.

4- Williams JV. The clinical presentation and outcomes of children infected with newly identified respiratory tract viruses. Infect Dis Clin North Am 2005; 19:569-584. doi: 10.1016/j.idc.2005.05.009

5- American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering LK. Baker CJ. Long SS. Kimberlin D. (Eds). Red Book: 2009 Report of the Committee on Infectious Diseases. 28th ed. Elk Gove Village. IL: American Academy of Pediatrics; 2009:560–569.

6- Aydın I, Agilli M, Aydin FN, Kurt YG, Cayci T, Tas A, et al. The reference ranges of neutrophil-lymphocyte ratio in different age groups. Gülhane Medical Journal 2015; 57:414-18. doi: 10.5455/gulhane. 166398.

7- Narcı A, Tuncer AA, Çetinkurşun S. Diagnostic importance of neutrophil/lymphocyte ratio in childhood appendicitis. The Medical Journal of Kocatepe 2009; 10:5-7.

8- Gayret ÖB, Erol M, Nacaroglu HT. The Relationship of Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio with Gastrointestinal Bleeding in Henoch-Schoenlein Purpura. Iran J Pediatr 2016;26(5):8191.

9- Han SY, Lee IR, Park SJ, Kim JH, Shin JI. Usefulness of neutrophil-lymphocyte ratio in young children with febrile urinary tract infection. Korean J Pediatr 2016;59(3):139-44.

10- Han Y, Kim SJ, Lee SH, Sim YS, Ryu YJ, Chang JH, et al. High blood neutrophil-lymphocyte ratio associated with poor outcomes in miliary tuberculosis. J Thorac Dis 2018;10(1):339-46.

11- Bekdas M, Ozturk H. Platelet to lymphocytes ratio in diagnosis of acute complicated appendicitis in childhood. J Clin Anal Med 2017; 8(4):299-301.

12- Wu Y, Chen Y, Yang X, Chen L, Yang Y. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were associated with disease activity in patients with systemic lupus erythematosus. Int Immunopharmacol 2016; 36:94-9.

13- Sayed AA, Allam AA, Sayed AI, Alraey MA, Joseph MV. The use of neutrophil-to-lymphocyte ratio (NLR) as a marker for COVID-19 infection in Saudi Arabia: A case-control retrospective multicenter study. Saudi Med J. 2021;42(4):370-376. doi: 10.15537/smj.2021.42.4.20200818

14- Ng WW, Lam SM, Yan WW, Shum HP. NLR, MLR, PLR and RDW to predict outcome and differentiate between viral and bacterial pneumonia in the intensive care unit. Sci Rep 2022;12(1):15974. doi: 10.1038/s41598-022-20385-3

Tables

Table 1. Demographic and clinical characteristics of the patients

Patients' characteristics	n (%)
Age Groups	
1 month-3 months:	6 (23.1%) (2 patients positive / 4 patients negative)
3 months-5 years:	18 (69.2%) (17 patients positive /1 patient negative)
≥5 years:	2 (7.7%) (2 patients positive)
Distribution by month of follow-up	
October:	3 (11.6%) (2 patients positive / 1 patient negative)
November:	8 (30.7%) (6 patients positive / 2 patients negative)
December:	2 (7.7%) (2 patients positive)
January:	1 (3.8%) (1 patients positive)
February:	2 (7.7%) (1 patients positive / 1 patient negative)
March:	4 (15.4%) (4 patients positive)
April:	6 (23.1%) (5 patients positive / 1 patient negative)
Detected Viral Pathogens	
Rhinovirus:	7 (26.9%)
H.bocavirus:	2 (7.7%)
RSV A/B:	7 (26.9%)
Enterovirus:	2 (7.7%)
Parainfluenza 4:	2 (7.7%)
Coronavirus OC43:	2 (7.7%)
H.metapneumovirus:	2 (7.7%)
Parechovirus:	1 (3.8%)
Parainfluenza 3:	1 (3.8%)
5 patients with co-infection	
1. patient:	Rhinovirus + H.bocavirus
2. patient:	Rhinovirus + Enterovirus
3. patient:	Rhinovirus + Parainfluenza 4
4. patient:	Rhinovirus + Enterovirus
5. patient:	RSV A/B + Coronavirus OC43
Co morbid Disease	Yes: 7(26.9%) None: 19 (73.1%)
Individual with respiratory tract infection at home	Yes: 13(50.0%) None: 13(50.0%)
History of recurrent respiratory infection	Yes: 7(26.9%) None: 19 (73.1%)
History of previous inhaler treatment	Yes: 10 (38.5%) None: 16 (61.5%)
High flow nasal cannula oxygen	Yes:25 (96.2%) None:1 (3.8%)
Noninvasive mechanical ventilation	Yes: 3 (11.5%) None:23 (88.5%)
Invasive mechanical ventilation	Yes: 3 (11.5%) None: 23 (88.5%)

Table 2. Results of whole blood parameters checked during the patients' admission to the pediatric intensive care unit

	Mean ± SD Median (minimum-Maksimum)
WBC $10^9/L$	12.05 ± 4.72 11.04 (4.28-23.35)
ANC $10^9/L$	6.66 ± 4.66 5.55 (1.40-20.77)
ALC $10^9/L$	4.18 ± 3.11 3.75 (0.90-13.30)
PLT $10^9/L$	331.50 ± 117.12 332.00 (152.00-582.00)
NLR	3.18 ± 3.65 1.38 (0.19-12.14)
PLR	142.42 ± 108.62 107.08 (12.48-350.90)
HGB *gr/dL*	10.93 ± 1.74 10.85 (8.4-15.6)
HCT *%*	32.22 ±5.58 32.30 (23.9-44.7)

WBC: White blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, NLR: Neutrophile-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HGB: Hemoglobin, HCT: Hematocrit.

Table 3. Examination of risk factors affecting respiratory tract viral panel positivity

	Viral Panel		Univariate		Multivariate 1		Multivariate 2	
	Negative	Positive	OR (%95 CI)	p value	OR (%95 CI)	p-value	OR (%95 CI)	p-value
WBC	11.83 ± 2.91	12.1 ± 5.14	1.013 (0.818 - 1.254)	0.907	---	---	---	---
ANS	6.09 ± 3.47	6.8 ± 4.96	1.037 (0.825 - 1.305)	0.754	---	---	---	---
ALS	4.33 ± 1.84	4.14 ± 3.38	0.981 (0.718 - 1.339)	0.903	---	---	---	---
PLT	402 ± 108.52	314.71 ± 115.12	0.993 (0.984 - 1.002)	0.148	---	---	---	---
NLR	2.08 ± 2.37	3.44 ± 3.9	1.141 (0.803 - 1.62)	0.461	**---**	**---**	0.953 (0.505 - 1.798)	0.882
PLR	140.73 ± 115.57	142.82 ± 109.89	1 (0.991 - 1.009)	0.968	0.993 (0.971 - 1.016)	0.556	**---**	**---**
HGB	9.82 ± 1.66	11.2 ± 1.69	1.895 (0.848 - 4.235)	0.119	0.876 (0.089 - 8.609)	0.910	1.119 (0.095 - 13.154)	0.929
HCT	28.78 ± 6.63	33.04 ± 5.14	1.198 (0.944 - 1.522)	0.138	---	---	---	---
MPV	8.18 ± 1.04	8.61 ± 1.19	1.495 (0.522 - 4.278)	0.454	3.699 (0.227 - 60.23)	0.358	3.526 (0.156 - 79.778)	0.428
CRP	4.58 ± 3.89	19.3 ± 29.01	1.153 (0.928 - 1.432)	0.200	1.342 (0.703 - 2.561)	0.373	1.222 (0.77 - 1.939)	0.395
AST	53.4 ± 46.72	54.55 ± 57.53	1 (0.982 - 1.019)	0.966	1 (0.869 - 1.151)	0.995	1.003 (0.869 - 1.157)	0.970
ALT	24.6 ± 20.67	41.41 ± 73.03	1.008 (0.976 - 1.041)	0.642	1.003 (0.831 - 1.211)	0.975	1.017 (0.811 - 1.274)	0.886
Urea	17.6 ± 9.81	21.38 ± 16.29	1.029 (0.914 - 1.159)	0.635	1.34 (0.764 - 2.351)	0.307	1.282 (0.808 - 2.035)	0.292
Krea	0.4 ± 0.42	0.29 ± 0.2	0.216 (0.007 - 6.39)	0.375	0 (0 - 87.539)	0.171	0 (0 - 58.187)	0.160
pH	7.3 ± 0.12	7.33 ± 0.09	27.961 (0.001 - 687284.597)	0.518	---	---	---	---
pCO_2	40.6 ± 7.13	42.29 ± 9.85	1.022 (0.911 - 1.146)	0.711	---	---	---	---
HCO_3	20.54 ± 5.12	21.67 ± 3.69	1.07 (0.851 - 1.346)	0.561	---	---	---	---
BE	-5.54 ± 6.96	-2.88 ± 5.27	1.079 (0.921 - 1.265)	0.347	---	---	---	---
Lactate	3.92 ± 4.4	2.14 ± 0.98	0.712 (0.429 - 1.181)	0.189	---	---	---	---

[1]Coc&Snell R^2: %40,3; [1]Nagelkerke R^2: %64,5; [2] Coc&Snell R^2: %39,3; [2]Nagelkerke R^2: %63

Alternative Treatment Methods and Application to Cardiovascular Areas with Summary of Some Related Studies

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Introduction

Heart attack is a heart disease with the highest morbidity and mortality rate among cardiovascular diseases and is very common in today's societies. The heart is a powerful organ that pumps blood an average of 60-80 times per minute during rest. It needs to be able to work on its own with the whole body and to feed itself for the continuity of this work. These self-feeding vessels are called coronary arteries (İlhan, 2016). If a deterioration occurs in these vessels, what we call ischemia occurs and the heart muscle, namely myocardium, cannot be fed adequately. And in this case, what we call myocardial infarction, that is, a heart attack occurs. Coronary insufficiency tables vary according to the type, degree and location of the stenosis in the coronary vessels. In some patients, there may be chest pain that occurs only during physical activity and is relieved by rest, as well as a heart attack (infarction) that sometimes develops as a result of sudden occlusion of the vessels and can lead to sudden death, starting with severe chest pain (Aybek, 2020).

When a part of the heart muscle undergoes ischemia and is deprived of oxygen, the myocardium cannot perform its function and a heart attack occurs. Patients' approach to alternative treatments has increased due to factors such as the expensiveness of the medical and surgical treatment used, the risky, the difficult accessibility for low and middle-income patients, and the greater confidence of people in natural methods. Although there are different methods in the treatment of heart attack in the world and in our country, research on alternative treatment has increased in many countries such as China and India, along with classical medical and surgical treatment. Among the alternative treatments, especially in many countries, especially in China, garlic, green tea, temple tree, licorice, cloves, ginger, St. John's Wort, pomegranate juice and cranberry juice are used as blood thinners, cholesterol-lowering, anti-hypertension, anti-atherosclerosis in the treatment of heart attack.

A heart attack usually has typical symptoms that manifest itself in the form of a pressing and compressive pain in the middle of the chest, radiating from the chest to the neck, spreading to the chin ears and arms, and especially numbness in the left arm and two little fingers (Şentürk, 2008). In humans, many factors such as age, obesity, genetic predisposition, alcohol and cigarette use, malnutrition, etc., form the basis for heart attack and many similar cardiovascular diseases and trigger disease formation.

However, among the important risk factors of heart attack can be counted as; Vascular diseases or heart diseases such as atherosclerosis, a previous heart attack, any rhythm disorder or faint, age (over 40 in men, over 50 in women), smoking, excessive alcohol consumption, some illegal drugs and derivatives; high levels of triglycerides, high bad cholesterol and low good cholesterol content, diabetes, high blood pressure, and chronic high stres (Jensen,1991). The diagnosis of heart attack is made by the symptoms seen in the patient, the patient's history, physical examination, EKG and blood tests (Şentürk, 2008).

Every year, approximately 18 million people die due to cardiovascular diseases and other diseases it causes. When the causes of death worldwide are examined, it has been determined that cardiovascular diseases are the number one cause of death in 2019 (Roth et al., 2020).

It is seen that most of these death rates are in middle-income countries such as Central Asian and Eastern European countries and some low-income countries. Heart attack is one of the diseases with the highest mortality and morbidity rates (Kılınç et al., 2021) According to the worldwide research statistics of the World Health Organization, the number of deaths from cardiovascular diseases is expected to reach 23 million in 2030 (https://nccih.nih.gov/research/statistics/2007/camsurvey_fs1.htm). According to the same report, 18 million people die every year due to heart diseases. According to the report of the American Heart Association, an increase of approximately 46% in heart failure is expected from 2012 to 2030; In 2030, it is predicted that more than 8 million people aged 18 and over in the USA (with 5.8 million in 2011) will have heart failure (Beştepe et al., 2020). It is understood that this estimated increase has triggered further research on heart attacks. Losses due to cardiovascular diseases in Turkey are equivalent to 30% of all deaths, and this rate represents half of the deaths from cancer (Coşkun et al., 2009). 31% of deaths in the world are due to cardiovascular diseases, and 85% of them are caused by heart attack and stroke. When we look at the 2018 Turkey data, 36.7% of the deaths are caused by cardiovascular system diseases, 39.1% of them are due to ischemic heart disease and 22.2% are due to cerebrovascular diseases (T.R. Ministry of Health, 2019).

Considering all these data and evaluations, it is seen that coronary heart disease and cerebrovascular disease are the most common causes of death among cardiovascular system diseases.

In addition, even though the deaths due to cardiovascular system diseases have decreased over the years, it is still seen that it is the most important cause of death both in the whole world and in Turkey, and the complications related to these diseases are increasing (Türk et al., 2021).

Considering the deaths caused by cardiovascular system disease, 36% of them are premature deaths occurring before the age of 75. In terms of chronic heart disease, cerebrovascular disease and peripheral arterial disease, atherosclerosis was found to be responsible for 71% of all deaths in 1997. When looking at the 35-45 age range in men, the incidence rate is 7% per year, while this rate rises to 68% at the age of 85-95. In women, the difference decreases as the age progresses, but if you look at the 10 years later, it is seen that the frequency increases at similar rates (İşlegen, 2007).

Many factors such as the increase in education level, increase in income levels, change in dietary habits and controlling infectious diseases in our country and throughout the world have led to an increase in life expectancy. The prolongation of the average life expectancy has significantly increased the percentage of individuals aged 65 and over in the general population in both developed and developing countries, and the calculations show that 20% of the world population will be 65 years and over in 2050 (Genç and Yiğitbaş, 2021). Among the alternative treatments, such as garlic, green tea, temple tree, licorice root, clove, ginger, St. John's Wort, pomegranate juice and cranberry juice, which are mainly preferred in many countries, especially in China, in the treatment of heart attacks, blood thinners, cholesterol lowering, antihypertensive herbal treatment sources used as a preventative against atherosclerosis. Despite all the developing opportunities, most of the patients die before they can even reach the health institution. Therefore, people should be informed about heart diseases, heart attack and its symptoms and should be directed to apply to the nearest health institution as soon as the symptoms are felt. Patients with heart attack symptoms apply to the emergency room, and treatment begins quickly. Oxygen support is provided by measuring the person's blood oxygen value. People with high pain can be benefited from various painkillers (Daşkapan, 2013). The main purpose of treatment; It is the removal and opening of the

blockage in the vessels feeding the heart and the immediate restoration of blood flow to that area. Tissue oxygenation is restored and myocardial function is restored. For this purpose, aspirin is given to remove blood clotting (blood thinner-anticoagulant therapy), nitroglycerin and oxygen therapy to relieve chest pain and improve blood flow.

CAM (complementary and alternative treatments)

In addition to all these medical treatments, as mentioned before, the tendency to alternative treatments has increased rapidly due to factors such as easier economic opportunities, accessibility for patients and their relatives, and the fact that natural methods create a more reliable impression on people. With this; Complementary and alternative medicine terms are generally gathered under one title and evaluated as a whole. Alternative medicine is defined as any health service that replaces medical treatments and is not included in modern biomedicine or treatments (Dömbekci et al., 2020). Complementary medicine, on the other hand, is defined as the treatment and care systems applied in addition to medical treatment (Ceyhan and Yiğit, 2016). According to the definition made by the United Nations National Institute of Health, "Complementary and alternative medicine; It is a broad field of health that encompasses all health services, methods, practices and accompanying theories and beliefs outside the politically dominant health system in a particular society or culture at a given time (Karayağız and Öztürk, 2008).

In other words, many definitions can be made about concepts such as complementary therapy, which are shaped by the culture, value judgments and beliefs of the society we live in. Traditional Medicine, or in other words, folk medicine, aims to evaluate the information to be obtained from the religious beliefs, socio-cultural values and other elements of the culture of the society in which it is transmitted from generation to generation and to use it in the treatment of diseases (Sütçü, 2018; Ersoy, 2014). An holistic approach to the concepts of health and disease is the essence of complementary alternative therapies. Observation, trial and error, and the use of datas obtained in this way as treatment have been transferred from each generation to the next and not only contributed to medical science, but also used as alternative treatment (Ersoy, 2014). The basis of alternative and complementary therapy applications is based on Chinese and Ayurvedic medicine treatments, and it has developed in many areas towards the end of the 19th century. The United States established alternative medicine offices within the national health institutes in 1993 and expanded its powers in 1998. It changed its name to the national center for alternative and complementary medicine (NCCAM, 2018). The purpose of this center; It is stated as to examine the reliability and effectiveness of complementary and alternative medicine applications and to ensure the participation of scientifically proven practices in traditional treatments (Karayağız and Öztürk, 2008).

However, when we summarize the factors that direct people to alternative treatments, the following come to forward;

*effective and high reliability

*The patient has a feeling that he can control the treatment

*does not include invasive methods

* therapists spend more time with the patient

*besides being accepted easily and getting depressed

* fear of side effects of applied medical treatments

*insecurity

*The patient has no hope of recovery.

(Ceyhan and Yiğit, 2016)

In general, the most common and most preferred complementary and alternative medical treatments from the past to the present; It covers ancient approaches such as Ayurveda, Acupuncture, Traditional Anatolian Folk Medicine, Herbalism and Yoga, and new approaches such as Oxygen Therapy, Ozone Therapy, Detoxification Therapy, Aromatherapy, Homeopathy, Dietary Support, Electromagnetic Fields, Massage, Reiki, Bioenergy and Hypnosis (http://tr.wikipedia.org/wiki/Alternatif).

Ayurveda treatment, which is the most preferred and widely used in countries such as China and India, is one of the oldest treatment methods (X century BC). It literally means 'science of wellness' and originates from the mystical philosophy of the Hindu religion.

It is widely practiced in Nepal, Sri Lanka and Bangladesh. It is accepted as focusing on healthy life instead of preventing the emergence of the disease or instead of intervening when the symptoms of the disease occur or curing the diseases. According to this system, the human body and the universe in which it lives are a whole, and human health is actually considered as a balance of various elements that make up this whole. Ways to live long and healthy; Recommendations on nutrition-lifestyle and physical-psychological health are presented in the form of complex systems such as massage, meditation and yoga techniques. Ayurveda practice can be summarized as maintaining a long and healthy life by reducing the effects of time and strengthening the body's immune system (Ozturk, 1990).

Another treatment method that attracts the most attention and has been the subject of research in recent years is herbal treatments. Since ancient times, plants have been used as medicine and serious successes have been achieved in the treatment of many diseases. Especially since the end of the 20th century, the tendency to treatments with herbs has increased rapidly due to reasons such as being more economical, easily accessible, and having less side effects and toxic effects. Herbal treatment sources such as garlic, green tea, ginger, Echinacea, Gingko biloba, Ginseng, Elderberry, Turmeric, St. John's Wort, Valerian, Chamomile are traditionally used to treat numerous illnesses, including heart disease, dementia, sleepping problems and some others. Because herbal remedies are derived from natural sources, they are generally considered safe, but this may not always be the case. Like traditional medicines, the use of herbal supplements may cause serious side effects or interact with other medicines taken.

When looking at complementary therapies in general, they are classified according to different criteria as follows:

1-Traditional Alternative Medicine: This field includes more common and accepted forms of therapy such as acupuncture, Ayurveda, naturopathy, homeopative, Oriental practices (Natural therapy, Chinese or Oriental medicine). Acupuncture is a kind of therapeutic model based on traditional Chinese medicine (includes Chinese herbal medicine, moxibustion, cupping, Chinese massage, mind-body therapies such as Qigong and tai chi, and dietary therapy). These therapies have been practiced around the world for centuries.

2- Biologically-Based Therapies

The biologically-based therapies include aromatherapy, diet-based therapies, chelation therapy, folk medicine, iridology, megavitamin therapy, phytotherapy/herbal medicine and neural therapy.

3-Body Therapies: Touch has been used in medicine since the early days of medical care. Tactile healing is based on the idea that illness or injury in one part of the body can affect all parts of the body. With manual manipulation, the body can fully focus on healing at the site of injury or illness if other parts can be brought back to optimum health. Body techniques are often combined with mind techniques. Examples of body therapies as chiropractic and osteopathic medicine; acupressure, Alexander technique, Bowen technique, chiropractic manipulation, Feldenkrais

method, osteopathic manipulation, reflexology, Rolfing, Trager bodywork, and Tui na, Meditation; Biofeedback and Hypnosis, Massage; including, Swedish massage, sports massage, deep tissue massage, and trigger point massage, body movement therapies; biofeedback, bioresonance, cognitive-behavioral therapies, deep-breathing exercises, tai chi, yoga, and shiatsu can be given. According to one of study; the use of frequency of this therapies change from 2% to 57%, with deep breathing and meditation most preferred. In this study, it was indicated that, Cardiac patients used these for stress, emotional health, and general wellness.

4-Dietary and Herbal Approach: For centuries, human beings have moved from a simple diet of meats, fruits, vegetables and grains to a diet consisting of foods usually rich in fats and complex carbohydrates. Both, as well as nutritional surplus and deficiency, have become problems that lead to certain chronic diseases in today's society. Many dietary and herbal approaches are used to balance the body's nutritional health. Within diet and herbal approaches, Dietary supplements; herbal medicines; Nutrition / Diet applications are included.

5-External Energy Applications: Some people believe that external energies from objects or other sources directly affect a person's health. Examples of external source energy therapys as Electromagnetic treatments; healing touch, light therapy, magnetic therapy, millimeter wave therapy, sound energy therapy, Reiki and Qigong can be given. Reiki include therapeutic touch and healing touch, known as together "hand-mediated" therapies and its aim to reduce pain and anxiety and increase health via healing energy aspect.

-Forms of Sense-Based Treatment: Some people believe that the senses, namely touch, sight, hearing, smell and taste, can affect overall health. Examples of therapies involving the senses are Art, dance and music;Visualization and auxiliary images also can be added (https://www.hopkinsmedicine. org/health/wellness-and-prevention/types-of-complementary-and- alternative-medicine; Rabito and Kaye, 2013).

In addition to all these, various researches on cardiovascular, lung and blood diseases and their treatments were conducted by the National Heart, Lung and Blood Institute and the National Institute of Complementary and Alternative Medicine in 2001.

In the workshop held on the general use of CAM applications, there were positive opinions on the fact that individuals began to prefer CAM applications to a large extent, and for this reason, a joint decision was taken to initiate large-scale clinical studies in order to maintain and protect the health of the population. It was emphasized that more serious research should be done to understand the basic functioning of CAM applications, that time and resources could be wasted and even risky. It is thought that meaningful basic and clinical studies are needed to standardize the use of procedural CAM interventions and herbal products in treatment (Lin, 2001).

Standart Conventional Treatment and Drugs for Cardiovascular Treatment

After the definitive diagnosis of heart attack is made, it is necessary to start treatment quickly to unclog the coronary vessels in the cardiovascular system, and for this purpose, two treatment methods are applied;

These are;

1. Clot Dissolving Drugs (Thrombolytic Therapy)

2. Percutaneous coronary intervention (coronary angiography and angioplasty)

If we look at the interaction between drugs used in the treatment of cardiovascular diseases and CAM;

-Warfarin and its use for antithrombotic therapy:

Many herbal uses have interactions with warfarin. During the use of herbal therapy, it increases the risk of bleeding in patients receiving warfarin or conventional antiplatelet therapy, etc. It can make positive or negative changes in platelet function, such as In many cases, it is thought to increase the likelihood of bleeding; however, numerous cases of increased bleeding tendency have been reported in patients using herbal supplements with or without warfarin.

-Digoxin: It has been explained that some plants can act as digoxin-like active substances and therefore, plants that are likely to act in this way can increase the digoxin effects of plants. Chinese silk vine (Periploca Sepium) is a poisonous plant containing cardiac glycosides. Uzarae radix (uzara root) has been noted to have cardiac effects like digoxin when used in high doses. Serious side effects of herbal treatments may result from lack of standardization and inadequacy in packaging and preparation.

-Cyclosporine: This substance, which is used for immunosuppression after heart transplantation, is exposed to many drug-drug interactions. For example, interaction with Diltizem causes increased levels of cyclosporine. If diltizem is used in patients with heart transplantation and hypertension, the dose of cyclosporine should be reduced. St. John's Wort is used as herbal treatment for those with depression and mood disorders. Unexpected interactions with cyclosporine have been reported in various sources. It is stated that it causes a decrease in the level of cyclosporine and its rapid elimination from the body (Miller et al., 2004).

After all these definitions of CAM and Cardiovascular area, we summarized many different CAM applications that have been tried in different geographies and including different applications in this field, and aimed to reveal the advantages and disadvantages of CAM applications in the Cardiovascular field, as well as being used in the treatment of many different diseases.

Summarized of some Studies on Cardiovascular Fields:

Studies to determine the use of CAM (complementary alternative therapy) in the field of treatment of heart disorders are also carried out in different countries.

One of them; It was conducted based on a questionnaire analysis between April 2013 and April 2014 to determine the use of CAM in patients in the Cardiac Surgery unit of Angers University Hospital in France. It was understood that 58% of the 154 patients included in the study used a complementary and alternative medicine at least once in their lifetime, 38% in the previous year before the disease appeared, and 14% between consultation and surgical intervention.

In total, 71% preferred alternative treatment at any stage of their illness, and only 29% of those who used it informed their doctors and paramedics about it. It has been understood that the use of complementary and alternative medicines is common among patients who apply to cardiac surgery units, but the authorities are not sufficiently informed about this issue (Dalmayrac et al., 2016).

Cardiovascular diseases (CVD) have traditionally been treated with medications and lifestyle changes. The use of complementary therapies in this field has been increasing in recent years. The use and success of CAM in the prevention and treatment of CVD, including hypertension, hyperlipidemia, coronary artery disease, heart failure, and arrhythmias, was discussed in this another study. Especially It has been suggested that Garlic, which is included in TAT herbal applications, may exert an antiatherosclerotic effect by interfering with the inflammatory and oxidative pathways and inhibiting the lipid shield in the vessels, and it may mediate decreased peripheral vascular radiation, vasodilation and smooth muscle expansion.

It has been concluded that alternative methods such as special nutrition programs, meditation, yoga, acupuncture, herbal herbs and vitamin supplements that can be used as an adjunct to traditional medical care to improve cardiovascular health and quality of life can be used in this area

as promising, and it has been emphasized that similar results have been obtained from different studies. But It was stated that physicians interested in the subject should be informed about the possible effects of CAM interventions such as diet, exercise, supplements, herbs and mind/body techniques (Aggarwal et al., 2017).

In the surgically created heart attack model, animals were administered T3 and T4 hormones via gavage as a treatment model in another study and the values of proapoptic proteins p53, JNK, BAX, Bcl2 were examined to determine the effect of the treatment given afterwards. It was determined that thyroid hormones improve cardiac function and decrease the expression of proapoptic proteins after heart attack since apoptosis is determined by genetically p53 evaluation. It was concluded that hormone therapy, which is one of the CAM methods, has advantages in terms of the therapeutic effect of heart attacks and the availability of alternative treatments against heart attacks (Castro et al., 2016).

In the heart attack model caused by doxorubucin carried out by Shahid et al. in 2021; It has been reported that doxorubucin was given every other day and intraperitoneally for two weeks, and oral administration was applied for 14 days to determine the cardioprotective effect of boswellic acids for treatment purposes. It was stated that blood lipid profile and cardiac biomarkers including LDH, CK-MB, CPK, SGOT and troponin T were measured and histopathological evaluations were made. According to the results obtained, it was stated that there was a significant increase in cardiac enzyme levels and full lipid profile parameters in the diseased group compared to the control group. On the basis of pretreatment with boswellic acids reduced the level of all measured parameters and reduced the severity of myocardial damage, as supported by histopathological studies as well (Shahid et al., 2021).

In a study conducted by Saravanan and Prakash in 2004 to investigate the effect of garlic (Allium sativum) on lipid peroxidation in heart attack; Iso was used to create a heart attack, and garlic oil was used as a treatment agent within herbal treatment CAM. As stated in the study; In myocardial necrosis caused by isoproterenol; A significant increase in serum iron content, a significant decrease in plasma iron binding capacity, ceruloplasmin activity and glutathione (GSH) level were observed, while a significant increase in lipid peroxide levels was obtained. It has been stated that the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and glutathione reductase (GRD) are significantly reduced in the heart with isoproterenol-induced myocardial necrosis. According to the results obtained; It has been stated that garlic oil provides a significant return with its curative effect in metabolic changes due to heart attack caused by isoproterenol. It is an important study in terms of using garlic-containing oil as a treatment agent and demonstrating the curative effect of garlic as an alternative supportive treatment (Saravanan and Prakash, 2004).

In the study of Mnafgui et al. in 2016; In the iso-induced heart attack model, the antithrombotic, anti-inflammatory and cardiac preventive effects of euganol were investigated. There were first negative and then positive changes in troponin-T, CK-MB and LDH and ALT plasma levels in all disease-induced and treated groups. An improvement was observed in the markers indicating cardiac damage in the eugonal treatment group, and that eugenol reduced inflammation by reducing inflammatory mediator proteins; indicating eugenol has a high level of protective effects in the cardiac formation process. It is important that the use of eujonal as a treatment agent and the subsequent improvements in biomarkers reveal the curative effect of eugenol, the active ingredient of garlic, as a herbal CAM application (Mnafgui et al., 2016).

In the study conducted by Ganguly and Alam in 2015 to determine homocysteine and its effects on the cardiovascular system; It has been stated that homocysteine can initiate an inflammatory response in vascular smooth muscle cells by stimulating the production of CRP. These findings provided new evidence for the role of homocysteine in the pathogenesis of the

disease and it was stated that it may be sufficient to prove the role of homocysteine in the pathogenesis of cardiovascular disease.

In this study, it was found that hyperhomocysteinemia may develop due to folate, vitamin B12 or choline deficiency, and It has been stated that the negative situation can be corrected and the risk of CVD caused by it can be prevented with supportive treatments such as nutrition and exercise. This study is important in terms of revealing the effect of homocysteine use and exposure to hyperhomocysteinemia on cardiovascular disease risk factors such as myocardial damage and also the effect of folate, vitamin B12 or choline deficiency on the occurrence of heart attack, which can be complemented by CAM supplementation applications. (Ganguly & Alam, 2015).

In other study; 96 male Wistar rats (16 weeks old) were used by dividing into 12 groups. Transthoracic echocardiography was performed on the rats. 4 weeks' treatment was appplied with using Allium sativum, Peganum harmala and Apium graveolens as CAM methods. Acording to findings, Allium sativum, Peganum harmala and Berberis vulgaris could significantly improve cardiac function by improvement of left ventricular remodeling, lowering hs-CRP and NT-ProBNP and echocardiographic indexes without toxic effect into rats, suggesting they can be used as an alternative treatment with no harmfull effect (Keihanian et al., 2021).

Oxidative stress is considered an important factor for several different disease including cardiovascular disease (CVD). An active components of Chinese herbal medicines (CHMs) have been searched and widely used for the treatment of CVD, including hypertension and coronary heart disease. In a different study, various active CHM components have been searched and It has been found that the active CHM components including astragaloside, ginsenoside and resveratrol can regulate and modulate the oxidative stress and the circulatory system, suggesting they can be used as an potential drugs for the treatment of various CVDs (Chang et al., 2020).

Gallic acid is a trihydroxybenzoic acid found in tea leaves and some plants. In a other study, the efect of gallic acid as an herbal treatment, on cardiac dysfunction and fibrosis in a mouse model of pressure overload-induced heart failure and in primary rat cardiac fibroblasts were searched. It was found that Gallic acid reduces cardiac hypertrophy, dysfunction, fibrosis and the expression of collagen type I and connective tissue growth factor; It decreases left ventricular end-diastolic and end-systolic diameter, perivascular fibrosis, and recovers the reduced fractional shortening and it suppresses the expression of atrial natriuretic peptide, brain natriuretic peptide, skeletal α-actin, and β-myosin heavy chain. According to these findings; gallic acid is a therapeutic agent and can be used safely for cardiac dysfunction and fibrosis in chronic heart failure (Jin et al., 2018).

In a different study, heart attack induced by experimentally giving the active substance (isopretrenol) to rats and to cause myocardial damage by adding homocysteine to the drinking water of the rats. Resveratrol, garlic extract and euganol as a complementary treatment were given. Regarding heart attack and damage, experimentally administered isopretrenol causes heart attack and homocysteine causes myocardial damage, the applied active substances of CAM methods had a protective and therapeutic effect at different doses and levels, suppresses apoptosis on the basis of genetic, molecular and biochemical analysis, indicating they can be used effectively and safetly (Kocaman, 2022; Kocaman and Dosay-Akbulut, 2021).

In different study, a cross-sectional survey was conducted to the patients of cardiovascular clinics at Singapore for 2 months in 2014. Information included subjects related to various aspects of CAM use and its effect. According to findings; The prevalence of CAM use was 43.4 % (333/768). Biologically-based systems (29.4 %) was the most preferred type of CAM. Some patients (19.0 %) used multiple types of CAM. Approximately half of patients (50.8 %, n = 169) found CAM effective in treating their heart condition, while a smaller proportion (25.2 %, n = 84) found CAM more effective than conventional western medicine in treating their heart condition. A small

number of CAM users (7.8 %, n = 26) come a cross with side effects from CAM which included gastrointestinal complaints, allergy and rash.These findings suggested that CAM methods are popular within and can be prefer and use safely by cardiovascular patients (Teo et al., 2016).

Another similar study was conducted to evaluate the effects of Mind-body practices (MBI) (eg, Tai Chi, yoga, meditation) included in CAM on individuals with heart failure. For this purpose, the questions were asked to 1314 participants for determining the use and effects of 9 CAM methods covering mind-body practice. The results were found as follows; Tai Chi (n = 7), yoga (n = 4), relaxation (n = 4), meditation (n = 2), acupuncture (n = 2), biofeedback (n = 2), stress management (n = 1) , Pilates (n = 1) and reflexology (n = 1). Most of the participants (n = 22, 95.8%) experienced quality of life (14/14), exercise capacity (8/9), depression (5/5), anxiety and fatigue (4/4), blood pressure (3/5), stated that there was an improvement in criteria ranging from low to moderate according to heart rate (5/6) and heart rate variability (7/9) criteria. According to these data; It has been stated that CAM applications can create moderate advantages in people with heart disease and can be applied and preferred as a supportive treatment. (Dosay-Akbulut, 2020).

In a similar different study, 10,572 respondents with cardiovascular disease were used and analyzed data on their CAM use. Among them, 36% had used CAM in a 12 months period. The most commonly used therapies were herbal products (18%) and mind-body therapies (17%). Among herbs, garlic, echinacea, ginseng, ginkgo biloba, and glucosamine with or without chondroitin were preferred mostly. Among mind-body therapies, deep-breathing exercises and meditation come forward. The patient that used CAM for their cardiovascular condition within this survey, reported the therapies as beneficial (80% for herbs, 94% for mind-body therapies) (Yeh et al., 2006).

Another study; based on determination of CAM using ratio after acute myocardial infarction (AMI). Patients with AMI were assessed using Angina Frequency and Quality of Life domains from the Seattle Angina Questionnaire and the Short Form-12 Physical and Mental Component scales for their CAM using ratio and their choosing categories (as mind-body, biological, and manipulative therapies) prior to and 1 year after AMI obtained from 24 US sites.

Among 1884 patients which they indicated not using CAM at the time of their AMI, 33% of them reported that started et least 1 forms of CAM therapy 1 year following AMI. 62% adopted mind-body therapies, 42% adopted biological therapies, and 15% started to use manipulative therapies. According to findings; they were not obtaine any association between CAM use and health-status recovery after AMI within patients of this study (Shafiq et al., 20016).

Coronary heart disease is determined and known with the formation of arterial plaque. If care is not taken, it can be cause important problems like myocardial infarction and heart failure. Zhishi Xiebai Guizhi Decoction can be use as a representative prescription in the treatment of coronary heart disease. This another study was dizayn to give the information related to mechanism of Zhishi Xiebai Guizhi Decoction in treatment of coronary heart disease. In this study, first of all the content of Zhishi Xiebai Guizhi Decoction was determined. Then, it was aimed to determine and analyze the molecular targets of Zhishi Xiebai Guizhi Decoction in coronary heart disease by using OMIM and GeneCards databases.

In the findings; by using network pharmacology analysis, they were found that different important core compounds of Zhishi Xiebai Guizhi Decoction (includes five herbs; as Aurantiifructus immaturus, Allii macrostemonis bulbus, Trichosanthisfructus, Magnoliae officinalis cortex, and Cinnamomi ramulusand) has therapeutic efficacy and anti-inflammatory effects especially agains to chest stuffiness and indirectly against to cardiovascular diseases.

According to results; they indicated that Zhishi Xiebai Guizhi Decoction as one of CAM types may play a role in treating coronary heart disease with successfully via several different signaling

pathways and it can be nomanated in using against to coronary heart disease (Liu et al., 2022; Gao et al., 2021).

Another study; carried out to determine the using ratio of CAM within hospitalized patients with acute coronary syndrome from March 1, 2001 to October 31, 2002. Because the complementary and alternative medicine (CAM) is generally preferred by patients with cardiovascular disease. Poisson regression models were used to assess the results.

In the findings; CAM using ratio was 19% in patients with coronary artery disease (CAD). Most of them who used CAM were non-Caucasian (31% vs 12%), uninsured (12% vs 7%), economically burdened (58% vs 29%), and had depression (13% vs 6%, $p<0.05$ for all). The most preferred CAM types in patients using CAM are beta-blockers (64% vs. 46%, $p=0.008$), aspirin (73% vs. 74%, $p=0.90$), and statins (68% vs. versus 71%, p=).

According the results, CAM users of this study have worse socioeconomic status but in comparison with standart therapies no evidence was obtained that using of CAM cause to worse situation (Decker et al., 2007).

There is unsufficient datas on the use of complementary and alternative medicine (CAM) by patients diagnosed with coronary heart disease (CHD). In this study; the using ratio of CAM among patients with CHD, the reasons and factors effecting their choise, the preffered types of CAM, and the relationship between CAM using situation and patient's demographics were searched.

A cross-sectional questionnaire descriptive study was carried out with Palestinian CHD patients from three different hospitals. Siahpush scale was used to determine the approach of CHD patients toward CAM use.

150 patients were interviewed and 128 (85.3%) of the them completed the questionnaire. The majority of CAM users indicated they use CAM for health problems other than CHD, while a total of 59 (45.9%) patients have used CAM for their heart problems. Also; religious practices were found to be the most preferable form of CAM used by patients, while body and traditional alternative methods were the less.

According to the findings; Participants of this study commonly prefer CAM. Also, Religious practices come to first (Salah et al., 2020).

Another study was carried out to determine the possible use of Traditional Chinese medicine (TCM) within the first 24 hours of hospitalization (early IV TCM) for acute myocardial infarction (AMI). Datas were collected from 175 modern Western medicine hospitals throughout China including total of 14 097 patients with AMI across the 3 years. Within the CAM techniques the most preferable ones was herbal medicines and divided into 6 main groups according to their contents. These are respectively, Salvia miltiorrhiza; Panax notoginseng; Folium ginkgo; puerarin; and breviscapinun. According to result, almost all (99%) hospitals tried early intravenous TCM. The most commonly used early intravenous TCMs were Salvia miltiorrhiza (danshen) (35.5%), after that Panax notoginseng (12.2%); Folium ginkgo (7.5%); puerarin (2.7%); and breviscapinun (2.2%) come to forward respectively. On the basis of analyses, no significant associations between early intravenous TCM and in-hospital bleeding or mortality was obtained (Spatzs et al., 2018).

Complementary and alternative medicine (CAM) is most commonly used in China in the treatment of coronary artery disease (CAD). In different study; to determine the CAM using ratio and types and related informations against to CAD, a questionare study was design in Beijing. The obtained datas were analyzed with Student's t-test and chi-square test. The datas wete obtained from 546 patients with a diagnosis of CAD in the period of May to July, 2009. According to findings; 69.1% of the patients with CAD used at least one of CAM types. Patent herbal medicine (90.7%) was the most preferred CAM type. Comparison to non-CAM users, It was reported that

CAM users were older ($p < 0.01$), had a longer disease course ($p = 0.02$), and had better current health status.

On the basis of results; there are several different and important factors which can affect the use of CAM by CAD patients (Chu et al., 20013).

In another study; the use of complementary and alternative medicine (CAM) possibilies, ratios, types and the reasons among people with type 2 diabetes and/or cardiovascular disease (CVD), and the effect onto the quality of life were searched.

For this study; a survey was applied via mail and internet in Victoria, Australia.

The applicants were adults with type 2 diabetes and/or CVD, questiones were asked about whether they used CAM therapy as well as conventional medical treatment.

In the findings; The answers were given as follows; from 2766 people, 45.1% had used CAM in last 1 year period with between 20-96 years old ages. CAM users reported lower (worse) quality of life data than nonusers. In contrast, CAM users reported more positive changes in their perception of illness and behavior towards it than nonusers.

Most CAM users (94.6%; n=1180) used CAM, primarily vitamins, minerals and other nutritional supplements; 96.9% used them in addition to prescription drugs in standard treatment. 54.5% (n=671) of CAM users received counseling from CAM practitioners.

The rate of recommendation for the first use of CAM by a doctor, pharmacist or another healthcare professional (42.7%); Belief that CAM will be good (39.4%); The belief that CAM is not harmful (33.3%); a desire to have more control over their health (31.3%); desire to use "natural" (27.8%); close circle advice (23.8%); results were obtained as dissatisfaction with the standard treatment (18.2%).

These findings show that the use of CAM has a positive effect on the perspectives of people with chronic conditions. Considering the general high use of CAM, there seems to be a belief that the use of CAM can support the prevention of chronic diseases (Canavay et al., 2013).

Complementary and alternative medical (CAM) therapies are growing in popularity. There is little information on the use and success of CAM therapy, especially in patients with cardiovascular disease (CVD). In another study; telephone questionnaires were applied to 107 randomly selected patients with CVD. In the findings; 64% of the surveyed patients reported using CAM. Nutritional supplements (40%) and vitamins (35%) were the most preferred ones. Most of the CAM users (65%) declared that they applied to CAM because of their heart conditions. Friends or relatives (43%) or their own doctors were reported as the sources of information about CAM. However, the majority of respondents stated that they also received an oral supplement at the same time as CAM.

According to findings; It is seen that the use of CAM treatments is high on the basis of patients participating in the survey. Therefore, more illumination and studies are needed on this subject (Wood et al., 2003).

The use of complementary and alternative medicine (CAM) is increasing day by day. People with cardiovascular diseases frequently use CAM, despite significant side effects and insufficient information to support its use. This study was planned to reveal the relationship between CAM use in heart patients, the types of CAM used, the reasons and factors affecting its use, patient demographics and CAM use.

This study was conducted with 329 adult heart patients in the South-West District of Trinidad and Tobago. A questionnaire was applied to the participants between July 1, 2012 and August 31, 2012. Data analysis included $\chi(2)$ tests and binary logistic regression.

In the result; One hundred and eighty-five (56.2%; standard error [SE] = 2.74%) patients were found to use CAM. Herbal medicines are the most common (85.9%; SE = 2.56%); Among the CAM methods used, spiritual therapy/mind-body systems (61.6%; SE = 3.58%), physical therapy/body manipulation (13.5%; SE = 2.51%), alternative systems (8%, 1; SE = 2.01%) and other methods (3.8%; SE = 1.41%) respectively.

The most frequently referenced CAM subtypes were determined as omega-3 fatty acids (54.6%), B vitamins (50.4%), special diet/nutritional supplements (41.1%), and faith healing (54.1%). Participants believe that CAM improves health and wellness (79.5%; SE = 2.97%), is supportive in the fight against diseases (78.9%), believes that it can overcome the limitations of standard treatment (69%), those who believed it relieved symptoms (21.6%), cost less than standard treatment (CM) (21.6%) and had symptoms those who stated that they improved less than those who stated that there were fewer adverse/damaging effects compared to standard treatment (CM) (29.7%) or those who were unsatisfied with CM treatment (12.4%) results are declared. Ethnicity has been found to have an impressively greater effect on CAM use than religion.

Acording to findings; In this study, the use of complementary and alternative medicine was found to be high in heart patients (56.2%, SE = 2.74%), and it was revealed that ethnicity and close environment affected the use of CAM (Bahall, 2015).

It would be beneficial for patients with coronary heart disease to learn how to manage their condition and make the necessary controls in order to maximize their quality of life and prevent recurrence or worsening of the disease. This study was planned to investigate the behaviors of patients towards the use of complementary and alternative drugs and therapies (CAM) within 1 year after experiencing a cardiac problem in England.

A questionnaire was applied to 463 patients who applied to 4 hospitals in the West Midland region of England during a 12-month follow-up period. In the results; 91.1% of the patients participated in the survey study and 29.1% stated that they used CAM (mostly, acupuncture, homeopathy, chiropractic, dietary supplements, massage and rubbing for aches and pains, exercises) and/or one of the test kits, but very few (8.9%) used both methods. According to the results, CAM was used more frequently in the treatment of other diseases than cardiovascular diseases. Self-test kit use (77.2%) was found to be more common than CAM (31.7%), the most common (80.0%) being recorded as blood pressure monitors. The majority of the patients (89.5%) declared that they bought these test kits from various sources on their own initiative.

On the basis of findings; It was stated that the patients tried new technologies to monitor their cardiovascular health, and the rate of CAM usage was less than that of test kits (Greenfield et al., 2008).

This another study was conducted to evaluate the frequency and types of CAM treatments in patients hospitalized with acute coronary syndrome arising from cardiac causes.

An attempt was made to reveal the profile of CAM users. Data were collected through interview and based on information on demographics, patient's medical history, CAM use, doctor visits, and patient-physician communication. 223 patients provided the necessary information by meeting the appropriate criteria and filling out the questionnaire. Sixty-three percent stated that they had used at least one CAM treatment in the past 1 year for general health purposes. Only 11.7% of the patients reported using CAM for cardiovascular problems. The rate of CAM use was found to be higher in women than in men, and it was observed that they tended to use more CAM treatments. More than one third (35.9%) of the patients using CAM did not inform their doctor about it (Barraco et al., 2005).

Complementary and alternative medicine (CAM) can provide both risks and benefits for people with cardiovascular disease. In this study,

A general systematic study was conducted, including studies on the use of CAM in patients with cardiovascular disease. Twelve of the 27 studies included in this overall evaluation were in the United States; six of them were made in Canada. The remaining nine were conducted in England, Hong Kong, India, Italy, Korea, Nigeria, Spain and Turkey. It was stated that sample sizes ranged from 65 to 10572 patient data.

When we look at the results; Twenty-seven studies were included in this study and the data were evaluated. It has been observed that the rate of CAM use in heart patients varies between 4-61%. Among the CAM types used, the rate of biologically based treatments varied between 22% and 68%, while herbal medicines were used between 2% and 46%. Within the biologialy based treatments; vitamins, minerals and other dietary supplements were preferred and used between 3% and 54%. The most used supplements were vitamin B/B12 or B complex, vitamin C, vitamin E, calcium, glucosamine/chondroitin, coenzyme Q10, calcium and magnesium. The most preferred herbs were echinacea (2% to 14%), garlic (1% to 69%), ginger (1% to 24%), ginkgo (1% to 9%), and ginseng. The using ratio of mind-body therapy (MBT) ranged from 2 to 57% with prayer and fasting. Aiming good health were the main reasons for taking vitamins and herbal therapy as well as specifically to treat cardiovascular disease and believe to get benefit from CAM. The majority of patients did not inform their healthcare professionals about their use of CAM.

According to these results; The use of CAM appeared to be common in patients with cardiovascular disease. The findings stated that the effects of CAM on the medical treatment of cardiovascular disease were not taken seriously and patient-physician communication should be strengthened in this regard (Grant et al., 2012).

Another study was conducted to examine the frequency and pattern of complementary and alternative medicine (CAM) use in patients with hypertension who applied to a hypertension clinic. 225 patients who applied to the hypertension clinic of Lagos University Teaching Hospital over a 3-month period were interviewed and data were collected. Information on socio-demographic data, hypertension, blood pressure, compliance with conventional drugs and use of CAM were obtained. Healing systems, mind-body connections, dietary supplements and herbs, energy therapy, and manipulation and touch therapy types were classified into questions to determine the CAM methods used.

When we look at the results; Data from 90 (40%) male and 135 (60%) female patients were evaluated. 88 (39.1%) of the participants stated that they use CAM. Herbal products were declared as the most preferred type of CAM. Garlic was preferred as the most frequently used herbal product (69.3%). Others are native plants (25%), ginger (23.9%), bitter leaf (Vernonia amygdalina) (9.1%) and aloe vera (4.5%). Spiritual therapy was used at a rate of 2.5%. None of the patients used healing systems, energy therapy, or manipulation and touch as CAM types. There was no difference between CAM users and non-users in terms of characteristics such as socio-economic status and blood pressure control.

According to these results, it has been revealed that a significant proportion of hypertensive patients use CAM therapies. Due to the high rate of use of herbal CAM methods among the hypertensive patients participating in this study, it is seen that it is important to inform the physicians about the benefits and harms of such practices for hypertension and related heart diseases that have the potential to ocur (Amira et al., 2007).

In another similar study; Information was obtained from 200 randomly selected adults by face-to-face interviews in Durban, South Africa. When we look at the results;

The prevalence of CAM use for the 2000/2001 period was found to be 38.5%. Spiritual healing and herbal/natural medicines, including vitamins, were the most common types, 42.8% and 48.1%, respectively, within the general CAM use. People stated that they use CAM for the treatment of

diseases such as diabetes mellitus, headaches, arthritis and joint pains, stress, skin disorders, backaches, hypertension and nasal disorders, especially cardiovascular disorders. Half of CAM users also preferred drugs. Analysis showed that age, gender, marital status, religion, education level and income did not affect the use of CAM. More than half of CAM users (51.9%) stated that they used it after a relative recommendation or a CAM-related advertisement in the press. Seventy-nine percent of users stated that they got positive results from their treatments. Fifty-four percent of CAM users (excluding those who only use spiritual healing) said that they did not inform their doctors about using CAM.

Although CAM is preferred to treat many different ailments, this practice is not specific to a particular demographic and has been found to be similar to the rest of the world in many ways. It was stated that since most CAM users are satisfied with the effects of CAM, it would be beneficial to conduct more research on this subject and reveal its possible positive and negative effects (Singh et al., 2004).

Another similar study was conducted to evaluate the current and prospective use of complementary and alternative medicine (CAM) therapies in outpatients with cardiovascular disease. This study was planned in response to the increasing interest in CAM treatments and the need to determine their use, especially by patients with cardiovascular disease. The data was created with a 17-question questionnaire. The questionnaire was completed by 1,055 patients (655 males, 351 females; mean age 63.5 years). Of the respondents, 36.8% had cardiac symptoms for more than 10 years, 48.2% had coronary artery disease, and 82.5% stated that they had used CAM therapy. In the use of CAM, as a variety; 75.4% stated that they use dietary supplements, 31.5% chiropractic treatment, 23.9% mind-body therapies and 19.2% massage. The top 5 dietary supplements used were multivitamins (52.8%), fish oil (45.2%), vitamin C (36.1%), vitamin E (29.0%), and fiber (27.1%). The top supplements used for heart-related conditions were fish oils, omega 3 fatty acids, multivitamins, folic acid, coenzyme Q10, and vitamin E. Only 14.4% said that they informed their doctors about the use of CAM. The most preferred practices for improving cardiac symptoms were relaxation techniques, stress management, meditation and guided imagery. Among patients with coronary artery disease, the greatest benefit from using these methods was reported to be chest pain (20.0%), sleep disturbance (17.9%), leg swelling (17.9%), and shortness of breath (13.9%). It was stated that the majority of the participating patients used CAM therapies and were warmly welcomed to these applications (Prasad et al., 2013).

Conclusion

One of the diseases with the highest mortality rate in the world is heart attack. The incidence of ischemic heart disease, hypertension, diabetes and other chronic diseases, increased consumption of foods high in fat and sugar, and sedentary behaviors cause an increase in cardiovascular problems. In order to correct this situation where many risk factors are effective, to increase the quality of life of the society, to reduce the morbidity and mortality rates, and most importantly, for people to access the treatment in the most effective, fastest, most economical and most accessible way, there are different methods for the treatment of Cardiovascular problems.

Due to reasons such as the increase in the cost of classical drug therapy and surgical interventions in the world, the difficulty of reaching the treatment on time and within the available possibilities, the increase in awareness about the increasing side effects of drug therapy, it is at the forefront in developed and developing countries and is preferred as an alternative to other treatments. The use of alternative treatments has become widespread.

It is known that there is a significant increase in the preference of alternative methods, especially in the treatment of heart attack, in addition to the known anticoagulant and antithrombotic treatments. It is stated that early diagnosis and treatment of a heart attack reduces the damage to the heart, and with this, before a definitive heart attack is diagnosed, various

treatments are applied even in case of doubt to reduce the negative effect. Because of the narrow therapeutic range of cardiac drugs, it has been stated that the possibility of adverse interactions with dietary supplements and herbal medicines is of concern, therefore it is important to understand the frequency and nature of CAM use in patients with CVD in order to promote beneficial CAM therapies and prevent possible herb-drug interactions.

In this study, myocardial infarction, within the Cardiovascular problems and its alternative treatments, the usability of these treatments, as well as the efficacy and causes of alternative treatments in the world with summarize of some related sudies are mentioned. It was concluded that complementary alternative treatment methods can be used in cases where conventional treatments are insufficient and under the control of a doctor.

References

1- Aggarwal, M. Aggarwal, B. Rao, J. (2017). Integrative Medicine for Cardiovascular Disease and Prevention. *The Medical Clinics of North America, 101(5)*, 895-923.

2- Amira, O.C. Okubadejo, N.U. (2007). Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care centre in Nigeria. *BMC Complementary and Alternative Medicine, (7),* 30. doi: 10.1186/1472-6882-7-30.

3- Aybek, T. (2020). *TOBB ETÜ Hastanesi,* Ankara. ta@tayfunaybek.com

4- Bahall, M. (2015). Complementary and alternative medicine usage among cardiac patients: a descriptive study. *BMC Complementary and Alternative Medicine, (15),*100. doi: 10.1186/s12906-015-0610-y.)

5- Barraco, D. Valencia, G. Riba, A.L. Nareddy, S. Draus, C.B. Schwartz, S.M. (2005). Complementary and alternative medicine (CAM) use patterns and disclosure to physicians in acute coronary syndromes patients. *Complementary Therapies in Medicine, 13(1),* 34-40. doi: 10.1016/j.ctim.2005.02.003.

6- Beştepe, N. Dönderici, Ö. Demirkan, B. (2020). Evaluatıon of major rısk factors and metabolıc syndrome crıterıa ın predıctıng coronary artery dısease rısk. *Ankara Medical Journal, (4),*844-857 // 10.5505/amj..93276

7- Canaway, R. Manderson, L. (2013) Quality of life, perceptions of health and illness, and complementary therapy use among people with type 2 diabetes and cardiovascular disease. *Journal of Alternative and Complementary Medicine, 19(11),* 882-90. doi: 10.1089/acm.2012.0617. Epub 2013 Jun 21.)

8-Castro, A.L.D. Fernandes, R.O. Ortiz, V.D. Campos, C. Bonetto, J.H.P. Fernandes, T.R.G. Conzatti, A. Siqueira, R. Tavares, A.V. Schenkel, P.C. Belló-Klein, A. Araujo, A.S.R. (2016). Thyroid hormones improve cardiac function and decrease expression of pro-apoptotic proteins in the heart of rats 14 days after infarction, *Apoptosis*, *(21)*, 184–194. 10. 1007/s10495-015-1204-3

9-Chang, X. Zhang, T. Zhang, W. Zhao, Z. and Sun, J. (2020). Natural Drugs as a Treatment Strategy for Cardiovascular Disease through the Regulation of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *(2020),* 5430407. https://doi.org/10.1155/2020/5430407

10- Ceyhan, D. Yiğit, T.T. (2016). Güncel Tamamlayıcı ve Alternatif Tıbbi Tedavilerin Sağlık Uygulamalarındaki Yeri. *Düzce Üniversitesi Sağlık Bilimleri enstitüsü dergisi*, 6(3), 178-189.

11- Chu, F.Y. Yan, X. Zhang, Z. Xiong, X.J. Wang, J. Liu, H.X.(2013). Features of complementary and alternative medicine use by patients with coronary artery disease in Beijing: a cross-sectional study. *BMC Complementary and AlternativeMedicine, (13), 287*. doi: 10.1186/1472-6882-13-287.)

12- Coşkun, M.Z. Tarı, E. Ateş, S. Kırma, C. Kılıçgedik, A. İzgi, A. Durduran, S. Kaya, A.B. (2009). *TMMOB Harita ve Kadastro Mühendisleri Odası 12. Türkiye Harita Bilimsel ve Teknik Kurultayı,* 11¬15 mayıs, Ankara İstanbul'da akut kalp krizi haritalarının coğrafi bilgi sistemleri ile üretilmesi ve geo¬istatiksel olarak incelenmesi.

13- Dalmayrac, E. Quignon, B. Baufreton, C. (2016).Complementary and Alternative Medicine in Cardiac Surgery: Prevalence and Modality of use. *Heart Lung Circulation, 25(7)*, 712-8.

14- Daşkapan, A. (2013). Kadın ve Kalp Damar Hastalıkları. *Aile ve Kadın Sempozyumu*, 16 Mayıs, Kırıkkale, 23.

15- Decker,C. Huddleston, J. Kosiborod, M. Buchanan, D.M. Stoner, C. Jones, A. Banerjee, S. Spertus, J.A. (2007). Self-reported use of complementary and alternative medicine in patients with previous acute coronary syndrome. *American Journal of Cardiology, 99(7),* 930-3. doi: 10.1016/j.amjcard.2006.11.041.

16-Dosay-Akbulut, M. (2020). *Bireysel Tıp ve Alternatif Tıp:* Tanım, Uygulamaları ile Kullanım Olasılıkları ve Kısıtlamaları. Nobel Tıp Kitabevleri. 68 pp.

17-Ersoy, R. (2014). Modernizm-Postmodernizm Bağlamında Geleneksel Tıp Uygulamalarının Güncelliği Üzerine Bir Değerlendirme. *Milli Folklor, 26(101*),182-192.

18- Ganguly, P. Alam, S.F. (2015). Role of homocysteine in the development of cardiovascular disease, *Nutrition Journal*, *(14)*, 6. 10.1186/1475-2891-14-6

19- Gao, J. Pan, Y. Zhao, Y. Li, H. Mi, Z. Chen, H. Tan, X.. (2021).Network Pharmacology Study on Molecular Mechanisms of Zhishi Xiebai Guizhi Decoction in the Treatment of Coronary Heart Disease. *Evidence Based on Complementeray and Alternative Medicine, (2021);* 3574321. doi: 10.1155/2021/3574321)

20- Genç, F. and Yiğitbaş, Ç. (2021). Hipertansif yaşlıların hipertansiyon algıları ve kontrollerine ilişkin davranışları. *Genel Tıp Dergisi;31(1)*, 1-11

56- Grant, S.J. Bin, Y.S. Kiat, H. Chang, D.H. (2012). The use of complementary and alternative medicine by people with cardiovascular disease: a systematic review. *BMC Public Health, (12), 299*.

21- İlhan, A.O. (2016). Investigation of the effect of chronic alcohol use on myocardial apoptosis and the cardioprotective role of calpain inhibitor 1'xxin (N-acetyl-leucine-leucine-norleucinal) in a heart attack model induced by isoproterenol in rats. *PhD thesis*, ESOGÜ, Eskişehir, 119 pp.

22- Greenfield, S. Pattison, H. Jolly, C. (2008). Use of complementary and alternative medicine and self-tests by coronary heart disease patients. *BMC Complementary and Alternative Medicine, (8),* 47 pp.

23- İşlegen, Ç. (2007). Fiziksel aktivite ve koroner kalp hastalıkları risk faktörleri. *Spor Hekimliği Dergisi*, (42), 157-180.

24- Jensen, G. Nyboe, J. Appleyard, M. Schnohr, P. (1991). Risk factors for acute myocardial infarction in Copenhagen, II: Smoking, alcohol intake, physical activity, obesity, oral contraception, diabetes, lipids, and blood pressure." *European Heart Journal, 12(3)*,298-308. doi: 10.1093/oxfordjournals.eurheartj.a059894.

25- Jin, L. Sun, S. Ryu, Y. Piao, ZH. Liu, B. Choi, SY. Kim, RG. Kim, HS. Kee, HJ. & Jeong, MH. (2018).Gallic acid improves cardiac dysfunction and fibrosis in pressure overload-induced heart failure. *Scientific Reports*, (8), 9302. DOI:10.1038/s41598-018-27599-4.

26- Karayağız, M. Öztürk, C. (2008). Tamamlayıcı ve alternatif tedaviler ve çocuklarda kullanımı. *Çocuk Sağlığı ve Hastalıkları Dergisi. 51(1)*, 62-7

27- Keihanian, F. Moohebati, M. Saeidinia, A. Mohajeri, S.A. Madaen, S. (2021).Therapeutic effects of medicinal plants on isoproterenol-induced heart failure in rats. *Biomedicine & Pharmacotherapy, (134),* 111101.

28- Kılınç, E. Metlek S. (2021). Su Altı Görüntülerinden Nesne Tespiti. *European Journal of Science and Technology, (23*), 368-375, https://doi.org/10.31590/ejosat.873540

29- Kocaman, S. Dosay-Akbulut, M. (2021). Heart Attack and Alternative Treatments. *AKU International Journal of Engineering and Applied Sciences 4(2),* 99-106.

30-Kocaman S. (2022). Ratlarda deneysel olarak oluşturulacak kalp krizi ve hasarı modeli ile farklı tedavi yöntemlerinin karşılaştırmalı olarak test edilmesi. Afyon Kocatepe Uni. Sağlık Bilimleri Enstitüsü. *PhD Thesis*. 144 pp.

31- Lin, M.C. Nahin, R. Gershwin, E. Long hurst, J.C. Wu, K.K. (2001). State of Complementary and Alternative Medicine in Cardiovascular, Lung and Blood Research. Executive Summary of a Workshop. *Circulation,(103),*2038- 2041.

32- Liu, Y. He, X. Di, Z. (2022). Study on the Active Constituents and Molecular Mechanism of Zhishi Xiebai Guizhi Decoction in the Treatment of CHD Based on UPLC-UESI-Q Exactive Focus, Gene Expression Profiling, Network Pharmacology, and Experimental Validation. *ACS Omega, 7(5),* 3925-3939 pp. DOI:10.1021/acsomega.1c04491.

33- Mnafgui, K. Hajji, R. Derbali, F. Gammoudi, A. Khabbabi, G. Ellefi, H. Allouche, N. Kadri, A. Gharsallah, N. (2016). Anti-inflammatory, antithrombotic and cardiac remodeling preventive effects of eugenol in ısoproterenol-ınduced myocardial ınfarction in wistar rat, *Cardiovascular Toxicoloy*, *(16)*, 336-44.10. 1007/s12012-015-9343-x.

34- Miller, K.L. Liebowitz, R.S. Newby, L.K. (2004). Complementary and alternative medicine in cardiovascular disease: A review of biologically based approaches. *American Heart Journal, (147),*401-11.

35- Ozturk, Y. (1990). İlaç ve Tıbbi Bitkiler Yönünden Hindistan'a Bakış. *Pharmacia-JTPA, 30(3),* 148-68).

36- Öztürk, Y.E. Dömbekci, H.A. Ünal, S.N. (2020). Geleneksel Tamamlayıcı ve Alternatif Tıp Kullanımı. *journal of integrative and anatolian medicine, 1(3),* 23 – 35

37- Prasad, K. Sharma, V. Lackore, K. Jenkins, S.M. Prasad, A. Sood, A. (2013). Use of complementary therapies in cardiovascular disease. *American Journal of Cardiology, 111(3),* 339-45. doi: 10.1016/j.amjcard.2012.10.010.

38- Rabito, M. and Kaye, A.D. (2013). Complementary and Alternative Medicine and Cardiovascular Disease: An Evidence-Based Review. Evidence Based on Complementary and Alternative Medicine, (2013), 672097. doi: 10.1155/2013/672097.

39- Roth, G.A. Mensah, G.A. Johnson, C.O. Addolorato, G. Ammirati, E. Baddou, L.M. Barengo, N.C. Beaton, A.Z. Benjamin, E.J. Benziger, C.P. Bonny, A. Brauer, M. Brodmann, M. Cahill, T.J. Carapetis, J. Catapano, A.L. Chugh, S.S. Cooper, L. T. Coresh, J. Criqui, M. Fuster, V.(2020). Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. *Journal of the American College of Cardiology, 76 (25),* 2982-3021.

40- Salah, A.O. Salameh, A.D. Bitar, M.A. Zyoud, S.H. Alkaiyat, A.S. Al-Jabi, S.W. (2020). Complementary and alternative medicine use in coronary heart disease patients: a cross-sectional study from Palestine. *BMC Complementary Medicine and Therapies, 20(1),* 231. doi: 10.1186/s12906-020-03028-w.

41- Saravanan, G. Prakash, J. (2004). Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats, *Journal of Ethnopharmacology*, *94(1)*, 155-8. 10.1016/j. jep.2004. 04. 029.

42- Shafiq, A. Jayaram, N. Gosch, K.L. Spertus, J.A Buchanan, D.M. Decker, C. Kosiborod, M. Arnold, S.V. (2016). The Association Between Complementary and Alternative Medicine and Health Status Following Acute Myocardial Infarction. *Clinical Cardiology, 39(8),* 440-5. doi: 10.1002/clc.22559.

43- Shahid, M.H. Anjum, I. Mushtaq, M.N. Riaz, S. (2021). Cardioprotective effect of boswellic acids against doxorubicin induced myocardial infarction in rats, *Pakistan Journal of Pharmacology Sci*, *34(1(Supplementary),* 359-365.

44- Spatz, E.S. Wang, Y. Beckman, A.L. Wu, X. Lu, Y. Du, X. Li, J. Xu, X. Davidson, P.M. Masoudi, F.A. Spertus, J.A. Krumholz, H.M. Jiang, L. (2018). Traditional Chinese Medicine for Acute Myocardial Infarction in Western Medicine Hospitals in China. *Circulation: Cardiovascular Quality and Outcomes, 11(3),* e004190.

45- Singh, V. Raidoo, D.M. and Harries, C.S. (2004). The prevalence, patterns of usage and people's attitude towards complementary and alternative medicine (CAM) among the Indian community in Chatsworth, South Africa. *BMC Complementary and Alternative Medicine, (4),*3. doi: 10.1186/1472-6882-4-3.

46- Sütçü, S. (2018). Sosyolojik Açıdan Alternatif Tıp Geleneği Ve Uygulamaları: Isparta Örneği, Süleyman Demirel Üniversitesi Sosyal Bilimler Enstitüsü Sosyoloji Anabilim Dalı, *Yüksek Lisans Tezi*, Isparta, 180 pp.

47- Şentürk, S. (2008). İsoproterenol ile miyokart infarktüsü oluşturulmuş ratlarda L-Lizin'in total sialik asit düzeylerine etkisinin incelenmesi, *Yüksek Lisans Tezi*, Trakya Üniversitesi Sağlık Bilimleri Enstitüsü Biyokimya Anabilim Dalı. 66 pp.

48-T.C. Sağlık Bakanlığı. (2019). Bilgilendirme yayinlari, ANKARA.

49-Teo, T.Y. Yap, J. Shen, T. and Yeo, K.K. (2016). Complementary and alternative medicine use amongst patients with cardiovascular disease in Singapore. *BMC Complementary Medicine and Therapies*, *(16),* 446.doi: 10.1186/s12906-016-1430-4

50- Türk, H. Akı, M. Karaca, M. (2021). Sigaraya Bağlı Artan Kardiyovasküler Hastalık Riskini Düşürme. *Sağlık okur yazarlığı Dergisi, 2(2)*,40-47.

51-Yeh, G.Y. Davis, R.B. Phillips, R.S.(2006). Use of complementary therapies in patients with cardiovascular disease. *American Journal of Cardiology, 98(5),* 673-80. doi: 10.1016/j.amjcard.2006.03.051.

52- https://nccih.nih.gov/research/statistics/2007/camsurvey_fs1.htm

53-https://www.hopkinsmedicine.org/health/wellness-and-prevention/types-of-complementary-and-alternative-medicine

54- http://nccam.nih.gov/health/whatiscamNCCAM, 2018

55- Wikipedia.org [Internet]. England: Wikipedia [Updated: 2015 Dec 21; Cited: 2014 May 10]. Available from: http://tr.wikipedia.org/wiki/Alternatif

56- Wood, M.J. Stewart, R.L. Merry, H. Johnstone, D.E. Cox, J.L. (2003). Use of complementary and alternative medical therapies in patients with cardiovascular disease. *American Heart Journal, 145(5),* 806-12. doi: 10.1016/S0002-8703(03)00084-X.).

Current Treatment Approach ın Mushroom Poisoning

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Introduction

Mushrooms are living organisms that are widely found in nature, some of them are consumed as food. Consumption of some types of mushrooms can lead to poisoning, which can even lead to death. Humid environments provide mushrooms to thrive. For this reason, mushroom poisoning is mostly caused by the consumption of mushrooms collected in rainy periods and regions.

90-95% of deaths due to mushroom poisoning are caused by amatoxins (Barbato, 1993; Diaz, 2018). Therefore, in this study, poisoning due to amatoxins and their treatment will be explained.

Amatoxins are oligopeptides and are selective inhibitors of RNA polymerases. Amatoxins are not inactivated by boiling or freezing. Alpha amanitin is the most hepatotoxic among amatoxins, and its lethal dose in humans is 0.1 mg/kg (Baumann, Münter & Faulstich, 1993).

Amatoxins absorbed from the gastrointestinal tract by ingestion of poisonous mushrooms come to the liver, which is the main target organ, via the portal vein (Karlson-Stiber & Persson, 2003). 60% of the alpha amanitine absorbed from the gastrointestinal tract is excreted in the bile, and some of it comes back to the liver by enterohepatic circulation (Busi et al., 1979). Amatoxins are taken to the liver by active transport, OATP (Organic anion transporting polypeptide) 1B3 and NTCP (Sodium-taurocholate cotransporter) (Letschert et al., 2006). Since messenger RNA cannot be synthesized due to RNA polymerase inhibition, protein synthesis and metabolism at the cellular level in the liver cease (Karlson-Stiber & Persson, 2003; Kröncke et al., 1986).

Most deaths caused by ingestion of mushrooms containing amatoxin are due to hepatocellular necrosis (Diaz, 2005). In the kidneys, amatoxin is filtered from the glomeruli and reabsorbed from the proximal tubules, so it can develop acute tubular necrosis in the kidneys (Gibbons, 1982). The rate of binding of amatoxins to proteins is quite low. They are excreted in urine and feces, and can be detected by high-performance liquid chromatography (Jaeger et al., 1993).

Most edible mushrooms are not toxic or cause mild or moderate gastrointestinal toxic effects. (Trestrail, 1991). The clinical manifestations of amatoxin poisoning are divided into four stages. The first stage is the latent phase, which is usually asymptomatic, lasting 0 - 24 hours. The second stage is the gastroenteritis phase, which starts approximately 6 hours after the mushroom is taken, and symptoms such as nausea, vomiting, diarrhea, abdominal pain, and hematuria are observed (6-24 hours). The third stage is the relative recovery phase in which asymptomatic elevations of hepatic enzymes are observed (24-72 hours). The last stage (4th - 9th day) is the hepatic failure phase in which acute liver failure develops, renal damage, encephalopathy, coma and death can ocur (Berger & Guss, 2005). Symptoms seen after a prolonged latent period are more suggestive of amatoxin poisoning (Diaz, 2005).

For the diagnosis of mushroom poisoning, identification of the eaten mushroom, the time elapsed between the mushroom ingestion and the onset of the specified symptoms, and laboratory tests are used (*Zilker, 2008*). In case of suspected amatoxin poisoning, first of all, examinations

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such as complete blood count, blood glucose, urea, creatinine, electrolytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubins, prothrombin time (PT), active partial thromboplastin time (aPTT) and complete urinalysis should be done. For diagnosis of amatoxin poisoning, alpha amanitin can also be checked in the urine (Butera et al., 2004).

Prevention from Mushroom Poisoning

Mushrooms that grow in nature are collected in many countries for consumption as food. For this reason, mushroom poisoning is common and important health problem, especially in rainy periods and in rural areas. People who do not have sufficient knowledge and experience to avoid mushroom poisoning should not collect mushrooms and should not consume the mushrooms they collect. People especially in rural areas should be educated on this issue.

Treatment

Symptomatic patients should be hospitalized, supportive treatments such as fluid and electrolyte support and symptomatic treatments should be given. In the stage of hepatic damage, glucose-containing fluids should be preferred because of the risk of hypoglycemia (Ward et al., 2013).

Gastric lavage should be performed in patients presenting at an early stage with suspected mushroom poisoning. After lavage, it is recommended to administer repeated doses of activated charcoal (initial dose 1 g/kg, maximum 50 g in adults and 0.5 g/kg repeated doses) (Diaz, 2005; Zellner et al., 2019). Maximum benefit is obtained if activated charcoal is started within the first 24 hours after mushroom ingestion. However, in the first 4 days, 0.5 grams/kg can be applied every 4 hours. The biliary excretion of amatoxins can take up to 5 days. Activated charcoal binds amatoxins and allows them to be excreted in the faeces (Jaeger et al., 1993). There was a significant decrease in mortality in those who received repeated doses of activated charcoal and supportive treatment compared to those who received only supportive treatment (mortality 10% and 47%, respectively) (Enjalbert et al., 2002).

The most frequently used drugs in treatment are n-acetylcysteine, intravenous benzathine benzylpenicillin (penicillin G benzathine), silymarin and cimetidine (Karlson-Stiber & Persson, 2003).

N-acetylcysteine is an antioxidant and free radical scavenger. However, it has been proven that it is not hepatoprotective in cases of acute liver failure other than paracetamol, such as mushroom poisoning (Karlson-Stiber & Persson, 2003; Sklar & Subramaniam, 2004). However, it can be used in treatment considering its low side effects and potential benefits. In one study, it was found that the combined use of silibinin and N-acetylcysteine increased survival (Bergis et al., 2012).

High-dose (300,000 - 1,000,000 units/kg/day) intravenous penicillin has been shown to inhibit the uptake of amatoxin into hepatocytes. Benzylpenicillin acts by inhibiting the carrier protein of alpha amanitin (OATP 1B3) in the hepatocyte membrane (Karlson-Stiber & Persson, 2003; Magdalan et al., 2009).

Silymarin is one of the most studied drugs for the treatment of poisoning by amatoxins. However, no randomized controlled trials have been conducted (Schneider, Borochovitz & Krenzelok, 1987). Silymarin is an antioxidant and free radical scavenger derived from thistle, which maintains hepatic glutathione levels such as n-acetyl cysteine. The main isomer and pharmacologically active moiety of silymarin is silibinin (Popp et al., 2020; Saller, Meier & Brignoli, 2001). A meta-analysis of patients poisoned with amatoxins showed that mortality was significantly reduced in patients treated with silibinin compared to those treated without silibinin administration (9.8% with silibinin versus 18.3% with standard treatment; $P<0.01$) (Saller, Meier & Brignoli, 2001). Silibinin also acts mainly by inhibiting the uptake of amatoxins into hepatocytes via OATP-1B3, like Penicillin G (Popp et al., 2020).

In amatoxin poisonings, silymarin can be administered as 1 g/day oral or as a continuous infusion of 20 mg/kg/day for 6 days after the first 1 hour of 5 mg/kg administration of silibinin intravenously (Mitchell, 2010). It was stated that silibinin (alone or in combination) or N-acetyl-cysteine should be used as the first choice treatment because of the low mortality rates (5.6% and 6.8%, respectively) (Mengs, Pohl & Mitchell, 2012). N-acetyl-cysteine can be given as an alternative if silibinin is not available or in addition to silibinin because of its different mechanism of action (Liu et al., 2020).

Cimetidine inhibits the hepatic microsomal cytochrome P450 enzyme system. For this reason, high dose (200 mg/hour iv) administration in amatoxin poisoning limits the formation of cytotoxic metabolites from amatoxin. Thus, it decreases mitochondrial damage and hepatocellular necrosis (Schneider, Borochovitz & Krenzelok, 1987; Salhanick, Wax & Schneider, 2008).

The combination of methylprednisolone, which inhibits the NTCP transporter, and polymyxin B, which inhibits the OATP 1B3 transporter, has been proposed as a potential antidote due to positive results in animal experiments. However, due to the adverse effects of corticosteroids, more studies are needed before this combination can be applied in humans (Garcia et al., 2019).

Since most of the excretion of amatoxins is via the urinary tract, forced diuresis (for 4 - 5 days with 100 - 200 mL/hr target diuresis) has been recommended to increase their clearance (Jaeger et al., 1993). Hemodialysis and hemoperfusion are used to remove toxins in poisoning. However, their efficacy in amatoxin poisoning is controversial. There was no clinical improvement in patients who underwent hemodialysis and hemoperfusion, and no toxin was found in the dialysis fluids of these patients (Mullins & Horowitz, 2000). The efficacy of hemodialysis, hemoperfusion, and plasmapheresis is limited due to rapid blood clearance (rapid uptake by tissue and low plasma concentrations) of amanitins (Berger & Guss, 2005; Jaeger et al., 1993). However, it has been reported that hemoperfusion may be effective in preventing cerebral edema due to fulminant liver failure (Berger & Guss, 2005). In amatoxin poisoning, hemodialysis is recommended only when acute renal failure develops (Bergis et al., 2012).

In those who develop acute hepatic failure, high-volume plasma exchange has been shown to improve survival without liver transplantation (Larsen et al., 2016). Although plasma exchange transfusions are partially beneficial, their efficacy has been found to be insufficient in controlled studies (Wittebole & Hantson, 2011).

The Prometheus fractionated plasma separation and adsorption (FPSA) system can also prevent liver failure by eliminating amatoxins (Bergis et al., 2012).

Molecular absorbent regeneration system (MARS) is a method that removes albumin-bound toxins with albumin-containing dialysate (Wittebole & Hantson, 2011).

Interruption of the enterohepatic cycle of amatoxins by performing biliary drainage in dogs reduced intestinal amatoxin absorption by more than 70%. Less severe signs of toxicity were observed in dogs with biliary drainage than in dogs without drainage. This could be a promising adjunctive treatment for amatoxin poisoning (Sun et al., 2018).

When treatment is applied in the first 2-3 days of poisoning, mortality decreases to 9% (Saviuc & Flesch, 2003). It was determined that hepatic coma followed the increase in AST and ALT in patients with hepatic failure, and there was a significant correlation between this increase and mortality (Eren et al., 2010).

Liver transplantation should be planned when a complete clinical recovery is not achieved despite all treatments (Enjalbert et al., 2002). It is stated that liver transplantation should be performed in patients accompanied by hepatic encephalopathy with high INR, ammonia and AST levels or patients with metabolic acidosis, persistent hypoglycemia and hypofibrinogenemia (Galler,

Weisenberg & Brasitus, 1992). Ganzert criteria have been developed for liver transplantation in amatoxin poisoning. They stated that at any time between the 3rd and 10th days after eating mushrooms, if the serum creatinine is 106 μmol/L or above with a decrease in the prothrombin index to 25% or less of normal, regardless of the presence of hepatic encephalopathy, urgent liver transplantation is required (Ganzert, Felgenhauer & Zilker, 2005).

Patients should be referred to a more advanced center center to avoid loss of time in suspected amatoxin poisoning. If possible, patients with a diagnosis of amatoxin poisoning should be followed up in healthcare facilities that are experienced in toxicology and have an organ transplant unit (Bernuau, 2004).

REFERENCES

Barbato, M. P. (1993). Poisoning from accidental ingestion of mushrooms. *The Medical Journal of Australia, 158* (12), 842-847. Doi: 10.5694/j.1326-5377.1993.tb137674.x.

Baumann, K., Münter, K. & Faulstich, H. (1993). Identification of structural features involved in binding of alpha-amanitin to a monoclonal antibody. *Biochemistry, 32* (15), 4043-4050. Doi: 10.1021/bi00066a027.

Berger, K. J. & Guss, D. A. (2005). Mycotoxins revisited: Part I. *The Journal of Emergency Medicine, 28* (1), 53-62. Doi: 10.1016/j.jemermed.2004.08.013.

Bergis, D., Friedrich-Rust, M., Zeuzem, S., Betz, C., Sarrazin, C. & Bojunga, J. (2012). Treatment of Amanita phalloides intoxication by fractionated plasma separation and adsorption (Prometheus®). *Journal of Gastrointestinal and Liver Diseases : JGLD, 21* (2), 171-176.

Bernuau, J. (2004). Acute liver failure: avoidance of deleterious cofactors and early specific medical therapy for the liver are better than late intensive care for the brain. *Journal of Hepatology, 41* (1), 152-155. Doi: 10.1016/j.jhep.2004.05.007.

Busi, C., Fiume, L., Costantino, D., Langer, M. & Vesconi, F. (1979). Amanita toxins in gastroduodenal fluid of patients poisoned by the mushroom, Amanita phalloides. *The New England Journal of Medicine, 300* (14), 800.

Butera, R., Locatelli, C., Coccini, T. & Manzo, L. (2004). Diagnostic accuracy of urinary amanitin in suspected mushroom poisoning: a pilot study. *Journal of Toxicology. Clinical Toxicology, 42* (6), 901-912. Doi: 10.1081/clt-200035472.

Diaz, J. H. (2018). Amatoxin-Containing Mushroom Poisonings: Species, Toxidromes, Treatments, and Outcomes. *Wilderness & Environmental Medicine, 29* (1), 111-118. Doi: 10.1016/j.wem.2017.10.002.

Diaz, J. H. (2005). Evolving global epidemiology, syndromic classification, general management, and prevention of unknown mushroom poisonings. *Critical Care Medicine, 33* (2), 419-426. Doi: 10.1097/01.ccm.0000153530.32162.b7.

Diaz, J. H. (2005). Syndromic diagnosis and management of confirmed mushroom poisonings. *Critical Care Medicine, 33* (2), 427-436. Doi: 10.1097/01.ccm.0000153531.69448.49.

Enjalbert, F., Rapior, S., Nouguier-Soulé, J., Guillon, S., Amouroux, N. & Cabot, C. (2002). Treatment of amatoxin poisoning: 20-year retrospective analysis. *Journal of Toxicology. Clinical Toxicology, 40* (6), 715-757. Doi: 10.1081/clt-120014646.

Eren, S. H., Demirel, Y., Ugurlu, S., Korkmaz, I., Aktas, C. & Güven, F. M. (2010). Mushroom poisoning: retrospective analysis of 294 cases. *Clinics (São Paulo, Brazil), 65* (5), 491-496. Doi: 10.1590/S1807-59322010000500006.

Galler, G. W., Weisenberg, E. & Brasitus, T. A. (1992). Mushroom poisoning: the role of orthotopic liver transplantation. *Journal of Clinical Gastroenterology, 15* (3), 229-232.

Ganzert, M., Felgenhauer, N. & Zilker, T. (2005). Indication of liver transplantation following amatoxin intoxication. *Journal of Hepatology, 42* (2), 202-209. Doi: 10.1016/j.jhep.2004.10.023.

Garcia, J., Costa, V. M., Bovolini, A., Duarte, J. A., Rodrigues, D. F., de Lourdes Bastos, M. & Carvalho, F. (2019). An effective antidotal combination of polymyxin B and methylprednisolone for α-amanitin intoxication. *Archives of Toxicology, 93* (5), 1449-1463. Doi: 10.1007/s00204-019-02426-5.

Gibbons, R. B. (1982). Mushroom poisoning. *Comprehensive Therapy, 8* (12), 33-39.

Jaeger, A., Jehl, F., Flesch, F., Sauder, P. & Kopferschmitt, J. (1993). Kinetics of amatoxins in human poisoning: therapeutic implications. *Journal of Toxicology. Clinical Toxicology, 31* (1), 63-80. Doi: 10.3109/15563659309000374.

Karlson-Stiber, C. & Persson, H. (2003). Cytotoxic fungi-an overview. *Toxicon, 42* (4), 339-349. Doi: 10.1016/s0041-0101(03)00238-1.

Kröncke, K. D., Fricker, G., Meier, P. J., Gerok, W., Wieland, T. & Kurz, G. (1986). alpha-Amanitin uptake into hepatocytes. Identification of hepatic membrane transport systems used by

amatoxins.
The Journal of Biological Chemistry, 261 (27), 12562-12567.

Larsen, F. S., Schmidt, L. E., Bernsmeier, C., Rasmussen, A., Isoniemi, H., Patel, V. C., Triantafyllou, E., Bernal, W., Auzinger, G., Shawcross, D., Eefsen, M., Bjerring, P. N., Clemmesen, J. O., Hockerstedt, K., Frederiksen, H. J., Hansen, B. A., Antoniades, C. G. & Wendon, J. (2016). High-volume plasma exchange in patients with acute liver failure: An open randomised controlled trial. *Journal of Hepatology, 64* (1), 69-78. Doi: 10.1016/j.jhep.2015.08.018.

Letschert, K., Faulstich, H., Keller, D. & Keppler, D. (2006). Molecular characterization and inhibition of amanitin uptake into human hepatocytes. *Toxicological Sciences, 91* (1), 140-149. Doi: 10.1093/toxsci/kfj141.

Liu, J., Chen, Y., Gao, Y., Walline, J. H., Lu, X., Yu, S., Zhao, L., Ge, Z. & Li, Y. (2020). N-acetylcysteine as a treatment for amatoxin poisoning: a systematic review. *Clinical Toxicology (Philadelphia, Pa.), 58* (11), 1015-1022. Doi: 10.1080/15563650.2020.1784428.

Magdalan, J., Ostrowska, A., Podhorska-Okołów, M., Piotrowska, A., Izykowska, I., Nowak, M., Dolińska-Krajewska, B., Zabel, M., Szelag, A. & Dziegiel, P. (2009). Early morphological and functional alterations in canine hepatocytes due to alpha-amanitin, a major toxin of Amanita phalloides. *Archives of Toxicology, 83* (1), 55-60. Doi: 10.1007/s00204-008-0376-9.

Mengs, U., Pohl, R. T. & Mitchell, T. (2012). Legalon® SIL: the antidote of choice in patients with acute hepatotoxicity from amatoxin poisoning. *Current Pharmaceutical Biotechnology, 13* (10), 1964-1970. Doi: 10.2174/138920112802273353.

Mitchell, S. T. (2010). New Comprehensive Amatoxin Mushroom Poisoning (AMP) Treatment Protocol. *Clinical Toxicology, 6* (48), 628.

Mullins, M. E. & Horowitz, B. Z. (2000). The futility of hemoperfusion and hemodialysis in Amanita phalloides poisoning. *Veterinary and Human Toxicology, 42* (2), 90-91.

Popp, T., Balszuweit, F., Schmidt, A., Eyer, F., Thiermann, H. & Steinritz, D. (2020). Assessment of α-amanitin toxicity and effects of silibinin and penicillin in different in vitro models. *Toxicology in Vitro, 67*, 104921. Doi: 10.1016/j.tiv.2020.104921.

Salhanick, S. D., Wax, P. M. & Schneider, S. M. (2008). In response to Tong TC, et al. Comparative treatment of alpha-amanitin poisoning with N-acetylcysteine, benzylpenicillin, cimetidine, thioctic acid, and silybin in a murine model. *Annals of Emergency Medicine, 52* (2), 184-185. Doi: 10.1016/j.annemergmed.2007.11.050.

Saller, R., Meier, R. & Brignoli, R. (2001). The use of silymarin in the treatment of liver diseases. *Drugs, 61* (14), 2035-2063. Doi: 10.2165/00003495-200161140-00003.

Saviuc, P. & Flesch, F. (2003). Intoxications aiguës par les champignons supérieurs et leur traitement [Acute higher funghi mushroom poisoning and its treatment]. *Presse Médicale (Paris, France), 32* (30), 1427-1435.

Schneider, S. M., Borochovitz, D. & Krenzelok, E. P. (1987). Cimetidine protection against alpha-amanitin hepatotoxicity in mice: a potential model for the treatment of Amanita phalloides poisoning. *Annals of Emergency Medicine, 16* (10), 1136-1140. Doi: 10.1016/s0196-0644(87)80472-9.

Sklar, G. E. & Subramaniam, M. (2004). Acetylcysteine treatment for non-acetaminophen-induced acute liver failure. *The Annals of Pharmacotherapy, 38* (3), 498-500. Doi: 10.1345/aph.1D209.

Sun, J., Zhang, Y. T., Niu, Y. M., Li, H. J., Yin, Y., Zhang, Y. Z., Ma, P. B., Zhou, J., Lu, J. J., Zhang, H. S. & Sun, C. Y. (2018). Effect of Biliary Drainage on the Toxicity and Toxicokinetics of Amanita exitialis in Beagles. *Toxins (Basel), 10* (6), 215. Doi: 10.3390/toxins10060215.

Trestrail, J. H. (1991). 3rd. Mushroom poisoning in the United States-an analysis of 1989 United States Poison Center data. *Journal of Toxicology. Clinical Toxicology, 29* (4), 459-465. Doi: 10.3109/15563659109025741.

Ward, J., Kapadia, K., Brush, E. & Salhanick, S. D. (2013). Amatoxin poisoning: case reports and review of current therapies. *The Journal of Emergency Medicine, 44* (1), 116-121. Doi: 10.1016/j.jemermed.2012.02.020.

Wittebole, X. & Hantson, P. (2011). Use of the molecular adsorbent recirculating system (MARS™) for the management of acute poisoning with or without liver failure. *Clinical Toxicology (Philadelphia, Pa.), 49* (9), 782-793. Doi: 10.3109/15563650.2011.624102.

Zellner, T., Prasa, D., Färber, E., Hoffmann-Walbeck, P., Genser, D. & Eyer, F. (2019). The Use of Activated Charcoal to Treat Intoxications. *Deutsches Ärzteblatt International, 116* (18), 311-317. Doi: 10.3238/arztebl.2019.0311.

Zilker, T. (2008). Vergiftungen durch Pilze. Thomas Zilker (Ed.), In: Klinische Toxikologie *für die* Notfall*-und* Intensivmedizin *(1st ed., pp. 247-270). Bremen*: *UNI-MED.*

Cognitive Communication Disorders in Alzheimer's Disease

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Introduction

Dementia is a chronic, often progressive syndrome and is the 7th leading cause of death worldwide. In individuals with dementia, cognitive functions are impaired more than would be expected from normal aging processes (Organization, 2017). The result is that dementia significantly reduces people's cognitive functions, activities of daily living, communication skills, and quality of life (Munis, Parlak, & Köse, 2021).

Alzheimer's disease (AD) constitutes 60–70% of the etiology of dementia (Organization, 2017). Although AD first affects the memory, it is a neurodegenerative disease in which cognitive functions such as attention and visual-spatial functions are increasingly impaired (Parlak, Altan, & Saylam, 2022; Parlak, Babademez, Tokgöz, Bizpınar, & Saylam, 2022). Later symptoms can thus include problems with language, mood swings, disorientation (including easily getting lost), self-neglect, loss of motivation, and behavioral issues. It is estimated that AD will become a worldwide public health problem that directly affects 100 million people by 2050. It has been stated that more than five million Americans have AD and that this number may increase to 16 million by 2050 (A. s. Association, 2018; Cao et al., 2020). According to the 2012 Alzheimer Europe Report, 331.512 individuals are currently suffering with AD in Turkey (Özbabalık & Hussein, 2017). Globally, the number of individuals with AD is expected to increase rapidly as the elderly population increases.

Language disorders appear early in AD and worsen as the disease progresses. As the disease progresses and the cognitive functions of individuals deteriorate, the effects on their communication skills increase and their independence level decreases (Parlak, Tokgöz, Bizpinar, Saylam, & Köse, 2022). That people with AD become increasingly dependent on others reduces the quality of life of their caregivers, along with increasing the burdens on families and health systems (Bayles & Tomoeda, 2007). In this study, the cognitive communication disorders seen in AD were examined.

Cognition

Cognition is a set of abilities that enable many mental processes and functions to work together and is a general term that refers to our information processing systems and stored knowledge (Baş B, 2022; Kathryn Bayles, McCullough, & Tomoeda, 2018). All situations that enable us to perceive and interpret patterns, to achieve goals, and to change our behaviors to ensure survival are realized through these cognitive functions. Cognitive functions play an important role in many daily living activities, such as chatting with a friend or finding a job, and in adapting an individual to daily life. To conceptualize cognition, we can liken it to a company. The mission of this company is analyzing the senses, detecting regularities in incoming sensory information, remembering, and using experience to guide behavior (Bayles et al., 2018; Bayles & Tomoeda, 2007).

Cognitive functions are generally evaluated with cognitive screening tests, such as Mini-Mental State Examination and Addenbrooke Cognitive Examination. Cognitive functions are examined in these tests in five sections: orientation, attention, executive functions, memory, visuospatial functions and language. Cognitive functions and their subsections are shown in Figure 1 (Baş B, 2022; Chung, Pollock, Campbell, Durward, & Hagen, 2013; Diamond, 2013; Hoffmann, Bennett, Koh, & McKenna, 2010; Karakaş & İrkeç, 2008; Molloy & Standish, 1997; Noone, 2015; Sohlberg & Mateer, 2001; Squire, 2004).

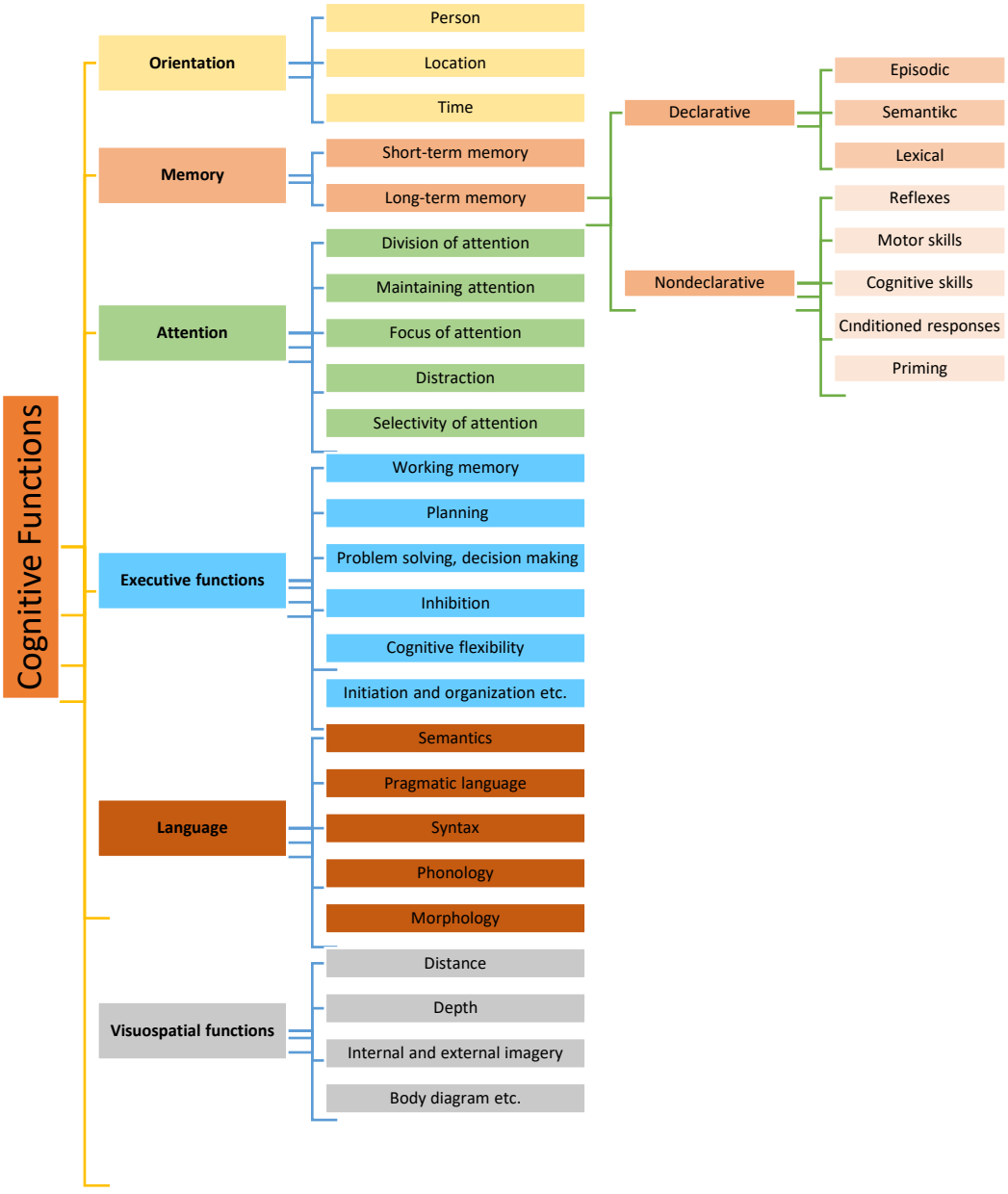

Figure 1: *Cognitive functions and their subsections (Baş B, 2022; Chung et al., 2013; Diamond, 2013; T. Hoffmann et al., 2010; Karakaş & İrkeç, 2008; Molloy & Standish, 1997; Noone, 2015; Sohlberg & Mateer, 2001; Squire, 2004)*

Communication

Communication is the sharing of information through a system of symbols. Communication starts with the formation of an idea in the speaker's mind. The speaker encodes the message he or she wants to convey in some way; in other words, he or she makes it communicable with the rules of whichever communication tool he or she will use. The receiver of the message analyzes it and makes sense of it (decoding). There are types of communication: verbal communication and nonverbal communication. People convey their thoughts, ideas, and feelings using spoken or written words in verbal communication. Nonverbal communication involves a variety of techniques, including body language, which includes facial emotions, gestures, and more. Communication types are shown in Figure 2 (Hans & Hans, 2015; Liu, 2016; Rocci & de Saussure, 2016).

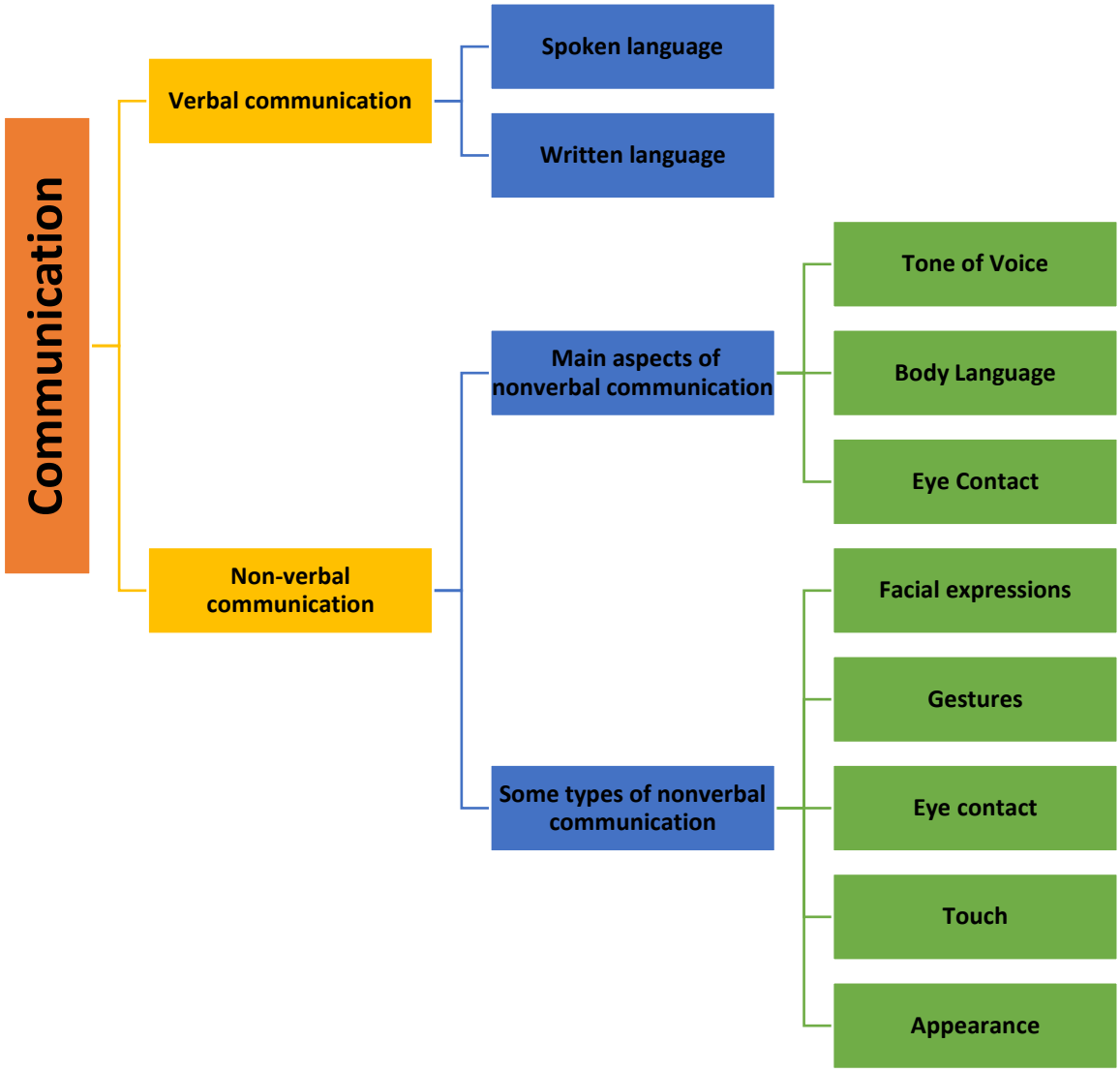

Figure 2: Communication types (Hans & Hans, 2015; Liu, 2016; Rocci & de Saussure, 2016).

To communicate verbally or non-verbally, an individual must have an idea to share and a system of symbols with which to express that idea. Both verbal and nonverbal communication are impaired in AD because both require cognitive processes to share and interpret information. The pathophysiological changes in AD interrupt the information processing process. Because verbal communication requires the conscious sharing of ideas through language, it is typically the most affected in AD (Bayles et al., 2018).

Cognitive Communication Disorder

Cognitive communication disorder is difficulty in any aspect of communication skills as a result of the influence on cognitive functions (A. S.-L.-H. Association, 2022c). The most common etiologies of cognitive communication disorders are dementia (especially AD), stroke, brain tumor, traumatic brain injury (TBI), and right brain injury. Cognitive communication disorders have negative effects such as difficulties in communicating people's needs and feelings and reducing their independence. Some potential effects of cognitive communication disorders are shown in Table 1 (A. S.-L.-H. Association, 2022c).

Table 1: *Impacts of cognitive communication disorder (A. S.-L.-H. Association, 2022c).*

Impacts of Cognitive Communication Disorder
• **Reduced awareness of and ability to initiate and effectively communicate one's needs**
• **Risk of injury due to inability to communicate and/or anticipate the consequences of one's own actions in an emergency**
• **Reduced memory, judgment, and ability to initiate and effectively modify routine information exchange**
• **Reduced social communication skills and/or ability to manage one's emotions, often resulting in loss of relationships**
• **Impairment of ability to fulfill educational or professional roles, resulting in potential loss of employment**
• **Difficulty effectively performing personal lifestyle management activities (i.e., paying bills)**
• **Decreased awareness of the disorder and its extent (i.e., loss of ability to assess one's own communication effectiveness)**
• **Decreased ability to predict possible outcomes with reasonable decision making and problem solving**

How does cognitive communication disorder occur in Alzheimer's disease?

The progressive deterioration in cognitive functions in individuals with AD leads to cognitive-communication disorder, preventing communication. The production and understanding of language are linked to information stored in the memory. When we think of the simple act of

naming an object, for example, a carrot. we first need to perceive the characteristics of the carrot. For that recognition to occur, a match must be made with the information in our long-term memory. After that, the intention to say the name of the object is created. The linguistic representations of objects are part of the long-term lexical memory and must be retrieved and brought into consciousness. Maybe you can't be sure of what a carrot looks like, and therefore can't be sure whether the object at hand is a turnip, radish, or carrot. In this case, a decision must be made whether to indicate your uncertainty. A motor plan should be created to express uncertainty about the object's name or properties. Therefore, the simple object naming actions are perception, access to long-term memory, association, recognition, lexical recall, decision-making, motor planning, and self-monitoring (Bayles et al., 2018; Klein & Mancinelli, 2021; Szatloczki, Hoffmann, Vincze, Kalman, & Pakaski, 2015).

Since the information processing abilities of the explicit and working memory systems of individuals with AD are affected, they have difficulty in producing verbal information (Bayles et al., 2018; Hornberger, Bell, Graham, & Rogers, 2009; Rogers & Friedman, 2008). In people with AD, information processing is impaired due to deficiencies in the cognitive processes of perception, recognition, attention, and memory, which causes patients to have difficulties in understanding language (MacDonald, Almor, Henderson, Kempler, & Andersen, 2001).

Cognitive Communication Disorders in Different Stages of Alzheimer Disease

The Clinical Dementia Rating (CDR) scale is frequently used in the clinic for staging AD (Gürvit, 2004; Parlak, Babademez, et al., 2022).The CDR is calculated by testing six different cognitive and behavioral domains: orientation, memory, judgment and problem solving, home and hobbies performance, community affairs, and personal care. The CDR is based on a scale of 0–3: no dementia (CDR = 0), questionable dementia (CDR = 0.5), mild dementia (CDR = 1), moderate dementia (CDR = 2), and severe dementia (CDR = 3) (Figure 2) (Hughes, Berg, Danziger, Coben, & Martin, 1982). In this article, cognitive communication disorders in mild, moderate and advanced stages of AD were examined. Impairments in cognitive functions according to Alzheimer's disease stages are shown in Figure 3 (Bayles et al., 2018).

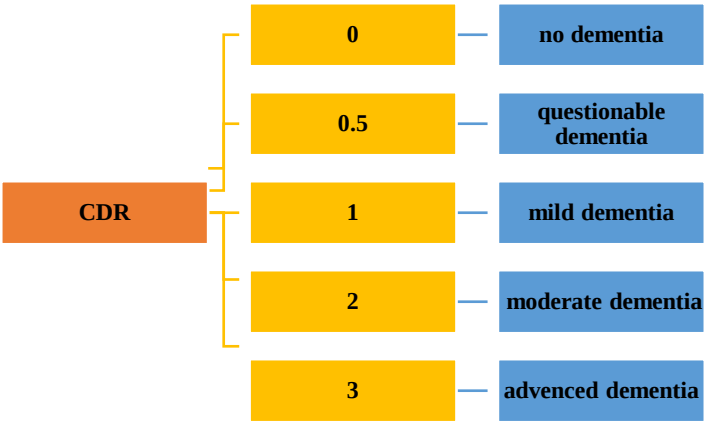

Figure 2: *Clinical Dementia Rating Scale (Hughes et al., 1982).*

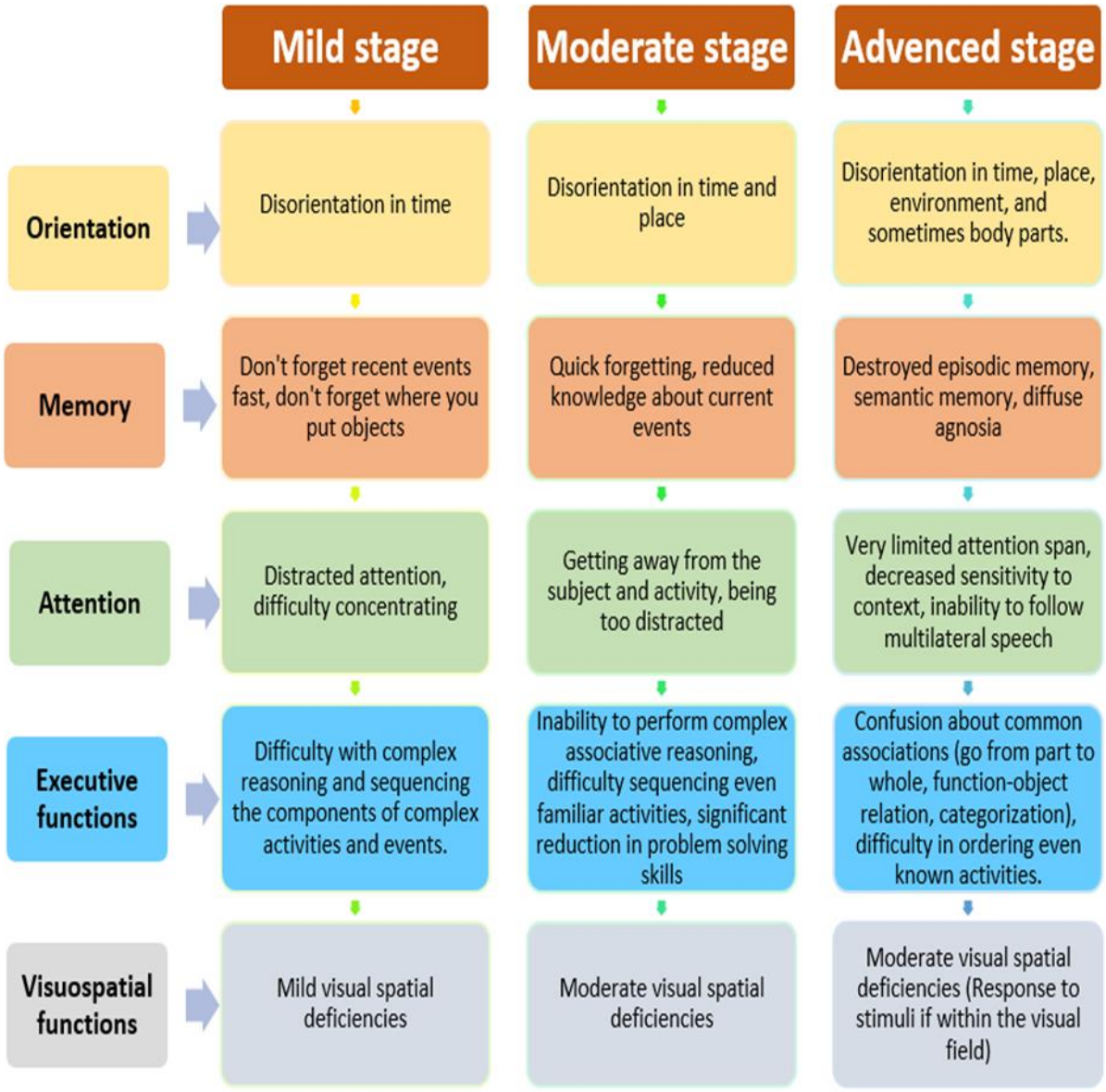

Figure 3: *Impairments in cognitive functions according to Alzheimer's disease stages (Bayles et al., 2018).*

Cognitive Communication Disorders in Mild Alzheimer's Disease

In mild-stage AD, speech is fluent, and the patients can understand most of what they read and hear, but they quickly forget recently acquired information. While the semantic and pragmatic components of the language are affected, phonology and syntax are generally better preserved over the course of the disease. During spontaneous speech, early-stage AD patients usually have longer hesitations and pauses and slower speaking rates (Kathryn Bayles et al., 2018; Gayraud, Lee, & Barkat-Defradas, 2011; I. Hoffmann et al., 2010; Jarrold et al., 2014; Satt, Hoory, König, Aalten, & Robert, 2014). However, their speech is fluent, without dysarthria or articulation errors (Weiner, Neubecker, Bret, & Hynan, 2008).

Grammar is preserved in speech, but there is a marked increase in the number of "empty words" (e.g., "thing" and "it") in the content of the language (Blanken, Dittmann, Haas, & Wallesch, 1987; Irigaray, 1967). Because early-stage AD patients often forget what they have just

heard or thought, their oral discourse includes more sentence fragments and repetitiveness. Their speech is less consistent than that of their healthy peers (Bayles, Tomoeda, Kaszniak, Stern, & Eagans, 1985; Bayles, Tomoeda, & Trosset, 1992). Dysnomia during speech is common, and a naming error is often semantically related to the target word (e.g., "sharp" for "knife") (Bayles & Tomoeda, 1983). Their performance on verbal fluency tests shows significant deficiencies compared to that of their healthy peers (Araujo et al., 2011; Henry, Crawford, & Phillips, 2004). In addition, one study found that the most frequently reported symptom in individuals with mild AD was difficulty naming things (K. A. Bayles & Tomoeda, 1991).

Individuals with mild AD can identify words and describe pictures but have difficulty following the context of speech. However, they may have the ability to follow three-step instructions. Reading and writing skills are generally preserved at this stage, but typos are common (Kathryn Bayles et al., 2018; Klein & Mancinelli, 2021; Szatloczki et al., 2015).

The written language of individuals with AD is more affected than their spoken language, and their written discourse contains more errors than that of their healthy peers (Groves-Wright, Neils-Strunjas, Burnett, & O'Neill, 2004). Grammar and spelling errors are common in written language. Although they generally understand what they hear and read, they quickly forget this (Bayles et al., 1992). They can answer most questions and define words, but they often miss the point of a joke, which can be mistaken for sarcasm (Bayles et al., 2018).

Cognitive Communication Disorders in Moderate Alzheimer's Disease

People with moderate stage AD are fluent in speech but usually speak more slowly and are more hesitant to speak. Their speech is filled with more frequently repeated words and longer pauses (Gayraud et al., 2011). Intellectual repetitions are common in their speech when they are asked to describe a picture or object. The morphology of the language usually remains intact, but the content is significantly affected (Bayles et al., 1992).

The oral expressions of individuals with middle-stage AD contain fewer nouns than verbs (Blanken et al., 1987). Vocabulary loss and deterioration in conceptual knowledge are observed to be more common in their performance on vocabulary tests (Bayles et al., 1992). Their ability to create and name examples of things falling under a certain category is significantly impaired compared to that of their healthy peers. Written languages also contain many errors.

Individuals with middle-stage AD exhibit a decrease in understanding both written and spoken language (Bayles et al., 1992; Rochon, Waters, & Caplan, 2000). The mechanics of reading are preserved at this stage, although most of them are at the level of words and expressions. However, reading comprehension is impaired, and what they understand from what they read is quickly forgotten. They are largely insensitive to context and may miss the main points of jokes. They also tend to interpret figurative language literally (Bayles et al., 2018).

Individuals with moderate AD produce significantly less opinionated discourses than those with mild AD do. During conversations, they often forget their intentions. This leads to many half-sentence fragments. In addition, they lack self-monitoring and self-correction. They are usually able to name items when given naming tasks but have difficulty participating in the conversation. Their verbal outputs tend to be less significant. They usually understand two-step instructions, but they have difficulty understanding three-step instructions. As in the early stage, they can write words for dictation, but they may have difficulty writing long phrases (Bayles et al., 2018; Klein & Mancinelli, 2021; Szatloczki et al., 2015).

Cognitive Communication Disorders in Advanced Alzheimer's Disease

In advanced-stage AD, which is the last stage of the disease, due to cognitive destruction, the patients' language outputs are greatly reduced, and their speech is often meaningless. There are significant differences in their ability to communicate with other individuals with advanced AD. Some of them cannot speak at all, while others produce meaningless language. In addition, some may have limited functional vocabulary. Most of the remaining language outputs are either common social expressions or nonsensical words. Many of them are still able to follow simple one-step instructions, but all of them can no longer follow instructions with more than one step. Some can contribute to speech, say their names, and maintain the characteristics of social language (Bayles & Tomoeda, 2007). However, almost all of them cannot express themselves in writing. Although some can read single words aloud, their reading comprehension is severely impaired (Bayles & Tomoeda, 2007). Cognitive communication disorders according to Alzheimer's disease stages are shown in Figure 4 (Bayles et al., 2018; Klein & Mancinelli, 2021; Szatloczki et al., 2015).

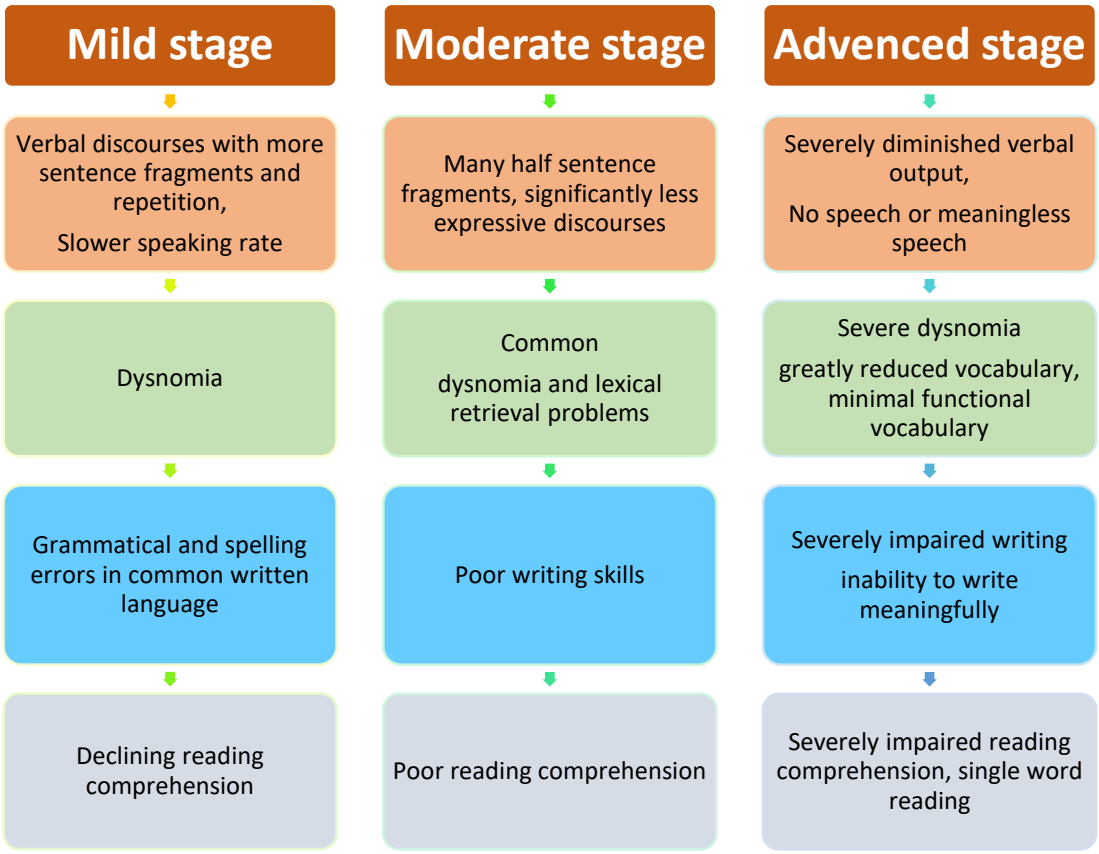

Figure 4: *Cognitive communication disorders according to Alzheimer's disease stages (Bayles et al., 2018; Klein & Mancinelli, 2021; Szatloczki et al., 2015)*

Conclusion

Attention, orientation, executive functions, visuospatial functions, memory, and language are our basic cognitive functions. The most common cause of dementia is Alzheimer's disease, and progressive deterioration in cognitive functions occurs during the disease process. Due to the relationship between communication and cognition, patients' communication skills are affected even in the disease's early stages. Therefore, cognitive communication disorders are accepted as an

important early symptom of Alzheimer's disease, which is a neurodegenerative disease, and communication ability gradually deteriorates over the course of the disease.

References

Araujo, N. B. d., Barca, M. L., Engedal, K., Coutinho, E. S. F., Deslandes, A. C., & Laks, J. (2011). Verbal fluency in Alzheimer's disease, Parkinson's disease, and major depression. *Clinics, 66*(4), 623-627.

Association, A. S.-L.-H. (2022c). Cognitivecommunication referral guidelines for adults. Retrieved from https://www.asha.org/slp/cognitive-referral/

Association, A. s. (2018). 2018 Alzheimer's disease facts and figures. *Alzheimer's & dementia, 14*(3), 367-429.

Baş B, Parlak. M.M (2022). *İşitme Kayıplı Çocuklarda İletişim ve Bilişsel Fonksiyonlar* (Ş. ÖA Ed.): Akademisyen Kitabevi.

Bayles, K. A, McCullough, K., & Tomoeda, C. (2018). *Cognitive-Communication Disorders of Dementia Definition, Diagnosis, and Treatment*: Plural Publishing,.

Bayles, K. A, & Tomoeda, C. (2007). Assessment of Cognitive-Communication Disorders of Dementia. *Cognitive-Communication Disorders of Dementia. San Diego: Plural Publishing*, 139-166.

Bayles, K. A., & Tomoeda, C. K. (1983). Confrontation naming impairment in dementia. *Brain and language, 19*(1), 98-114.

Bayles, K. A., & Tomoeda, C. K. (1991). Caregiver report of prevalence and appearance order of linguistic symptoms in Alzheimer's patients. *The gerontologist, 31*(2), 210-216.

Bayles, K. A., Tomoeda, C. K., Kaszniak, A. W., Stern, L. Z., & Eagans, K. K. (1985). Verbal perseveration of dementia patients. *Brain and language, 25*(1), 102-116.

Bayles, K. A., Tomoeda, C. K., & Trosset, M. W. (1992). Relation of linguistic communication abilities of Alzheimer's patients to stage of disease. *Brain and language, 42*(4), 454-472.

Blanken, G., Dittmann, J., Haas, J.-C., & Wallesch, C.-W. (1987). Spontaneous speech in senile dementia and aphasia: Implications for a neurolinguistic model of language production. *Cognition, 27*(3), 247-274.

Cao, Q., Tan, C.-C., Xu, W., Hu, H., Cao, X.-P., Dong, Q., . . . Yu, J.-T. (2020). The prevalence of dementia: A systematic review and meta-analysis. *Journal of Alzheimer's Disease, 73*(3), 1157-1166.

Chung, C. S., Pollock, A., Campbell, T., Durward, B. R., & Hagen, S. (2013). Cognitive rehabilitation for executive dysfunction in adults with stroke or other adult non-progressive acquired brain damage. *Cochrane database of systematic reviews*(4).

Diamond, A. (2013). Executive functions. *Annual review of psychology, 64*, 135-168.

Gayraud, F., Lee, H.-R., & Barkat-Defradas, M. (2011). Syntactic and lexical context of pauses and hesitations in the discourse of Alzheimer patients and healthy elderly subjects. *Clinical linguistics & phonetics, 25*(3), 198-209.

Groves-Wright, K., Neils-Strunjas, J., Burnett, R., & O'Neill, M. J. (2004). A comparison of verbal and written language in Alzheimer's disease. *Journal of communication disorders, 37*(2), 109-130.

Gürvit, İ. (2004). Demans sendromu, Alzheimer hastalığı ve Alzheimer dışı demanslar. *by Öge AE. İstanbul, Nobel Tıp Kitap Evleri Ltd. Şti*, 367-415.

Hans, A., & Hans, E. (2015). Kinesics, haptics and proxemics: Aspects of non-verbal communication. *IOSR Journal of Humanities and Social Science (IOSR-JHSS), 20*(2), 47-52.

Henry, J. D., Crawford, J. R., & Phillips, L. H. (2004). Verbal fluency performance in dementia of the Alzheimer's type: a meta-analysis. *Neuropsychologia, 42*(9), 1212-1222.

Hoffmann, I., Nemeth, D., Dye, C. D., Pákáski, M., Irinyi, T., & Kálmán, J. (2010). Temporal parameters of spontaneous speech in Alzheimer's disease. *International Journal of Speech-Language Pathology, 12*(1), 29-34.

Hoffmann, T., Bennett, S., Koh, C.-L., & McKenna, K. (2010). A systematic review of cognitive interventions to improve functional ability in people who have cognitive impairment following stroke. *Topics in stroke rehabilitation, 17*(2), 99-107.

Hornberger, M., Bell, B., Graham, K. S., & Rogers, T. (2009). Are judgments of semantic relatedness systematically impaired in Alzheimer's disease? *Neuropsychologia, 47*(14), 3084-3094.

Hughes, C. P., Berg, L., Danziger, W., Coben, L. A., & Martin, R. L. (1982). A new clinical scale for the staging of dementia. *The British Journal of Psychiatry, 140*(6), 566-572.

Irigaray, L. (1967). Approche psycho-linguistique du langage des dements. *Neuropsychologia, 5*(1), 25-52.

Jarrold, W., Peintner, B., Wilkins, D., Vergryi, D., Richey, C., Gorno-Tempini, M. L., & Ogar, J. (2014). *Aided diagnosis of dementia type through computer-based analysis of spontaneous speech*. Paper presented at the Proceedings of the Workshop on Computational Linguistics and Clinical Psychology: From Linguistic Signal to Clinical Reality.

Karakaş, S., & İrkeç, C. (2008). *Kognitif nörobilimler*: MN Medikal & Nobel.

Klein, E. R., & Mancinelli, J. M. (2021). *Acquired language disorders _ a case-based approach* Plural Publishing.

Liu, M. (2016). Verbal communication styles and culture. In *Oxford research encyclopedia of communication*.

MacDonald, M. C., Almor, A., Henderson, V. W., Kempler, D., & Andersen, E. S. (2001). Assessing working memory and language comprehension in Alzheimer's disease. *Brain and language, 78*(1), 17-42.

Molloy, D. W., & Standish, T. I. (1997). A guide to the standardized Mini-Mental State Examination. *International psychogeriatrics, 9*(S1), 87-94.

Munis, Ö. B., Parlak, M. M., & Köse, A. (2021). Analysis of the consistency of information received from Alzheimer's disease patients and their families in the quality of life and depression scales. *Turkish Journal of Clinics and Laboratory, 12*(4), 372-378.

Noone, P. (2015). Addenbrooke's cognitive examination-III. *Occupational Medicine, 65*(5), 418-420.

Organization, W. H. (2017). Global action plan on the public health response to dementia 2017–2025.

Özbabalık, D., & Hussein, S. (2017). Demans Bakım Modeli Raporu. *Aile ve Sosyal Politikalar Bakanlığı*.

Parlak, M. M., Altan, E., & Saylam, G. (2022). Dysphagia in Individuals with Dementia. *Journal of Ear Nose Throat and Head Neck Surgery*(30(2)), 88-96. doi:DOI: 10.24179/kbbbbc.2021-86783

Parlak, M. M., Babademez, M. A., Tokgöz, S. A., Bizpınar, Ö., & Saylam, G. (2022). Evaluation of Swallowing Function according to the Stage of Alzheimer's Disease. *Folia Phoniatrica et Logopaedica, 74*(3), 186-194.

Parlak, M. M., Tokgöz, S. A., Bizpinar, Ö., Saylam, G., & Köse, A. (2022). Investigation of cognition, nutrition, independence and swallowing difficulty, relationship with quality of life, and effect levels in elderly people with Alzheimer's disease living with their families. *Neurology Asia, 27*(3), 701-708.

Rocci, A., & de Saussure, L. (2016). *Verbal communication* (Vol. 3): Walter de Gruyter GmbH & Co KG.

Rochon, E., Waters, G. S., & Caplan, D. (2000). The relationship between measures of working memory and sentence comprehension in patients with Alzheimer's disease. *Journal of Speech, Language, and Hearing Research, 43*(2), 395-413.

Rogers, S. L., & Friedman, R. B. (2008). The underlying mechanisms of semantic memory loss in Alzheimer's disease and semantic dementia. *Neuropsychologia, 46*(1), 12-21.

Satt, A., Hoory, R., König, A., Aalten, P., & Robert, P. H. (2014). *Speech-based automatic and robust detection of very early dementia.* Paper presented at the Fifteenth Annual Conference of the International Speech Communication Association.

Sohlberg, M. M., & Mateer, C. A. (2001). *Cognitive rehabilitation: An integrative neuropsychological approach*: Guilford Press.

Squire, L. R. (2004). Memory systems of the brain: a brief history and current perspective. *Neurobiology of learning and memory, 82*(3), 171-177.

Szatloczki, G., Hoffmann, I., Vincze, V., Kalman, J., & Pakaski, M. (2015). Speaking in Alzheimer's disease, is that an early sign? Importance of changes in language abilities in Alzheimer's disease. *Frontiers in aging neuroscience, 7*, 195.

Weiner, M. F., Neubecker, K. E., Bret, M. E., & Hynan, L. S. (2008). Language in Alzheimer's disease. *The Journal of clinical psychiatry, 69*(8), 1223.

Lumbar Dısc Degeneratıon-Lumbar Dısc Hernıa

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Necati ÜÇLER
Uğur Taşkın KAPLAN

Introduction

The human lumbar spine, like the entire spine, consists of thin, compressible, fibro-cartilaginous intervertebral discs to support upright posture and allow a wide range of motion. With age, the discs begin to degenerate by losing their water. In certain circumstances, disc damage can accelerate these changes, drastically alter the load-bearing properties of the disc, and lead to significant disc degeneration. In severe cases, this degeneration can cause significant morbidity, including pain and neurological symptoms.

To begin with, disc degeneration as a biochemical phenomenon associated with aging and the accompanying radiological changes differ from disc disease as a symptomatic clinical entity. In fact, radiographic signs of disc degeneration are very common in the general population, even if they are not symptomatic. In the literature, magnetic resonance imaging (MRI) of 67 asymptomatic patients was found in the literature by Boden et al. showed significant abnormalities consistent with its degeneration (Boden et al, 1990). Paajanen et al. in their study, they found abnormal lumbar discs on MRI in 35% of asymptomatic control patients (Paajanen et al, 1989). Disc degeneration and lumbar disc herniation are very common in the community, with or without symptoms.

Symptoms associated with disc disease are often nonspecific; therefore, they do not provide much diagnostic benefit over symptoms. The most common symptom directly related to lumbar disc disease is low back pain, usually without radiculopathy. It should be noted that because back pain is such a common symptom, the presence of back pain is not in itself a diagnosis of disc disease and is not necessarily associated with evidence of disc degeneration on imaging in a particular patient.

Diagnosis

As in all patients, a detailed history should be taken and a comprehensive history should be taken including the onset of symptoms, duration and severity. The symptoms associated with the disease should be identified by identifying aggravating and extenuating causes. It is also important to evaluate the patient's social history in terms of contributing to the patient's treatment. Fever, night sweats, night pain, pain that does not go away with position changes, involuntary weight loss, use of anticoagulants, immunocompromised status, active/previous malignancy, age over 60 years, long-term corticosteroid use, low back pain accompanying severe trauma history are red flag symptoms (Downie's). A et al, 2008, Henschke, Maher & Refshauge, 2008:).Additional investigations should be considered in patients with multiple red flag symptoms. In these patients, symptoms indicating emergencies such as cauda equina syndrome should be sought.

Although not every patient with lumbar disc degeneration shows symptoms, the most common complaint of patients with disc disease is low back pain, usually radiating to the sacroiliac joints (Madigan, 2009). It results in increased sensitivity to inflammatory mediators such as prostaglandin E2 and interleukin-6-8 in the vascularized granulation tissue of annular tears of diseased discs, which affects the severity of pain (Peng, 2006). Pain is usually exacerbated by positions that increase axial loading on the discs and increase intradiscal pressure, such as flexion, standing, and sitting.

Patients who experience protrusion or herniation of the nucleus as a result of degenerative disc disease may develop symptoms of nerve root compression or, in rare cases, neurogenic claudication. The anatomy of the posterior longitudinal ligament is stronger in the midline but weaker laterally. This may facilitate posterolateral herniation and cause compression in the nerve roots (13 Peng 2006). The nerve root affected by the posterolateral herniation will usually be the root that emerges from under the pedicle of the caudal vertebra. These patients may experience symptoms associated with nerve root compression, including dermatomal sciatic pain, distal paresthesias, and myotomal motor weakness.

Physical examination

Since the differential diagnosis of low back pain is wide, it is important to examine not only the waist but also the abdomen and hips. On examination, the patient's gait and standing should be observed. Visual inspection of the lumbar region; and the greater trochanters of the femurs, as well as the spinous processes and paraspinal muscles are important. Spinal range of motion should be evaluated while the patient is standing, especially in the anterior-posterior plane. Patients with pain from disc pathology will typically experience more pain when the axial loading of the discs is greatest and in positions that increase stretching of the lumbar nerve roots. Lower extremity strength, reflexes, and sensation should be tested. Motor and sensory defects of certain muscle groups may indicate the level affected.

Straight leg raise or Lasègue tests are applied for radicular symptoms caused by nerve root compression caused by the lumbar disc. With the patient lying on his back, the affected leg is straightened and slowly passively raised. The test is considered positive if the patient's sciatic pain recurs between 30 and 70 degrees. A meta-analysis by Deville et al. in 2000 revealed the sensitivity and specificity of straight leg lift to be 91% and 26%, respectively, in detecting lumbar disc herniation (Devillé, 2000). The onset of symptoms with contralateral leg elevation - the so-called cross straight leg raise or Fajersztajn's cross sciatic sign, while not particularly sensitive (29%) is much more specific (88%) (Devillé, 2000).

Treatment

Non-Surgical Treatments

The initial treatment for acute low back pain, including those caused by lumbar disc disease, is usually nonsurgical. The primary treatment for acute and subacute low back pain in adults is patient education, activity modification to limit disc loading, nonsteroidal anti-inflammatory drugs (NSAIDs), and gradual return to physical activity. However, some patients may find relief with heat therapy. Muscle relaxants can provide relief, but their side effects should be kept in mind. Limited dose and limited duration opioid use may be considered in a selected group of patients with severe acute pain that cannot be adequately controlled with NSAIDs and acetaminophen (Goertz, 2012, Modic, 1984).

The results of epidural steroid injections for acute low back pain in the literature are not consistent with each other. There is some evidence that they may provide short-term subjective improvement in low back pain, but this is not consistent with a functional improvement or change

in surgical planning. Epidural injections are not recommended in patients without symptoms of radiculopathy. There is no indication for oral steroid use in the treatment of uncomplicated acute low back pain (Engers, 2008, Heymans, 2008).

Physical therapy applications such as acupuncture, spinal manipulation, transcutaneous electrical nerve stimulation or lumbar traction may also play an active role in pain. However, surgical intervention may be considered for patients with prolonged symptoms or progressive or particularly motor weakness of pain and neurological symptoms (Engers, 2008).

Surgical Treatments

Since most cases of low back pain (>85% in 6 weeks) (including those with disc herniation) are self-limiting and will resolve with non-surgical treatment in the first place, they can be followed up with non-surgical treatments, except in emergencies. It is important to select patients who will require surgical intervention. (1) patients must be symptomatic; (2) symptoms must match examination and imaging findings; (3) symptoms must be resistant to a long course of appropriate non-surgical treatment (5-8 weeks); (4) there should be a reasonable expectation that surgery will improve the patient's symptoms and functional status, (5) patients with persistent, debilitating low back pain due to painful disc herniations or disc degeneration and who do not respond to nonsurgical treatment may be considered for surgery. In patients with one or two levels of degenerative disc disease and chronic low back pain for at least 2 years, research has shown better benefit of lumbar spinal fusion versus intensive physical therapy (Engers, 2005).

References

1. Boden SD, McCowin PR, Davis DO, et. al. (1990) Abnormalmagnetic-resonancescans of thecervicalspine in asymptomaticsubjects. A prospectiveinvestigation. J Bone Joint Surg Am; 72: pp. 1178-1184.
2. Devillé WL, van der Windt DA, Dzaferagić A, et. al. (2000) The test of Lasègue: systematic review of the accuracy in diagnosing herniated discs. Spine; 25: pp. 1140-1147.
3. Downie A, Williams CM, Henschke N, et. al. (2013) Red flags to screen for malignancy and fracture in patients with low back pain: systemati creview. BMJ 2013; 347: pp. f7095.
4. Engers A, Jellema P, Wensing M, et. al. (2008) Individual patient education for low back pain. Cochrane Database Syst Rev; CD004057
5. Fager CA. (1994) Observations on spontaneous recovery from intervertebral disc herniation. Surg Neurol; 42: pp. 282-28.
6. Goertz M, Thorson D, Bonsell J, et. al. (2012) Adult Acute and Subacute Low Back Pain.15th ed. Institute for Clinical Systems Improvement Bloomington, MN
7. Henschke N, Maher CG, Refshauge KM. (2008) A systematic review identifies five "red flags" to screen for vertebral fracture in patients with low back pain. J Clin Epidemiol; 61: pp. 110-118.
8. Heymans MW, vanTulder MW, Esmail R, et. al. (2004) Back schools for non-specificlow-backpain. Cochrane Database Syst Rev; CD000261.
9. Paajanen H, Erkintalo M, Kuusela T, et. al. (1989) Magnetic resonance study of disc degeneration in young low-back pain patients. Spine; 14: pp. 982-985.
10. Madigan L, Vaccaro AR, Spector LR, et. al. (2009) Management of symptomatic lumbar degenerative disk disease. J Am Acad Orthop Surg; 17: pp. 102-111.
11. Modic MT, Hardy RW, Weinstein MA, et. al. (1984) Nuclear magnetic resonance of the spine: clinical potential and limitation. Neurosurgery; 15: pp. 583-592.
12. Peng B, Hao J, Hou S, et. al. (2006) Possible pathogenesis of painful intervertebral disc degeneration. Spine; 31: pp. 560-566.
13. Peng B, Wu W, Hou S, et. al. (2005) The pathogenesis of discogenic low back pain. J Bone Joint Surg Br; 87: pp. 62-67.
14. Engers DK, Choudhri TF, Dailey AT, et. al. (2005) Guidelines for the performance of fusion procedures for degenerative disease of the lumbar spine. Part 7: intractable low-back pain without stenosis or spondylolisthesis. J Neurosurg Spine; 2: pp. 670-672.

Effects of Melatonin on Physiological Functions

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INTRODUCTION

Melatonin is a derivative of the serotonin molecule produced in our body as a result of the metabolism of the amino acid tryptophan. The serotonin molecule, which is widely produced in the brain, is converted into melatonin in the pineal gland and given to the blood. Studies have shown that the hormone melatonin plays a very important role in protecting and improving body health. Melatonin is responsible for circadian regulation and sleeps control, which is encountered in the organism at the highest concentration during the night (Tamura et al., 2009). With ageing, there is a significant decrease in melatonin production among the elderly, which may decrease sleep quality (Kennaway et al., 2002). In humans, as in other mammals, pineal melatonin is the nocturnal hormone. It shows a circadian rhythm in the blood, and nighttime values are considerably higher than daytime levels (Waldhauser et al., 1984; IIInerova, 1988). Melatonin has many important functions in metabolism. Melatonin is also known to affect the depth and quality of sleep. Accordingly, it has been determined that people who secrete enough melatonin have a more restful and relaxing sleep (Tsuno et al. 2005). In this way, melatonin; is also a regulatory factor for cardiovascular health, blood pressure, immune system functions and hormone levels (Pieri 2013). Normally, various oxidizing and harmful substances are also produced as a result of chemical reactions occurring in the physiological order of our body. These substances are called reactive oxygen radicals. Radicals play a critical role in the development of various diseases such as ageing, cardiovascular diseases, infections and cancer (Reiter et al. 2000). Melatonin; protects the health of the body by cleaning and removing the radicals produced in the body during the night

(Maestroni et al.1999). Studies show that melatonin secretion alleviates the effects of depression, is beneficial in relieving reflux and supports eye health (Konturek et al. 2007). Melatonin has also been reported to trigger growth hormone production from the pituitary gland during secretion. Growth hormone plays a critical role in the regulation of body metabolism. There are studies that the secretion of melatonin prevents the development of diabetes. It has been observed that the level of fat and cholesterol in the blood decreases in proportion to its production, therefore it plays a supporting role in heart health (Arendt 2000).

DISCUSSION

1. Effect of melatonin on the reproductive system

Many studies have shown that melatonin secretion affects the reproductive system (Öztekin et al., 2006; Özturk et al., 2003). Although exogenous melatonin varies according to species, age, dose and application time, it modifies reproduction (Turgut et al., 2002).

In seasonally breeding hamsters, long-term darkness inhibits reproduction due to more melatonin secretion, causing testicular regression in males and anoestrus in females. Although humans do not show seasonal reproduction, epidemiological studies reveal that the pregnancy and

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birth rates show the seasonal distribution in different geographical areas. In regions with long winters, activation of the hypothalamic-gonadal system and pregnancy are lower than in summer (Ölmez et al., 2000). The pineal gland is an upper centre that regulates the endocrinological activity in the organism through the pituitary, thyroid, adrenal gland and gonads. Therefore, it is closely related to events related to dysfunctions in endocrine organs (Turgut et al., 2002).

When the melatonin level drops, the hypothalamogonadol system is activated. During childhood and adolescence, melatonin synthesis gradually declines. Serum melatonin concentration was found to be high in hypothalamic ammonites. With rapid and continuous (if prolonged) exercise, the serum melatonin concentration rises, which can cause ammonia. Melatonin inhibits gonadotropin-releasing hormone (GnRH) in experimental animals. Similar data in humans have not yet been fully elucidated (Brzezinski, 1997). It has been shown that when 300 mg of oral melatonin is given to young women for 4 months, it inhibits LH release and ovulation, and progestin administration increases this effect (Olcese, 2020). It can also directly affect the functions of the ovaries. The presence of melatonin receptors in the granulosa cell membrane has been demonstrated (Voordouw et al., 1992).

In a study of mice, mice administered melatonin was able to maintain sexual interest and performance similar to much younger mice; It has been reported that both male and female mice show regeneration of the sex organs (Gunn et al.. 2016)

2. Effect of melatonin on the immune system

The first finding of the immunological role of melatonin belongs to Mills et al. (2005). Carrillo et al. reported that suppression of immune functions as a result of soft tissue trauma and hemorrhagic shock in mice was reversed with melatonin and that chronic melatonin treatment increased leukocyte natural killer (NK) activity in humans (Carrillo-Vico et al. 2013).

Melatonin increases the immune response. When applied in immunodeficiency states, it leads to significant immune activation. Acute stress, which is known to have negative effects on immune parameters, and immunodeficiency conditions caused by the application of immunosuppressive pharmacological agents can be controlled with melatonin (Ölmez et al., 2000).

Innate immunity can be modulated by the exogenous administration of melatonin. NK cell activity is reduced following pinealectomy in mice. The presence of melatonin in the bone marrow indicates its modulating effect on the growth of NK cells and monocytes. An increase in both NK cells and monocyte counts was observed 7-14 days after the administration of exogenous melatonin to young male mice. In addition, melatonin mediates macrophage production and function. The production of cytokines such as TNF-α and IL-1β, known as macrophages, can be altered by the external administration of melatonin (Hotchkiss et al., 2002).

The immunomodulatory effect of melatonin is mediated by opioid peptides, lymphokines and pituitary hormones originating from T-helper lymphocytes. It also causes a significant increase in interleukin-2 (especially the production of T-lymphocytes, which play an important role in the body's fight against cancer) and γ-interferon levels.

Melatonin also increases the in vivo antibody response (IgM and IgG) (Lissoni et al., 1994). Since the inhibitory effect of melatonin on the immune response is related to the antioxidant property of the molecule, it has been suggested that it may be useful in organ transplantation. The lack of toxicity also supports that this agent can be used safely in transplantation (Reiter et al., 1999).

Another effect of melatonin on the immune system is its anti-inflammatory effect. Some studies suggest that melatonin may affect diseases that may contain viruses and be contagious, such as HIV, bacterial infections, and cancer (Maestroni, 1999).

3. Effect of melatonin on ageing

The pineal gland synthesizes melatonin, a powerful free radical scavenger and antioxidant agent, and its functions decrease with ageing; This gland has increased the interest of researchers dealing with ageing (Reiter et al., 1998).

Decreased antioxidant capacity and damage caused by free radicals are also mentioned among the causes of anatomical and functional degeneration occurring in organs during the ageing process, and it is reported that the reason is melatonin, which decreases with age (Mollaoğlu et al., 2005; Kılıç et al. 2020). Because melatonin is thought to have a stimulating effect on antioxidant enzymes, reducing lipid peroxidation and protecting brain tissue from oxidative damage. Increased free radicals due to decreased melatonin have been blamed for many of the neurodegenerative damages that occur in the brain (Kerman et al., 2005).

Many scientific articles report that plasma melatonin concentration in humans decreases with age (Touitou, 2001; Turgut et al., 2002). With ageing, there is a decrease in the synthesis and release of melatonin. Parallel to this, the circadian rhythm of melatonin is disrupted. According to Touitou (2001), the plasma melatonin concentration measured in elderly people is approximately 40-50% lower than the normal value. In experimental animals, it was determined that the circadian rhythm deteriorates as the animal ages, and the serum melatonin levels of day and night become almost equal (Turgut et al., 2002).

4. Effect of melatonin on sleep

Sleep is indispensable for humans. The key mechanism in sleep regulation is the light-dark cycle, and exposure to light leads to stimulation of a neural pathway that extends from the retina to the hypothalamic area in the brain. The suprachiasmatic nucleus, located in the hypothalamic area, works like the "biological clock" responsible for regulating activities that affect the whole body, by initiating signals that control hormones, body temperature, and the feeling of sleep or wakefulness to other areas of the brain (Macchi and Bruce, 2004). Until the dark hours come, the release of sleep-related melatonin-like hormones is suppressed due to the inactivity of the pineal gland. When the sun's rays disappear, the pineal gland is stimulated and melatonin production begins, as the suppressive signals that prevent the release of melatonin created by the suprachiasmatic nucleus disappear. As melatonin levels increase, fewer stimuli are perceived and the feeling of sleep increases (Arendt, 2000; Arendt et al.,2005).

In a study by Garfunkel et al.; It has been reported that melatonin deficiency has an important role in the frequency of sleep disorders in elderly people, that there is no change in total sleep time with controlled-release melatonin at a dose of 2 mg/night, and sleep quality effectively improves in these patients with sleep disorders (Garfunkel et al., 1990). In another study, it was reported that melatonin supplementation given to patients with melatonin deficiency and sleep disorder may be beneficial in falling asleep (starting sleep) and in maintaining sleep (Haimov et al., 1995).

Riemann et al. (2002); showed that the nocturnal melatonin concentration decreased in patients with insomnia. Mac Farage et al., (1991); investigated the effect of using high-dose melatonin (75 mg) in the treatment of chronic insomnia and found that total sleep times increased in the group given melatonin. Low doses of melatonin have also been shown to be effective in treating insomnia. Volunteer patients were given 0.3 mg and 1 mg doses of melatonin, and it was shown that melatonin at both dose levels reduced the time needed to fall asleep (Birdsall, 1996).

Jet lag is a disorder that manifests itself with insomnia and flu-like symptoms that develop due to the deterioration of body rhythm after overseas flight travel. Concomitant concentration and disorientation, a decrease in blood pressure, and blood sugar levels, and changes in energy, alertness and hormone levels can be observed (Rohr and Herold, 2002; Ölmez et al., 2000). It has

been reported that the administration of melatonin minimizes the effects of jet lag in overseas travellers (Birdsall, 1996).

5. Effect of melatonin on cancer

It has been found that melatonin stops cell proliferation in cancerous tissues, inhibits mitotic activity, and has an antiestrogen effect in breast tissue (Macchi and Bruce, 2004). These effects are likened to chemotherapeutic agents. Therefore, melatonin reduces the proliferation of cancer cells, tumour growth and the number of metastases. In addition, melatonin levels were found to be low in patients with prostate and breast cancer (Macchi and Bruce, 2004; Özgüner et al., 1995).

In some studies, it has been reported that pinealectomy increases tumour formation, while melatonin decreases it (Özgüner et al., 1995). Similarly, melatonin has been found to reduce the frequency of chemical-induced tumours in animals. Pinealectomy; It has been determined that while it increases experimental cancer growth and metastasis of melanoma, leukaemia, lung, liver, ovary, pituitary gland, and prostate, high melatonin amount suppresses cancer growth in these tissues (Regelson and Pierpaoli, 1987; Karasket and Fraschini, 1991).

In a study conducted with the addition of melatonin to the treatment in patients receiving immunotherapy and chemotherapy; In addition to the regression in the tumour, a decrease in the severity of side effects and a prolongation of the life span has been reported (Lissoni et al., 1996).

It is reported that the probability of cancer is low in visually impaired people (Coleman and Reiter,1992; Bartsch et al., 1993). In this statement; It has been shown as a possible mechanism that melatonin is secreted more in visually impaired individuals. In this context, in the studies of Feychting et al. in Sweden and Verkasalo et al. in Finland, it was determined that completely blind women have a lower risk of breast cancer compared to the general female population (Feychting et al., 1998; Verkasalo et al., 1999).

6. Effect of melatonin on the cardiovascular system

Recent findings have shown that the effects of melatonin on the cardiovascular system are receptor and non-receptor mediated. Melatonin causes vasoconstriction in cerebral arteries and vasodilation in peripheral vascular beds. Melatonin levels were found to be low in coronary heart patients with myocardial infarction risk and sudden death risk (Sewerynek, 2002; Dubocovich and Markowska, 2005). Similarly, melatonin levels are low in patients with high LDL cholesterol levels and also in hypertensive patients, and it has been shown that melatonin administration reduces blood pressure (Paulis and Simko, 2007).

7. Effects of melatonin on feeding behaviour

There are different types of studies with conflicting results regarding the effect of melatonin on food intake. While some researchers have shown that melatonin reduces food intake in rats, chickens, hamsters and fish, others have suggested that melatonin has no effect on food intake in rats. However, studies in rodents have reported that food intake increases in response to the exogenous administration of melatonin or its agonists (Angers et al., 2003). It was observed that the blood glucose level, which increased after intravenous glucose administration, decreased again during the sleep period. Melatonin is thought to play an important role in this decrease in blood glucose levels (Rohr and Herold, 2002).

CONCLUSION

The basic duties of the hormone melatonin in the body can be summarized as follows: Melatonin is primarily responsible for the regulation of the body's sleep pattern. Accordingly, thanks to melatonin, whose secretion rate changes in the day-night cycle, the need for sleep occurs at night when daylight is lost. Again, thanks to melatonin, awakening from sleep is provided at certain times of the day through regulated sleep time. It is also known that melatonin secretion suppresses the programmed cell death (apoptosis) mechanism in cells (Yu Q et al. 2000). In this way, it is ensured that the life span of sensitive cells, which are of great importance for the body, especially neurons, which are the basic cellular elements of the nervous system, is prolonged and their loss is prevented. melatonin; helps to slow down the ageing process of the skin by increasing the sleep pattern and quality. Ageing is associated with oxygen radicals and wear of body tissues Melatonin creates an opportunity for tissues to repair themselves during sleep.

REFERENCE

Angers, K., Haddad, N., Selmaoui, B,, Thibault, L. (2003). Effect of melatonin on total food intake and macronutrient choice in rats. *Physiol Behav*, 80(1), 9-18.

Arendt, J. (2000). Melatonin, circadian rhythms and sleep. *New Engl J Med,* 343, 1114-1116

Arendt, J., Skene, D.J. (2005). Melatonin is a chronobiotic. Sleep Med Rev, 9, 25-39.

Bartsch, C., Bartsch, H., Fluchter S.H., Lippert T.H. (1993). *Depleted pineal melatonin production in primary breast and prostate cancer is connected with circadian disturbances; possible role of melatonin for synchronization of circadian rhythmicity. In: Melatonin and the Pineal Gland–From Basic Science to Clinical Application* (Eds Y Touitou, J Arendt, P Pevet):311-316. New York, Elsevier,

Birdsall, TC., (1996). The biological effects and clinical uses of the pineal hormone melatonin. *Alternative Medicine Review*, 2, 94-102.

Brzezinski, A. (1997). Mechanisms of disease: Melatonin in humans. *N England J Med*,

336(3), 186-95

Carrillo-Vico. A., Lardone, P.J., Alvarez-Sánchez, N., Rodríguez-Rodríguez, A., Guerrero, J.M. (2013). Melatonin: buffering the immune system. *Int J Mol Sci*, 22, 14(4), 8638-83.

Coleman, M.P., Reiter R.J. (1992). Breast cancer, blindness and melatonin. *Eur J Cancer Clin Oncol*, 28,501-503.

Dubocovich, M.L., Markowska, M. (2005). Functional MT1 and MT2 melatonin receptors in mammals. *Endocrine*, 27, 101-110.

Feychting, M., Osterlund B., Ahlbom, A. (1998). Reduced cancer incidence among the blind. *Epidemiology*, 9, 490-494.

Gunn, P.J., Middleton, B., Davies, S.K., Revell, V.L., Skene, D.J. (2016). Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. *Chronobiol Int*, 33(1), 39-50.

Garfunkel, D., Laundon M., Nof D., Zisapel N. (1990). Improvement of sleep quality in elderly people by controlled release of melatonin. *Lancet*, 346, 541-543.

Haimov, I., Lavie P., Laudon M., Herer P., Vigder C., Zisapel N. (1995). Melatonin replacement therapy of elderly insomniacs. *Sleep*, 18, 598-603.

Hotchkiss, AK., Nelson R.J. (2002). Melatonin and immune function: Hype or Hypothesis? *Clinical Reviews in Immunology*, 22(5&6), 351-371.

IIInerovi, H. (1988). Entrainment of mammalian circadian rhythms in melatonin production by light. *Pineal Res Rev*, 6,173-217.

Karasket, M., Fraschini F., *Is there a role for pineal gland in neoplastic growth? In Role of Melatonin and Pineal Peptides in Neuroimmunomodulation* (Eds F Fraschini, RJ Reiter), 243-251. New York, Plenum,

Konturek, S.J., Konturek, P.C., Brzozowski, T., Bubenik, G,A. (2007). Role of melatonin in upper gastrointestinal tract *J Physiol Pharmacol*, 58(6): 23-52

Kennaway, DJ., and Wright H. (2002). Melatonin and circadian rhythms. *Curr Top Med Chem*, 2,199-209.

Kerman,,M., Cirak B., Özgüner, M.F. Dağtekin, A. (2005). Does melatonin protect or treat brain damage from traumatic oxidative stress? *Exp Brain Res*, 163, 406-410.

Kılıç, E., Çağlayan, B., Beker Mustafa, C. (2020). Physiological and pharmacological roles of melatonin in the pathophysiological components of cellular injury after ischemic stroke. *Turkish Journal of Medical Sciences*, 50, 10-9.

Lissoni, P., Meregalli, S., Nosetto, L., et al. (1996). İncreased survival time in brain glioblastomas by a radioneuroendocrine strategy with radiotherapy plus melatonin compared to radiotherapy alone. *Oncology*, 53, 43-6.

Macchi, M.M., Bruce, J.N. (2004). Human pineal physiology and functional significance of melatonin. *Front Neuroendocrinol*, 25, 177-195.

MacFarlane, JG., Cleghorn, J.M., Brown, G.M., Streiner, D.L. (1991). The effects of exogenous melatonin on the total sleep time and daytime alertness of chronic insomniacs: a preliminary study, *Biol Psychiatry*, 30, 371-376.

Maestroni, GJ. (1999). Therapeutic potential of melatonin in immunodeficiency states, viral diseases, and cancer. Adv. Exp. Med. Biol. *Advances in Experimental Medicine and Biology*, 467, 217-26.

Mills, E., Wu, P., Seely, D., Guyatt, G. (2005). Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. *J Pineal Res*, 39, 360-366.

Mollaoğlu, H., Özgüner, M.F. (2005). Yaşlanma sürecinde melatoninin rolü. *Süleyman Demirel Üniversitesi Tıp Fakültesi Dergisi*, 12, 52-56.

Olces, J. M. (2020). Melatonin and Female Reproduction: An Expanding Universe. *Frontiers in Endocrinology*. https://doi.org/10.3389/fendo.2020.00085

Ölmez, E., Şahna, E., Ağkadir, M., Acet, A. (2000). Melatonin: Emeklilik yaşı 80 olur mu? *Turgut Özal Tıp Merkezi Dergisi*, 7(2), 177-187.

Özgüner, F., Özcankaya, R., Delibaş, N., Koyu, A., Çalışkan, S. (1995). Melatonin ve klinik önemi. *Süleyman Demirel Üniversitesi Tıp Fakültesi Dergisi*, 2, 1-6.

Öztekin, E., Mogulkoc, R., Baltaci, A.K., Tiftik, A.M. (2006). The influence of estradiol and progesterone and melatonin supplementation on TNF-alpha levels in ovariectomized and pinealectomized rats. *Acta Biol Hung*, 57(3), 275-81.

Özturk, A., Baltaci A.K.., Bediz, C.S., Mogulkoc, R., Gungor, S. (2003). Effects of Zinc and Melatonin on Testicular Tissue of Rats. *Biol Trace Elem Res*, 96 (1-3)2, 55-262.

Paulis, L., Simko, F. (2007). Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. *Physiol Res*, 56, 671-684.

Pieri, C., Marra, M., Moroni, F., et al. (2013). Impact of melatonin on immunity: a review. *Central European Journal of Medicine*, 8 (4), 369-376.

Regelson, W., Pierpaoli MD. (1987). Melatonin A rediscovered antitumor hormone? Its relation to surface receptors, sex steroid metabolism, immunologic response, and chronologic factors in tumour growth & therapy. *Cancer Invest*, 5, 379-385.

Reiter, R.J., Tan, D.X., Osuna, C., Gitto, E. (2000). Actions of melatonin in the reduction of oxidative stress. A review. *J Biomed Sci*, 7 ,444-58.

Reiter, RJ., Maestroni JM., Melatonin in related to the antioxidative defence and immune systems: possible implications for cell and organ transplantation. *J Mol Med* 1999; 77:36-39

Reiter, R.J., Guerrero, J.M.İ., Garda, J.J., Castroviejo, D.A. (1998). Reactive oxygen intermediates molecular damage and ageing: *Relation to melatonin.Ann NY Acad Sci*, 854, 410-24.

Riemann, D., Klein ,T, Rodenbeck, A., Feige, B., Horny, A., Hummel, R., Weske, G., Al-Shayla, A., Vonderholzer, U. (2002). Noctural cortisol and melatonin secretion in primary insomnia. *Psychiatry Research*, 113, 17-27.

Rohr, UD., Herold, J. (2002). Melatonin deficiencies in women. *Maturitas*, 41(1), 85-104.

Tsuno, N., Besset, A., Ritchie, K. (2005). Sleep and depression.J Clin Psychiatry, 66, 1254-69.

Sewerynek, E. (2002). Melatonin and the cardiovascular system. *Neuro Endocrinol Lett*, 23(1), 79-83,

Tamura, H., Nakamura, Y., Korkmaz, A., Manchester, L. C., Tan, D. X., Sugino, N., & Reiter, R. J. 2009. Melatonin and the ovary: physiological and pathophysiological implications. *Fertility and Sterility*, 92(1), 328–343.

Touitou, Y. (2001). Human ageing and melatonin: clinical relevance. Exp Gerontol, 36, 1083-1100.

Turgut, M., Baka, M., Yurtseven, M. (2002). Pineal gland'dan salgılanan bir nörohormon olan melatoninin etkileri, *Arşiv*, 11, 453-470.

Verkasalo, P.K., Pukkala, E., Stevens, R.G., Ojamo, M., Rudanko, S.L. (1999). Inverse association between breast cancer incidence and degree of visual impairment in Finland. *Br J Cancer*, 80, 1459-1460.

Voordouw, B.C.G., Euser, R.,Verdank, R.E.R,.,et al. (1992). Melatonin and melatonin progestin combinations alter pituitary ovarian function in women and inhibit ovulation. *J Clin Endocrinol Metab*, 74:108-17.

Waldhauser, F., Dietzel M., Daily and annual rhythms in human melatonin secretion: role in puberty control. *Ann NY Acad Si*, 1985, 53-205-44.

Yu, Q., Miller, S.C., Osmond, D.G. (2000). Melatonin inhibitors apoptosis during early B-cell development in Mouse bone marrow. *J Pineal Res*, 29, 86-93

Importance of Milk in Nutrition

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Introduction

The history of milk begins in the Neolithic Age when people transitioned from nomadic hunting to a settled society. With the establishment of a fixed dwelling, it was possible to collect and domesticate animals for work and food, thereby improving agricultural practices. The first animals to be domesticated were goats and sheep from the Middle East, which made it easier for humans to manage due to their size, robustness, adaptability, behaviour and social nature. For a long time, goats and sheep were a source of food (meat and milk) and clothing (wool). For centuries, milk has become a desirable and valuable source of food; thus, herds were formed and dairy breeds were selected (Yildiz,2010; Barłowska et al., 2011). It is seen that the themes of milk and milk curd are worked in the Babylonian reliefs of the 26th century BC. Again, in the 8th century BC, there are expressions about milk, curd and cheese in Homer's writings (Jain, 1998).

Humans have developed close contact with nature, and milk has become the 1st food source after birth. Breast milk has always been the sole source of food for children. Milk was introduced only after the domestication of animals, starting from goats and sheep about 13000 years ago (Haenlein, 2007; Yildiz, 2010), after nursing, followed by the domestication of cows about 4000 years later (Yıldız, 2010). Therefore, milk has become an indispensable food in human life.

Milk contains more vital nutrients than other foods. The nutritional value of a food is measured by the content of nutrients that the body needs to perform its normal functions. Almost all of the nutrients required by the body in milk have been collected in a sufficient and balanced way. Therefore, it is a foodstuff with superior properties (Gerhardt and Thomas, 2006).

Importance and Properties of Milk

Milk is a secretion of the mammary gland, the physical properties and composition of which vary between species. If a section of the breast is taken and examined; It is seen that a large number of channels, the diameter and number of which vary according to species and race, come to each udder chamber, which is separate and independent in terms of function (Demirci, 2001). Milk is a mixed oil/water emulsion containing fat, protein, lactose, minerals, enzymes, cells, hormones, immunoglobulins and vitamins. (Selvaggi et al., 2014b).

The blood vessels in the breast must supply sufficient blood to the cells and tissues that make up the milk. Because an average of 350-500 litres of blood is required for the formation of 1 litre of milk. It is synthesized in milk alveolar cells. While some components of milk pass directly through the blood, most of them are re-synthesized with the cornerstones in the blood (Metin, 2005). Worldwide, cow's milk is the most consumed, dominating world milk production with 782 million tons in 2013. Therefore, 85% of world milk production comes from cattle, followed by milk from other species such as Buffalo (11%), Goat (2.3%), Sheep (1.4%) and Camel (0.2%)

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(FAO, 2015). However, farms producing sheep milk constitute an important part of agricultural economies in many countries, especially in neighbouring countries to the Mediterranean and the Middle East. The world's largest producer of sheep's milk is China (12.2%) and the leading producers in Europe are Greece (8.7%), followed by Romania (7.2%) and Italy (6.1%) (Barłowska et al., 2011). Sheep milk is slightly less important in Near East and North Africa with 7.5% production and in Sub-Saharan Africa (5.6%) and East and Southeast Asia (3.9%). Milk production from small ruminants such as sheep and goats has increased over the years and new consumer markets are now sought (Selvaggi et al., 2014a). Milk is a good source of minerals such as calcium, phosphorus, magnesium, potassium, and zinc. However, milk with low iron content and iron bioavailability cannot make a significant contribution to the iron requirement in childhood. The mineral content of milk is affected by many conditions such as the physiological condition of the animal, lactation status, environmental and genetic factors, and some processes applied to milk (Baysal, 2004).

Under normal conditions, milk has a light consistency and a homogeneous fluidity. However, in some cases, this appearance may change; creeping, smearing, and the sticky structure may occur. If it shows a very thick consistency, it indicates that the milk has been mixed with colostrum or maybe late lactation milk. Milk has a slightly sweet, pleasant flavour provided by lactose, fat and minerals. The taste and smell of milk with high dry matter are perceived more strongly. The taste and odour in milk are revealed by the effect of some aroma substances. It is known that there are traces of flavour substances such as acetone, acetaldehyde, butyric acid and other free acids in fresh milk. As a result of increased chlorine ions and decreased lactose in breast diseases, milk also has a slightly salty taste. Excess globulin and mineral substance in colostrum give the milk a bitter and salty taste, as seen at the end of lactation (Metin, 2001).

Milk normally has a white or creamy colour. The natural colour of milk is affected by the breed and diet of the dairy animal. Milk is perceived as porcelain white with the effect of colloidal substances such as opaque calcium caseinate and milk fat reflecting light. After the casein is separated, the remaining whey appears in greenish-yellow colour, and in skimmed milk, it appears white with a slight blueish colour (Kırdar, 2001).

Almost all of the essential vitamins for humans are found in milk. Vitamins A, D, E and K are associated with milk fat. It is the carotenoids that give milk fat its yellowish colour and the riboflavin that gives it its fluorescent colour. As milk fat decreases, the content of fat-soluble vitamins also decreases. Unenriched milk has very little vitamin D and K. Milk also contains water-soluble vitamins. It is considered a good source of folate because it contains folate-binding proteins and whey protein that increase absorption (Miller et al., 2000).

Milk fat affects the appearance, taste, flavour and durability of milk. It is also a source of essential fatty acids, fat-soluble vitamins and energy. The oil exists as microscopic globules in the water emulsion. Milk, triglycerides (97-98%), phospholipids (0.2-1.0%), free sterols (0.22-0.41%), free fatty acids, fat-soluble vitamins (A, D, E, K)), contains more than 400 different fatty acids and fatty acid derivatives (Miller et al., 2000).

The specific gravity of Cow's Milk is 1.028-1,037 g/cm3, slightly higher than that of water. The reason for this difference; is lactose, protein and minerals found in milk with specific weights varying between 1.6-3.0 g/cm3 (Kırdar, 2001).

Due to the lactose and minerals found in real solution in its composition, milk freezes at a lower temperature, at about -0,55ºC, compared to distilled water (Oysun, 1991).

Lactose and soluble minerals, which are in the structure of milk and form a real solution, increase the boiling point. Due to these substances, the boiling point is 100.16 °C (Besler and Ünal, 2006).

DISCUSSION

Minor Components of Milk

Milk is often described as a colloidal suspension containing emulsified fat globules, a heterogeneous family of major and minor proteins, carbohydrates lactose, minerals, vitamins, enzymes (Huppertz and Kelly, 2009) and many minor components with important physiological and metabolic properties. It includes technological roles such as cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides (Haug et al., 2007).

The high content of saturated fatty acids in milk has been reported to be responsible for adverse effects that contribute to heart disease, weight gain and obesity (Insel et al., 2004). However, this is controversial as many milk components support health benefits, including oleic acid, conjugated linoleic acid, omega-3 fatty acids, proteins, vitamins, minerals and bioactive compounds, and various milk proteins and their peptides. -It has been suggested that it has cancer activity (Duarte et al., 2011; Rodrigues et al., 2009).

Salts and Minerals

Milk salts are mainly phosphates, citrates, chlorides, sulfates, carbonates and bicarbonates of sodium, potassium, calcium and magnesium. Since milk contains organic and inorganic salts, the salt level is by no means equivalent to the ash content (Huppertz and Kelly, 2009).

Some elements enter the milk from food, but milking equipment and equipment are important sources of elements such as copper, iron, nickel and zinc. The mineral and vitamin contents of goat and sheep milk are mostly higher than cow's milk (Park et al., 2007). Vitaminler

While milk's fat-soluble vitamins A, D, E, and K are related to the fat ratio of milk, water-soluble vitamin B complex and vitamin C are related to the water phase. Vitamins are variable and therefore their processing can reduce the effective vitamin content in milk. During processing, fat-soluble vitamins are retained by the cream, while water-soluble vitamins remain in skim milk or whey (Michaelidou and Steijns, 2006).

İmmune Components

Milk plays an important role in the defence of the mammalian host (Stelwagen et al., 2009). Immunoglobulins (Ig) found in the colostrum and milk of all lactating species provide immunological protection for the offspring against microbial pathogens and toxins. Depending on the species, it can find different immunoglobulins and concentrations. In colostrum, the concentration of immunoglobulins is particularly high, in contrast to IgG, the important class of immunoglobulins in ruminant milk, and the main immunoglobulin IgA found in human milk. Immunoglobulins are transported to mammary secretions via special receptors. In addition to immune globules, both colostrum and milk contain living cells, including neutrophils and macrophages, which secrete some of the immune-related components. These include cytokines and antimicrobial proteins and peptides such as lactoferrin, defensins and cathelicidins. Mammalian epithelial cells themselves contribute to hosting defence by secreting several innate immune effector molecules. A detailed understanding of these proteins and peptides offers great potential to add value to the dairy industry. This is demonstrated by the widespread commercial applications of lactoferrin isolated from bovine milk (Rodrigues et al., 2009).

Bioactive Peptides

Bioactive peptides can be obtained from precursor proteins by enzymatic hydrolysis with digestive enzymes derived from microorganisms or plants, or by fermentation of milk with proteolytic starter cultures (Korhonen and Pihlanto-Leppala, 2006).

Among the bioactive peptides from milk, those with blood-pressure-lowering effects are of particular interest (FitzGerald et al., 2004). Some antihypertensive products based on milk peptides with clinically proven health benefits are commercially available (Lopez-Fandino et al., 2006). Sheep and goat milk proteins are important sources of bioactive ACE-inhibiting peptides and antihypertensive peptides (Park et al., 2007). Goat milk is seen as an attractive area of research as it is less well studied than bovine milk, and also because new peptide angiotensin-converting enzyme (ACE) inhibitors are found in goat milk hydrolysates (Geerlings et al., 2006).

Polyamines

Polyamines consist of flexible polycations that are fully charged under physiological pH conditions. It fulfils several roles in cell metabolism and is necessary for cell growth and proliferation (Löser, 2000; Eliassen et al., 2002; Gugliucci, 2004; Larqué et al., 2007). In addition to participating in DNA, RNA and protein synthesis, the most important function of polyamines is to mediate the interaction of all known hormones and growth factors.

Polyamine requirements that cannot be met by biosynthesis must be met with exogenous polyamines consumed from food (Jeevanandam et al., 1997). It has been suggested that maturation of the gut is maintained by dietary polyamines; therefore, supplementation may be beneficial in formula-fed infants.

Moreover, polyamines are important for the reliability of enhanced DNA transcription and RNA translation that occur in response to infection and during tissue repair, during intestinal growth after surgery, and in intestinal barrier functions (Grimble and Grimble, 1998).

Hormones

Milk contains varying levels of hormones that have limited nutritional or diagnostic value. However, many studies have been conducted on the physiological roles of hormones in human and bovine milk (Koldovsky and Thornburg, 1987; Grosvenor et al., 1993).

Estrogen concentrations, namely 17β-estradiol, estrone and estriol concentrations in milk and a few dairy products, have been reported (Wolford and Argoudelis, 1979). Cow's milk fat contained 65% 17p-estradiol and 80% estrone. The occurrence of estrogen in both butter and skim milk indicates the distribution of these steroids between the milk fat phase and the serum phase. Estrone is found in milk as the predominant estrogen and estrogen concentrations are well known to correlate with the pregnancy and reproductive cycle. Progesterone levels in milk, it is associated with pregnancy and childbirth (Comin et al., 2005).

Cow Milk

When you think of milk, the first thing that comes to mind is cow's milk. Due to the high yield and the length of the lactation period, only cows are used as dairy animals in many countries today (Kırdar, 2010).

Most cows are born just before spring and lactation stop for 8-10 weeks during the winter. This production system is characterized by irregularities in the milk supply to the processor, both in quantity and composition, accompanied by seasonal variations in milk production potential (Auldist et al., 1998). In particular, milk coming from the late hours of the production season (autumn and winter) may have different production characteristics than early and mid-season milk, and some products cannot be produced at all (Lucey, 1996).

Sheep Milk

The nutritional value of sheep milk is higher than that of goat and cow milk, with proteins, lipids, minerals and vitamins important for human health and a caloric value corresponding to 5932 kJ/kg (Haenlein, 2001; Kaminarides et al., 2007; Park et al., 2007; Barłowska et al., 2011).

Since approximately 80% of the proteinaceous substances in its composition are made up of casein, it is included in the group of casein milk. Due to its high dry matter, its caloric value is also high. Its colour is whiter than cow's milk. The average lactation period of sheep is 7 months and their milk yield is between 400-700 litres in a lactation period, depending on the breeds (Akçapınar, 2000).

Sheep milk is a rich source of minerals. Calcium, phosphorus, magnesium, zinc, manganese and copper levels are higher in sheep compared to cow's milk, while the reverse is seen for potassium and sodium (Park et al., 2007; Wijesinha-Bettoni and Burlingame, 2013). Calcium and phosphate, which are essential elements for growth and bone maintenance and necessary for newborns, are abundant in sheep's milk (Al-Wabel, 2008). The bioavailability of these minerals makes sheep milk a valuable source of these elements. Calcium, which is bound to casein in both organic and mineral form, exhibits significant availability during the milk digestion process (Gueguen and Pointillart, 2000); therefore, the calcium bioavailability of sheep milk is strongly associated with high casein levels (Gaucheron, 2005).

Sheep milk's vitamin content is mostly higher than cow's and goat's milk, except for low concentrations of carotene and folate, and pantothenic acid and vitamin D, which have equal concentrations to cow's milk (Park et al., 2007; Wijesinha-Bettoni and Burlingame, 2013). Sheep milk is high in riboflavin, while cow, goat and buffalo milk are adequate sources of riboflavin. As a reference, nutrient intakes of riboflavin (0.5 mg/d) can be achieved with 2 cups of sheep's milk (300 mL). Sheep's milk can also be considered a source of vitamin C, containing an average of 4.6 mg/100 g (Wijesinha-Bettoni and Barbara Burlingame 2013). Since it is not common to drink sheep's milk, likely, two glasses of sheep's milk yoghurt or the equivalent of milk in 90 g of sheep cheese will meet the daily requirements (Recio et al., 2009). Sheep's milk is also a good dietary source of vitamin A and vitamin E. Vitamin A is only found in sheep's milk as retinol because dietary β-carotene is completely converted to this form (Raynal-Ljutovac et al., 2008); However, vitamin E exists in 3 forms, with α-tocopherol being the most abundant form (α-, β-, and γ-tocopherols) (Revilla et al., 2017).

Goat Milk

In terms of composition, it has values close to cow's milk. Since approximately 75% of the proteinaceous substances in its composition are made up of casein, it is included in the group of casein milk. Since the amount of carotene is low, goat's milk is whiter than cow's milk and this is how it is distinguished from cow's milk. The dry matter of goat milk varies between 13-14%. While the amount of non-protein nitrogenous substances is 0.19% in cow's milk and 0.12% in women's milk, it is as high as 0.44% in goat's milk (Uysal and Kılıç, 2005).

The ratio of capric, caprylic and capric fatty acids is high in goat milk fat. Goat milk is 2-3 times richer in vitamin A than other milk. The reason for this is that goats eat more green fodder in winter and the thyroid glands, which play a role in the conversion of carotene to vitamin A, are larger and more active in goats (Veral, 2005).

The lactation period of dairy goats varies between 6 and 10 months. The annual milk yield of culture breeds is around 1,000 litres. This amount increases up to 1,500 – 2,000 litres in specially fed goats. Goats are considered to be the animals that give the most milk in proportion to their body weight, and culture-breed goats give milk approximately 10 times their body weight in a

lactation period. As the yield increases, there is a decrease in the milk-fat and protein ratio (Dellal, 2005).

Goat milk contains a large amount of phosphate. Goat milk is a good source for eliminating phosphate deficiency seen in people who do not have the habit of eating meat and fish. Because it keeps stomach acidity under control, it is recommended that people with stomach problems drink goat's milk. Goat milk is poor in terms of some vitamins, especially vitamin B12, manganese and iron. For this reason, anaemia can be seen in those who are fed goat milk for a long time (Haenlein, 2004).

Breast Milk

The mineral content of breast milk is low and is compatible with the kidney functions of immature newborns. In addition to amino acids, glucose, fatty acids and vitamins, minerals are also needed for the reproduction, regeneration and growth of organisms. Milk protein is of good quality and its utilization rate in the body is 90%. The known contribution of milk proteins to growth and development in the body, as well as their effectiveness in tissue differentiation; It is known that it has positive effects on calcium absorption and immune system functions, reduces blood pressure and cancer risk, is effective in controlling body weight, and is protective against dental caries (Balcı, 2011). Lactose, a milk carbohydrate, is the source of milk energy. Lactose is helpful in the formation of brain and nerve cells and in regulating bowel movements. It develops beneficial gut bacteria by providing a suitable environment (pH). Mild nausea, gas in the stomach, heartburn and mild diarrhoea can be seen in those who do not have the habit of drinking milk. These findings go away as you continue to drink milk (Balcı, 2011).

Milk fat is another source of milk energy. It provides absorption of fat-soluble vitamins (A, D, E, K). In special cases and adulthood, semi-skimmed or skimmed milk can also be consumed. It has been reported that fatty acids in milk fat are a variable that enables the development of the nervous system and intellectual capacities of children (Samur, 2012).

Milk is rich in minerals (calcium, phosphorus, iodine, sodium, and magnesium). Milk contains more and more available calcium minerals than any other food. Calcium, phosphorus and magnesium are essential components of bone tissue. From childhood to the age of 20-25, with a balanced diet, bone mineral tissue increases. In old age, bone mineral tissue decreases due to inactivity and changes in hormonal balance. Milk, which is rich in calcium, phosphorus and protein content, provides the development of bone tissue in childhood and youth and reduces loss in old age. Milk protein not only increases calcium absorption but also ensures the formation of bone tissue cells. Lactose, a milk carbohydrate, is also an important factor that increases calcium absorption from the small intestines (Coşkun, 2003).

Milk contains a large number of vitamins that have a function in growth and development, the proper use of nutrients in the body, the fulfilment of the functions of the nervous system, and the development of body resistance and blood production. Riboflavin (vitamin B2), B12, vitamin A, B6, B1, niacin and folic acid are vitamins found in sufficient amounts in milk. The vitamins in the composition of milk are extremely sensitive to many physical and chemical effects such as heat and light. Loss of nutrients due to exposure to ultraviolet rays during the processing of milk, especially during heat treatment and transportation, is an undesirable situation in terms of health (Baysal, 2007; Samur, 2012). It is a natural food that is easy to digest and contains fluid, nutrients and energy elements that the newborn needs for ideal development and growth, with high biological benefits. Breast milk in terms of baby and mother; It has many benefits in terms of nutrition, health, immunity, development, psychological and socioeconomic aspects (Hancıoğlu Aytaç and Yazıcı, 2020).

The presence of cells, enzymes, hormones, lysozymes, different growth factors, lactoferrin, peptides and special immunoglobulins in the content of breast milk and the high level of taurine

make breast milk a unique compound. In addition to these substances in breast milk, the amounts of iron, zinc, copper, calcium and phosphorus are also adjusted according to whether the baby is premature or not. When the amount of these is adjusted at the beginning, end and different meals and the secretion of special immunoglobulins according to the needs of the baby is taken into account, there is no other explanatory source other than believing that this is made especially for babies by nature (Bilir, 1984; Erbersdobler et al.1984).

CONCLUSION

The amount of milk recommended being consumed for adequate and balanced nutrition of healthy individuals varies according to age, gender and physiological status (growth and development period, pregnancy, lactation, old age). 2-4 (400-800 ml) servings in the Nutrition Guide published by the National Milk and Dairy Products Council and 2 servings for adult individuals in the Turkey-Specific Dietary Guidelines [one serving: one medium glass (200 ml)] It is recommended that children, adolescents, pregnant and lactating women and postmenopausal women consume 3-4 (600-800 ml) portions. Milk contains a large number of vitamins that have a function in growth and development, the proper use of nutrients in the body, the fulfilment of the functions of the nervous system, and the development of body resistance and blood production. When milk and dairy products are considered a source of some important minerals, especially calcium and phosphorus, and some B group vitamins such as protein and riboflavin, it is understood that they are an important food group in terms of public health.

REFERENCES

Akçapınar, H. (2000). *Koyun Yetiştiriciliği Ders Kitabı*, İsmat Matbaacılık, Ankara.

Auldist, M.J., Walsh, B.J., Thomson, N.A. (1998). Seasonal and lactational influences on bovine milk composition in New Zealand. *J Dairy Res.* 65(3):401-11.

Balcı, E. (2011). Anne sütünün çocuk büyüme ve gelişmesine etkisi. *Türk Aile Hek. Derg,* 15(3), 135-138,

Barłowska, J., Szwajkowska M., Litwinczuk Z., Kr´ol, J. (2011). Nutritional value and technological suitability of milk from various animal species used for dairy production. *Compr Rev Food Sci Food Safety,* 10, 291-302.

Baysal, A. (2004). *Beslenme.* 10.baskı. Ankara, Hatiboğlu Yayınları, Bölüm II Besinler, Süt.

268-275.

Besler, H., Ünal, S. (2006). *Ankara'da satılan sokak sütlerinin bazı vitaminler açısından değerlendirilmesi ve ev koşullarında uygulanan kaynatmanın süreye bağlı olarak vitaminlere olan etkisi.* IV Uluslararası Beslenme ve Diyetetik Kongresi Bildiri Kitabı.

Bilir, Ş. (1984). *Ana ve Çocuk Sağlığı*, Hacettepe Üniversitesi Yayınları, A/14, Dördüncü Baskı, 222-226,

Comin, A., Renaville, E., Marchini, E., Maiero, S., Cairoli, F., Prandi, A. (2005). Technical note: Direct enzyme immunoassay of progesterone in bovine milk whey. *J Dairy Sci,* 88, 4239-4242.

Coşkun, T. (2003). Anne Sütü ile Beslenme. *Katkı Pediatri Dergisi*, 2:163-183.

Dellal, İ., Dellal, G. (2005). *Türkiye Keçi Yetiştiriciliğinin Ekonomisi, Süt Keçiciliği.* Ulusal Kongresi, 26-27 Mayıs, İzmir, 39-48.

Eliassen, K.A., Reistad, R., Risoen, U., Ronning, H.F. (2002). Dietary polyamines. *Food Chem* 78, 273-280.

Erbersdobler, H.F., Trautwein, E., Grenlich, H.G. (1984). Determinations of Taurine in Milk and Infant Formule Diets. *Eur. J. Pediatr*, 142, 133.

FitzGerald, R.J., Murray, B.A., Walsh, G.J. (2004). Hypotensive peptides from milk proteins. *J Nutr,* 134, 980-988.

Gaucheron, F. (2005). The minerals of milk. *Reprod Nutr Dev,* 45,473-83.

Geerlings, A, Villar, IC, Hidalgo-Zarco, F, Sánchez, M, Vera, R, Zafra-Gómez, A, Boza, J & Duarte, J. (2006). Identification and characterization of novel angiotensin-converting enzyme inhibitors obtained from goat milk. *Journal of Dairy Science,* 89, 3326-3335

Gehardt, S.E., Thomas, R.G. (2006). *Nutritive Value of Foods.* United States Department of Agriculture (USDA). Agricultural Research Service. Home and Garden Bulletin. Number 72.

Grimble, R.F., Grimble, G.K. (1998). Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. *Nutrition,* 14, 605-610.

Grosvenor, C.E., Picciano, M.F., Baumrucker, C.R. (1993). Hormones and growth factors in milk. *Endocr Rev,* 14, 710-728.

Gueguen, L., Pointillart A. (2000). The bioavailability of dietary calcium. J Am Coll Nutr 19:119-136.

Gugliucci, A. (2004). Polyamines as clinical laboratory tools. *Clin Chim Acta,* 344, 23- 35.

Haenlein, GFW. (2007). About the evolution of goat and sheep milk production. *Small Ruminant Res,* 68, 3-6.

Haenlein, GFW. (2001). Past, present and future perspectives of small ruminant dairy research. *J Dairy Sci*,84, 097-115.

Hancıoğlu Aytaç, S., Yazıcı, S. (2020). The Importance of Breastfeeding in Postpartum Period And Traditional Methods. *Necmettin Erbakan University Faculty of Health Sciences Journal,* 3,1.

Haug, A., Hostmark, A.T., Harstad, O.M. (2007). Bovine milk in human nutrition - a review. *Lipids Health Dis,* 6, 25.

Huppertz, T., Kelly, A.L. (2009). Properties and constituents of cow's milk. In: Milk Processing and Quality Management (eds A.Y. Tamine), pp. 24-47, Willey.

Insel, P., Turner, R.E., Ross, D. (eds) (2004). *Nutrition. American Dietetic Association, Jones and Bartlett*, USA.

Jain M. (1998). Dairy foods, dairy fats, and cancer: A review of epidemiological evidence. *Nutrition Research*, 18(5), 905-937.

Jeevanandam, M., Holaday, N.J., Begay, C.K., Petersen, S.R. (1997). Nutritional efficacy of spermidine supplemented diet. *Nutrition* 13, 788-794.

Kaminarides ,S., Stamou P., Massouras T. (2007). Comparison of the characteristics of set-type yoghurt made from ovine milk of different fat contents. *Int J Food Sci Tech,* 42,1019-28.

Kırdar, S. (2001). *Süt ve ürünleri analiz metodları - Uygulama Klavuzu*. 5-7. Bölüm. Ankara, Süleyman Demirel Üniversitesi, Süt Yayınları.

Koldovsky, O., Thornburg, W. (1987). Hormones in milk. *J Ped Gastroenterol Nutr,* 6, 172-196.

Korhonen, H., Pihlanto-Leppala, A. (2006). Bioactive peptides: production and functionality. *Int Dairy J* 16, 945-960.

Larqué, E., Sabater-Molina, M., Zamora, S. (2007). The biological significance of dietary polyamines. *Nutrition* 23, 87-95.

Lopez-Fandino, R., Otte, J., van Camp, J. (2006). Physiological, chemical and technological aspects of milk-protein-derived peptides with antihypertensive and ACE-inhibitory activity. *Int Dairy, J* 16, 1277-1293.

Loser, C. (2000). Polyamines in human and animal milk. *Br J Nutr,* 84, 55-58.

Lucey, J. (1996).Cheesemaking from grass-based seasonal milk and problems associated with late-lactation milk. *Journal of the Society of Dairy Technology,* 49, 59-64.

Michaelidou, A., Steijns, J. (2006). Nutritional and technological aspects of minor bioactive components in milk and whey: Growth factors, vitamins and nucleotides. *Int Dairy J,* 16, 1421-1426.

Miller, GD., Jarvis KJ., McBean, L.D. (2000). *Handbook of Dairy Foods and Nutrition. In: Jensen RG, Kroger M, editors. The Importance of Milk and Milk Products in the Diet.* CRC Press, New York.

Oysun, G. (1991). Süt Ürünlerinde Analiz Yöntemleri. Ege Üniversitesi Yayınları, İzmir.

Park, Y.W., Juarez, M., Ramos, M., Haenlein, G.F.W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Res,* 68, 88-113.

Recio, I., de la Fuente A, Ju´arez M, Ramos M. (2009). Bioactive compounds in sheep milk. In: Park YW, editor. Bioactive compounds in milk and dairy products. Iowa: Wiley-Blackwell, p 83-104, Chapter 4.

Revilla, I., Escuredo O., Gonz´alez-Mart´ın M I., Palacios C. (2017). Fatty acids and fat-soluble vitamins in ewe's milk predicted by near-infrared reflectance spectroscopy. Determination of seasonality. *Food Chem,* 214,468-77.

Rodrigues, L.R., Teixeira, J.A., Schmitt, F., Paulsson, M., Lindmark Masson, H. (2009). Lactoferrin and cancer disease prevention. *Critical Reviews in Food Science and Nutrition,* 49, 203-217.

Samur, G.E. (2012). *Anne Sütü*, 2. Baskı. Ankara, Reklam Kudu Ajansı,13-17.

Selvaggi, M., Laudadio V., Dario C., Tufarelli V. (2014a). Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. *J Sci Food Agric,* 94, 3090-9.

Selvaggi M, Laudadio V, Dario C, Tufarelli V. (2014b) Major proteins in goat milk: an updated overview on genetic variability. *Mol Biol Rep,* 41, 1035-48.

Uysal, H., Kılıç S. (2005). *Türkiye'de Keçi Sütü Üretimi ve Değerlendirme Olanakları, Süt Keçiciliği*. Ulusal Kongresi Bildirisi, İzmir.

Veral ,S. (2005). *Keçi sütünün değerlendirilmesi, keçi sütünden beyaz peynir üretim teknolojisi, Süt Keçiciliği* .Ulusal Kongresi Bildirisi, İzmir.

Wijesinha-Bettoni, R., Burlingame B. (2013). Milk and dairy product composition. In: Muehlhoff E., Bennett A., McMahon D., editors. Milk and dairy products in human nutrition. Rome, Italy: Food and Agriculture Organization of the United Nations. (2009). Lactoferrin and cancer disease prevention. *Crit Rev Food Sci Nutr*, 49, 203-217.

Wolford, S., Argoudelis, C. (1979). Measurement of estrogen in cow's milk, human milk and dietary products. *J Dairy Sci,* 62, 1458-1463.

Yildiz, F. (2010). *Advances in Food Biochemistry*. New York: CRC Press.

Immunosensors: Immobilization Methods, Transduction Mechanisms And Current Studies

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Introduction

Biosensors are analytical devices that transfer and interpret signals generated by the immobilization of biorecognition elements (enzyme, hormone, nucleic acid, cell, antibody) on a transducer surface. Biosensors consist of an analyte that is wanted to identify, a biorecognition element or a bioreceptor, and a transducer that convert the reaction which depends on the analyte's specific concentration to a signal (Sharma, 2016). The biocomponents that sense the analytes may be enzymes, antibodies, DNA probes, microorganisms, cells, and tissues while electrochemical, optical, piezoelectric, thermal or magnetic systems are used as transducers. Electrochemical, optical, piezoelectric and thermometric transducers are the four main types of transducers that are used in biosensors. Electrochemical devices usually measure a fixed voltage in a current (amperometry), a voltage in zero current (potentiometry), conductivity or impedance. Impedance can be defined as the resistance to current in an electrical circuit (Tothill, 2001). Optic transducers use some effects like light absorption, fluorescence or retractive index (Tothill & Turner, 2001). Thermometric biosensors work by using enthalpy changes during biochemical reactions. Sensors working with the piezoelectric principle measure the resonant frequency of wave propagation along the piezoelectric material. These principles are used to evaluate mass, viscosity and density changes at the sensor surface (Tothill, 2001). Immunosensors can operate in two ways, according to direct measurement methods, where the detection mechanism is directly dependent on the antigen-antibody interaction, or indirect measurement methods, where a label such as an enzyme or fluorescent molecule is used to detect the binding reactions (Karunakaran, Pandiaraj & Santharaman, 2015). According to the operation of the detection mechanism within the transduction process, immunosensors may be classified as electrochemical, optical, and piezoelectric immunosensors, or based on the use of labels they can be classified as labeled (indirect) and unlabeled (direct) (Holford, Davis & Higson, 2012).

Immunoassays are quantitative analysis methods that utilize antibodies as immunological agents. The selectivity of antibodies is extremely high and antibodies can bind to their specific targets even if there is a high diversity of different molecules in the sample. Immunoassays consist of a group of immunochemical methods and are frequently used in the field of clinical diagnosis (Karunakaran, Pandiaraj & Santharaman, 2015).

Antibodies are produced as the immune system response of hosts exposed to foreign invaders such as bacteria, viruses and parasites called antigens. Antibodies, also called immunoglobulins, are heavy globular plasma proteins. Antibodies can be classified into five classes: IgG, IgM, IgA, IgE and IgD. IgG is the most abundant antibody in serum and also the most commonly used antibody in immunosensor applications. Antibodies bind to the target antigen with

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high affinity, allowing rapid detection of a wide-range analyte spectrum from pathogens to toxins (Karunakaran, Pandiaraj & Santharaman, 2015).

An antibody can be fixed directly to the surface of an electrode or an optical waveguide in the transducer so that it can provide a detectable signal when binding with the analyte occurs. In this case, the antibodies are referred to as primary or captured antibodies. However, antibodies can be used for labeling when they carry labels such as radioisotopes, enzymes, cells, radiant probes, chemiluminescent probes, metal labels or nanoparticles. Antibodies used in this way are also called secondary antibodies or detection antibodies (Li, 2006). Both primary and secondary antibodies in biosensors are generally used in conjunction with electrochemical, optical and piezoelectric transducers. An immunosensor developed for online measurement of progesterone in bovine milk (Delwiche et al., 2001), an unlabeled quartz crystal microbalance (QCM) immunosensor studied for rapid detection of E.coli O157:H7 and Salmonella typhimurium in food samples (Su & Li, 2004), and an optical immunosensor for the detection of antibiotic and pesticide residues in water (Tschmelak, Proll & Gauglitz, 2005) can be counted among the samples of immunosensors.

Immobilization Methods of Antibodies

The performance of an immunosensor depends on three critical factors. These factors are: biosensing material immobilization without activity loss; analyte accessibility to biosensing materials; and low nonspesific adsorption with the solid substance (Sharma, Byrne & O'Kennedy, 2016). In order to ensure conformational stability and bioactivity during the immobilization procedure, the main goal is to make the active part accessible to the targets. Proper immobilization should be quick and convenient and should prevent biomolecule leaks. In addition, there are several parameters that affect immobilization such as biomolecule size, shape, working area, polarity, functional group presence, and storage conditions. Chemical and physical properties of transducers are also effective on immobilization (Asal et al., 2019).

The compatibility of antibodies to the sensor surfaces can be controlled by interactions between the antibody and surface reagents. Various approaches aim to make the sensor surface more suitable for immobilization with antibodies of specific orientation, such as functionalizing the surface with specific groups (glutaraldehyde carbodiimide, succinimide esters, malenimide or periodate), or designing it with nanostructures (Kierny, Cunningham & Kay, 2012).

Antibodies can be adsorbed on the sensor surface by noncovalent forces such as electrostatic or ionic bonds, hydrophobic interactions, and van der Waals forces. Adsorption may be classified as physisorption and chemisorption. While physisorption includes van der Waals forces and hydrophobic interactions, chemisorption basically includes the electrostatic interactions between the antibody and the surface (Sharma, Byrne & O'Kennedy, 2016). Antibody trapping on conductive polymer films is another noncovalent approach. The capture of antibodies against human serum albumin on galvanostatically polymerized pyrrole was reported (John et al., 1991).

Polyacetylene, polythiophene, polyaniline, polyindole and polypyrrole are common conductive polymers used in immunosensor manufacturing due to their superior biocompatibility against neutral flowing solutions. Polyquinon is another smart material that is used for the immobilization of biomolecules. In addition to their biocompatibility, they have conjugation and transduction capabilities. Thus, it makes it unnecessary to add a redox label to the solution or to the antibody (Sharma, Byrne & O'Kennedy, 2016).

Chemical immobilization is provided by primary bonds such as covalent bonds. Covalent bonds provide stable and irreversible immobilization and are widely used in antibody immobilization. Most covalent immobilizations use functional groups naturally found in

antibodies. Sugar domains and disulfide bonds between two heavy chains are the most frequently used functional groups to ensure correct orientation (Park, 2019). Covalent bonds are formed by surface modifications that enable the interaction of subsequent antibodies with reagents such as hydroxy, thiol, carboxy and amino groups on the surface. Various surface modification techniques have been developed by chemical modification, photochemical grafting, plasma gas discharge and ionized radiation graft polymerization methods. Binding of antibodies to the sensor surface using amine groups is often performed in the form of covalent bonding with the amino acid lysine to the side chains of antibodies, since these groups are relatively easy to access (Sharma, Byrne & O'Kennedy, 2016).

The primary amine groups of antibodies are covalently bound to the reactive succinimide esters. Feyysa et al. (Feyssa et al., 2013) reported that immobilization of anti CRP antibody via amine covalent bond in a microfluidic platform, enhanced the sequence signal compared to passive binding. The manufacturability of this biochip appears to be similar to that of the human CRP ELISA detection kit. Rahman et al. (Rahman et al., 2007) immobilized a polyclonal antibody onto a carboxylic acid group functionalized on the nanoparticle with the quartz crystal microbalance principle for the detection of bisphenol A.

Antibodies are glycoproteins and contain polysaccharides in their heavy chains. These sugar chains are positioned in the Fc region and immobilization over these regions may be the right choice. Saccharide molecules contain many hydroxyl groups and these groups can be converted to aldehyde groups by oxidation. Therefore, sugar chains that contain active aldehyde groups can be used as an immobilization site. (Park, 2019).

Another common method of immobilization on sugar chains is boronic acid immobilization. Boronic acid forms boronate esters with cis-diols of saccharide molecules at room temperature (Park, 2019). Lin et al. provided antibody conjugation on magnetic nanoparticles with the help of boronic acid by covalent binding of the carbohydrate part of the constant region (Lin et al., 2009).

The method of using disulfide bonds between two heavy chains is also used to increase the accessibility of the active sites of antibodies. The disruption of the disulfide bond occurs by agents such as beta-mercaptoethanol or dithiothreitol. Thus immobilization can be provided by utilizing the disulfide bond (Park, 2019).

Covalent immobilization of antibodies over chemical binders such as glutaraldehyde can damage the antigen binding site and reduce antigen binding affinity (Park, 2019). To overcome this problem, molecules with an affinity for antibodies have been studied. Among these molecules, biotin-avidin/streptavidin, protein A and G are frequently used (Sharma, Byrne & O'Kennedy, 2016; Park, 2019). De Juan Franco et al. developed a new, simple and fast orientation method by utilizing a fusion protein region called protein A gold binding site (PAG). Biotin-labeled antibodies can easily be captured by functionalized surfaces with proteins produced by avidin, streptavidin or a combination of these two proteins and form conjugates (De Juan-Franco et al., 2013). Ionescu et al. developed a highly sensitive direct impedimetric immunosensor for the detection of the herbicide atrazine. Affinity-based immobilization of anti-atrazine antibodies on polypyrrole film was performed using a gold electrode with nitrilotriacetic acid. They were able to detect the charge transfer resistance even with atrazine in the amount of 10 pg/ml, and they were able to gain functionality in the range of 10 pg/ml-1 μg/ml (Ionescu et al., 2010).

One of the most promising methods is the molecularly imprinted polymer method. Molecular imprint-based receptors are designed to selectively identify target molecules, including proteins. Molecularly imprinted polymers are generally formed around the template molecule by a

matrix polymerization method. After polymerization, the template is extracted and a porous structure is produced with the dimensions and shape of the molecule to be immobilized, providing an ordered orientation and recognition with high specificity (Kalecki et al., 2020).

Transduction Mechanisms of Immunosensors

Electrochemical

Antigen-antibody interactions occurring in electrochemical transducers cause changes in ion concentration, potential, current, conductivity, capacitance and impedance measurements. Electrochemical biosensors that take advantage of these changes have many advantages, such as being economical, easy to apply, portable, reliable, fast, powerful, mass-produced and functional with small amounts of analyte. Electrochemical immunosensors have become interesting and sought-after biosensing devices with the use of modern and developing technology (Hayat, Catanante & Marty, 2014).

Changes in the dielectric properties of the electrode surfaces can be monitored by potentiostatic procedures. With this discovery, amperometric biosensors have become more popular. Amperometry measures both the magnitude and intensity of the current at a fixed potential value at a given time in an electrochemical cell. When a regulated potential is applied between the electrodes, redox reactions occur that produce a measurable current proportional to the concentration of the relevant analyte (Hayat, Catanante & Marty, 2014). The electrodes used in amperometric sensors have the property of exhibiting high stability over long time periods and showing a linear response in the physiological range. Resulting from molecules such as potassium ferrocyanide being used to mediate the efficient transfer of electrons between the electrode surface and the labels, the electrodes are not affected by the oxygen flow, dissolved gas effects are avoided, and thus stable and accurate results are obtained (Grieshaber et al., 2008). Pinacho et al. developed an enzyme-labeled amperometric immunosensor immobilized on a magnetic bead that detects 7 different fluoroquinolones in milk (Pinacho et al., 2014). When the reaction takes place with the fluoroquinolone samples, the magnetic beads are easily captured by the magnetic graphite-epoxy composition (m-GEC) electrodes. Magnetic beads work specifically for fluoroquinolones from the multi-component structure of milk, hence there is no need for an extra extraction process. Voltammetry is the process of measuring the corresponding change in current while the potential is changed over a predetermined range. As a result, the measured current is translated into peaks and plateaus that represent the target of interest and where the height of the peak gives the amount of analyte. Detection of different multiple analytes in a single sample is possible because each analyte has a characteristic peak position, making the voltammetry technique extremely valuable for immunosensors (Su et al., 2011). This method is extremely sensitive due to low background noise generation, especially when applied after sample preconcentration (Velusamy et al., 2010). Cyclic voltammetry is an important technique that works with the mechanism of charge transfer reactions of redox labels, enabling the determination of biomolecule concentrations depending on oxidation/reduction pathways. In cyclic voltammetry, the potential of an electrode is drawn from the initial potential to the final potential and back to the initial potential. The changing current is measured during these potential transitions (Karunakaran, Pandiaraj & Santharaman, 2015). Singh and Krishnan developed a voltammetric immunosensor that can measure serum insulin level with 5 pM limit of detection. By exposing the analyte to magnetite nanoparticles, they ensured high sensitivity capture of serum insulin conjugates by the electrodes, thus developing the first voltammetric diabetes sensor (Singh & Krishnan, 2015).

Potentiometric measurement records and displays ion activities in an electrochemical reaction. It indicates the mass charge potential of an indicator electrode versus a reference electrode where the current is zero. The Nernst equation can be used to determine the relationship between

the potential and the amount of analyte. The most commonly used components in potentiometry are pH electrodes. Other electrodes like ion (F^-, I^-, CN^-, Na^+, K^+, Ca^{2+}, NH_4^+) or gas (CO_2, NH_3) selective electrodes may also be used for potential measurements (Bobacka, Ivaska & Lewenstam, 2008). The antibodies and antigens in solution each show a net polarity of electrical charge that correlates with the ionic composition of the solution and the molecules it contains. If the antibody complex combines with the antigen, a different electrical charge arises from the charge that the antibody alone has. This change can be measure by potentiometric methods (Karunakaran, Pandiaraj & Santharaman, 2015). Tarasov et al. developed a potentiometric immunosensor that detects BHV-1 specific antibody in bovine serum with a microfluidic chip immobilized with Bovine Herpes Virus-1 viral protein gE. This method, which takes less than 10 minutes to give results, has similar detection limits with ELISA and it is advantageous because it gives fast results (Tarasov et al., 2016).

Conductometric immunosensors measure the changes in conductivity of sample solutions due to biorecognition reactions. Most reactions produce ionic species concentration changes that cause electrical conductivity or current changes. A conductometric immunosensor consists of two electrodes placed at a certain distance from each other and an alternating voltage is applied which causes a current to be generated across the electrodes. If the immunosensor has been exposed to the analyte, the change due to the receptor-analyte combination in the identification layer causes a significant change in the total capacitance (Karunakaran, Pandiaraj & Santharaman, 2015). Mutlaq et al. developed a conductometric-based immunosensor for the detection of E. coli O157:H7 bacteria, one of the most common foodborne pathogens. They coated the gold electrode with polyaniline/zinc oxide (PANI/ZnO) nanocomponents, immobilized monoclonal anti-O157:H7 antibody as a biorecognition element, and measured in the detection range of 101-104 CFU/ml in 30 minutes (Mutlaq et al., 2021).

Impedance is basically the opposite of current in an electrical circuit. The difference between resistance and impedance is that the resistance obeys Ohm's law and opposes the current of electrons in alternating and direct current circuits. Impedance is a resistance that changes according to inductive and capacitive effects seen only in alternating current circuits (Leva-Bueno, Peyman & Millner, 2020). In electrochemical impedance spectroscopy (EIS), changes in current are measured after the application of a voltage with a specific frequency to the interface. The current/voltage ratio gives the impedance (Velusamy et al., 2010). C-reactive protein (CRP) is a routinely used biomarker in the clinical diagnosis of liver or cardiovascular inflammation. The amount of CRP in the blood plasma can also be used in the diagnosis of diseases such as diabetes and cancer (Lim & Ahmed, 2019). Jampasa et al. produced an electrochemical biosensor for the detection of CRP with a sandwich model, using a serigraphic graphene electrode modified with cysteine and gold. Anthraquinone was chosen as a redox label because of its stability, simplicity and compatibility with biomolecules. The produced immunosensor measured CRP in the range of 0.01-150 μg/ml with 1.5 ng/ml limit of detection (Jamasa et al., 2018).

The sensitivity of immunosensors can be increased by the modification of the surfaces of electrodes by nanomaterials. Because electrode surface area of would increased (Ahmed et al., 2014). Li et al. used gold nanoparticles (AuNP) to detect human epidermal growth factor 2 (HER2), a breast cancer biomarker. In this design, AuNPs are used as support surfaces for immobilization of HER2 specific peptide sequences and since they form a wide surface area they produce a powerful electrochemical current. The group reported 0,5 pg/ml limit of detection and 1 pg/ml to 1 ng/ml detection range (Li et al., 2018).

Optical

Optical immunosensors utilize methods such as chemiluminescence, light absorbance, florescence, phosphorescence, light polarization, and rotation for the detection of analyte. Optical sensors work on the principle of detecting changes in light reflections and emission caused by the formation of the Ab/Ag complex, using light as a stimulus. A typical optical sensor receives the light from the laser, diode or white bulb and allows the changes to be observed while reflecting it from the sensor. Measurements can be made sequentially or simultaneously by sending light to the sensor at various angles. Many publications also focus on the use of nanoparticles to improve the performance of optical immunosensors. Nanoparticles generally lower the detection limit and increase signal amplification depending on the increased surface area (Karunakaran, Pandiaraj & Santharaman, 2015).

Optical change can be created with transducer types such as surface plasmon resonance (SPR), interferometry, reflectometric interference spectroscopy, ellipsometry and total internal reflection fluorescence (TIRF) (Abdolrahim et al., 2015).

Optical immunosensors are basically classified into two groups as direct and indirect. A direct optical immunosensor consists of a physical component, a biological component and a detector. While the prism and light source are physical components, the recognition element or biochemical content is biological. These systems generally include an optical reader, a biorecognition material, and sample processing systems, as well as four subsystems: light source, detector module, data processing unit, and optical connections. In the biological component, biomolecules are used as molecular probes for bioconjugation with the analyte. In the physical component, excitation light creates a resonance in the surface plasmons in the film and the reflection intensity of the light reflected at certain angles is observed. The electronic signal which is converted from the reflected light by a linear array detector makes possible monitoring the the biochemical interaction between the analyte and the transduction surface. Such tests are completed in about ten minutes (Lee et al., 2018).

A surface plasmon is an excitable electron oscillation that occurs between two surfaces whose insulating constants are of opposite signs. In a surface plasmon resonance (SPR) immunosensor, antibodies are immobilized on a thin metal sheet, usually gold, and detection is made by observing the reflection angle of polarized light with a prism depending on the formation of the target ligand. The metal film acts as a mirror, reflecting this light, increasing the power of reflection and making it calculable and measurable. When immobilized antibodies bind to targets, a deviation in SPR angle is observed depending on the concentration of the target (Lim & Ahmed, 2019). Battaglia et al. developed two SPR-based immunosensors for the detection of procalcitonin (PCT), which is used as a sepsis marker in both human and veterinary medicine, for use in canines and equidae. They utilized the molecular imprinting method using dopamine and norepinephrine to create PCT binding sites on the gold chip surface. In the detection of markers in plasma, these two biosensors developed for canine and equidae reached the of 30 ng/ml and 15 ng/ml limit of detection, respectively (Battaglia et al., 2021).

In indirect methods, fluorophores, luminophores, chromophores, quantum dots and nanoparticles can be used as labels. Total internal reflection fluorescence (TIRF) microscopy can be used to detect excited fluorophores within a 200 nm long field. A laser beam with an angle of incidence greater than the critical angle travels through the silica layer and is reflected from the glass coating towards the inside. Due to the splitting between the transmitted and reflected rays in this way, a standing wave is produced from the silica layer, resulting in an area where waves with decreasing amplitude are seen (Lee et al., 2018). Käppel et al. developed a TIRF-based immunosensor that measures progesterone from milk samples taken from oestrus-cycled cows.

They covalently immobilized the monoclonal antiprogesterone antibody to the sensor surface as a biological identification element and aimed to detect progesterone. As a result, they were able to measure at the picogram level in less than 5 minutes, thus making it possible to monitor critical parameters such as estrus and pregnancy follow-up, which are important for milk production (Käppel, Pröll & Gauglitz, 2007).

Optical fiber immunosensors are another method that allows monitoring of antigen-antibody complexes immobilized on an optical surface. Optical fiber immunosensors do not require reference electrodes, are convenient, and are widely used in clinical diagnosis, in vivo observations, and the detection of hazardous materials. The total internal reflection principle is the basis of optical fiber biosensors. The rays are transferred along the center of the fiber with negligible light loss (Lee et al., 2018). Immunochemical agents are immobilized at one end of the optical fiber and measured by light absorbance or fluorescent emission. In other words, antigen-antibody binding causes a colorimetric change. In this way, measurements can be made depending on the amount of antigen present (Karunakaran, Pandiaraj & Santharaman, 2015). Yang et al. developed a dual channel optical immunosensor that measures SARS-Cov-2 and Influenza A viruses in 10 minutes on a single sensor with a dual channel system. This sensor can recognize nucleoproteins of influenza A H1N1, H3N2 and H7N9 and SARS-CoV-2 Omicron and Delta variants (Yang et al., 2022).

One of the most important nanostructural detection tools is quantum dots, which are nanoparticles consisting of fluorescently labeled probe biomolecules. Quantum dots are semiconductor nano-sized (2-10 nm) crystalline structures. They are brighter and more photostable than conventional fluorophores due to their high quantum efficiencies. In addition, their color is directly related to their size. The larger size emits a larger wavelength, giving a color close to red, while those with a smaller diameter give a color close to blue. Therefore, broad absorption spectra and narrow and symmetrical emission scales can be combined and excited by a single wavelength and functional light source to exhibit a multicolored character in disease mapping and detection (Singh et al., 2018). Kerman et al. developed a quantum dot-based sandwich biosensor for prostate-specific antigen (PSA) detection. They immobilized the primary antibodies with protein A on the carbon surface and ensured that the target antigen was captured by these antibodies. After adding secondary biotinylated antibodies to the assembly, a sandwich type immunoassay was created. Then, they added streptavidin-conjugated quantum dots to the system, enabling them to bind with secondary antibodies and detect the presence of PSA due to the presence of emission of quantum dots (Kerman et al., 2007).

In optical detection, functionalized metal nanoparticles with relatively higher stability and lower background noise can be used as an alternative to fluorescent labeling of antibodies. Gold nanoparticles are the most studied and frequently used particles among these metals. It has easy fabrication, high biocompatibility, conductivity, compatibility with surface modification, and optoelectronic properties based on size and shape, which are useful in biosensor development (Li et al., 2010). Mradula et al. used gold nanoparticles in the biosensors they developed for the detection of thyroxine hormone. They immobilized anti-thyroxine antibodies on AuNPs that they functionalized with cysteamine and were able to measure thyroxine with this bioconjugate with a 9.11 pg/ml limit of detection (Mradula et al., 2020).

Piezoelectric

Piezoelectric immunosensors work with the principle of measuring the mass sensitivity of piezoelectric quartz crystals, which varies depending on the antigen-antibody complexes formed. These types of immunosensors are popularly preferred in many sectors due to their simplicity, high sensitivity, specificity, stability, speed and safety (Lim & Ahmed, 2019). The mass change is

detected by quartz crystals, the main component of these sensors. Piezoelectric crystal oscillates at a specific frequency, if there is coupling, when an electrical signal is used at a certain frequency (Terry, White & Tiqwell, 2005). When voltage is applied to the quartz crystal by two electrodes, the orientation of the crystal changes and mechanical oscillation is observed at a characteristic vibrational frequency. With the formation of immunocomplexes, the crystal's mass increases because of the immunocomplexes extra mass load, the oscillation frequency of the crystal changes, and in this way, mass change can be detected electrically. Measurement methods used in immunosensors can be direct or indirect. In direct measurement of the analyte, the mechanical oscillation frequency decreases with increasing analyte concentration. In indirect measurements, the analyte first interacts with the free elements in the solution. This causes the frequency to change inversely with the amount of analyte (Lim & Ahmed, 2019).

QCM immunosensors are formed from a thin quartz plate with an electrode attached to its edges and an antigen or antibody immobilized on its surface. As piezoelectric QCM works by creating an electric field between two electrodes and oscillating the quartz crystal (Kurosawa et al., 2006). The applied electric field creates an acoustic wave that comes perpendicular to the crystal surface and passes through it. These waves provide the necessary impedance at the point where the width of the device reaches several half wavelengths. As a result, the quartz disc undergoes mechanical resonance where the resonant frequency occurs in the space between the two electrodes, depending on the thickness of the quartz disc and the natural velocity of the acoustic wave (Aberl, Kösslinger & Wolf, 1998). Immunological detection of the target analyte causes an increase in mass, which in turn causes a decrease in the resonance frequency in the oscillation. The change in mass can be determined by calculating the difference between the frequency measurement made before the analyte is applied and the measurement to be made after conjugation (Terry, White & Tiqwell, 2005). Kim et al. developed an immunosensor for the detection of canine parvovirus by immobilizing a monoclonal anti-CPV IgG antibody on a gold-plated quart crystal surface over thiol groups. They successfully detected CPV after stool sample loading (Kim et al., 2015).

SAW immunosensor setups generally consist of a piezoelectric material placed on a silicon substrate and metal electrodes combined with this material to generate mechanical acoustic waves and make them visible in case of a reaction response (Lim & Ahmed, 2019). This type of sensor is based on the production of a specific acoustic vibration with concentrated acoustic energy around the outer surface of a solid material that provides high sensitivity to surface adsorption. The electrical signal is confined by an independent layer to ensure the concentration of acoustic energy and transforms into polarized lateral waves parallel to the surface of the piezoelectric crystal. Since any material in contact with the surface will significantly change the speed and amplitude of the waves, the transducer can precisely detect all specific biological interactions on the surface of the piezoelectric material. When there is an increase in mass as a result of the formation of immunocomplexes, the excited waves traveling along the crystal surface are subject to frequency changes in the motion path. Changes in the resonance frequency proportional to the mass change on the transducer surface can be measured in this way (Fogel, Limson & Seshia, 2016). Toma et al. developed a SAW immunosensor supplemented with gold nanoparticles and used it for the detection of house dust mites. Gold nanoparticles conjugated with streptavidin affinity bind to the biotinylated detection antibody forming a sandwich format with the capture antibody and antigen immobilized on the surface. With the measurements made in this way, the detection limit of 2.5 ng/ml was reached (Toma et al., 2017).

Thermometric

Heat emission or absorption is often observed when biochemical reactions take place. Based on this phenomenon, thermal immunosensors have been developed based on the concept

of measuring thermal change with the formation of a specific antigen-antibody complex (Ramanathan & Danielsson, 2001). All temperature changes caused by the heat released or absorbed can be measured by measuring the molar enthalpy and by summing the energy of the products formed as a result of biochemical reactions. Heat-based detection methods are generally used in combination with enzymes with thermistor properties, since all reactions with the enzyme are accompanied by enthalpy changes (Lim & Ahmed, 2019). It is common practice to combine the flow injection assay (FIA) method, which pioneered the development of thermometric sensors, and the detection of thermal change in enzymatic reactions. Xu et al. developed an FIA-based thermometric ELISA (TELISA) for the detection and measurement of diazepam (DZP). DZP is used by mixing with beverages in various criminal cases, such as theft and sexual abuse. In this study, they produced a stable temperature change signal that was correlated with temperature changes as a result of enzymatic catalysis. The TELISA design is based on the competitive format determination of the thermal response generated by the catalytic degradation of analyte-enzyme conjugates by the respective products. With this principle, the detection limit of 33.71 ng/ml was reached (Xu et al., 2017).

Conclusions

Immunosensors have become popular tools since they meet the need for a sensitive, specific, fast, miniaturized size and inexpensive diagnostic device that can detect a variety of analytes. Significant progress has been made in this area by developing new protocols using up-to-date technology and modern tools. It has been observed that proper orientation and antigen-binding activity increase significantly with the development of recombinant antibodies and the application of appropriate immobilization techniques The major challenge in the development of immunosensors goes beyond the laboratory environment to end-user applications. The prevalence of these sensors will increase with the advances in nanomaterials, protocols, versatile designs, powerful sensors and signal generation.

References

Abdolrahim M, Rabiee M, Alhosseini SN, Tahriri M, Yazdanpanah S, Tayebi L. Development of optical biosensor technologies for cardiac troponin recognition. Anal Biochem 2015, 485:1–10. https://doi.org/10.1016/j.ab.2015.06.003

Aberl F, Kösslinger C, Wolf H. The quartz crystal microbalance (QCM) as an immunosensor. Methods Mol Med 1998, 13:519-29. https://doi.org/10.1016/j.ab.2015.06.003

Ahmed MU, Saaem I, Wu PC, Brown AS. Personalized diagnostics and biosensors: A review of the biology and technology needed for personalized medicine. Crit Rev Biotechnol 2014, 34(2): 180–196. https://doi.org/10.3109/07388551.2013.778228

Asal M; Özen Ö; Şahinler M; Baysal H.T; Polatoğlu İ. An overview of biomolecules, immobilization methods and support materials of biosensors. Sensor Review 2019; 39(3):377–386. https://doi.org/10.1108/SR-04-2018-0084

Battaglia F, Baldoneschi V, Meucci V, Intorre L, Minunni M, Scarano S. Detection of canine and equine procalcitonin for sepsis diagnosis in veterinary clinic by the development of novel MIP-based SPR biosensors. Talanta 2021, 230. https://doi.org/10.1016/j.talanta.2021.122347

Bobacka J, Ivaska A, Lewenstam A. Potentiometric ion sensors. Chem Rev 2008, 108(2):329–351. https://doi.org/10.1021/cr068100w

De Juan-Franco E, Caruz A, Pedrajas JR, Lechuga LM. Site-directed antibody immobilization using a protein A-gold binding domain fusion protein for enhanced SPR immunosensing. Analyst 2013, 138(7): 2023–2031. https://doi.org/10.1039/c3an36498d

Delwiche M; Tang X; Bondurant R; Munro C. Improved biosensor for measurement of progesterone in bovine milk. Transactions of the ASAE 2001, 44(6): 1997–2002.

Feyssa B, Liedert C, Kivimaki L, Johansson LS, Jantunen H, Hakalahti L. Patterned immobilization of antibodies within roll-to-roll hot embossed polymeric microfluidic channels. PLoS ONE 2013, 8(7) : e68918. https://doi.org/10.1371/journal.pone.0068918

Fogel R, Limson J, Seshia AA. Acoustic biosensors. Essays Biochem 2016, 60(1): 101–110. https://doi.org/10.1042/EBC20150011

Grieshaber D, Mackenzie R, Vörös J, Reimhult E. Electrochemical biosensors-sensor principles and architectures. Sensors 2008, 8:1400–1458. https://doi.org/10.3390/s80314000

Hayat A, Catanante G, Marty JL. Current trends in nanomaterial-based amperometric biosensors. Sensors 2014, 14(12): 23439–23461. https://doi.org/10.3390/s141223439

Holford T.R.J; Davis F; Higson S.P.J. Recent trends in antibody based sensors. Biosens Bioelectron 2012, 34(1):12–24. https://doi.org/10.1016/j.bios.2011.10.023

Ionescu RE, Gondran C, Bouffier L, Jaffrezic-Renault N, Martelet C, Cosnier S. Label-free impedimetric immunosensor for sensitive detection of atrazine. Electrochimica Acta 2010, 55(21):6228–6232. https://doi.org/10.1016/j.electacta.2009.11.029

Jampasa S, Siangproh W, Laocharoensuk R, Vilaivan T, Chailapakul O. Electrochemical detection of c-reactive protein based on anthraquinone-labeled antibody using a screen-printed graphene electrode. Talanta 2018, 183: 311–319. https://doi.org/10.1016/j.talanta.2018.02.075

John R, Spencer M, Wallace GG, Smyth MR. Development of a polypyrrole-based human serum albumin sensor. Analytica Chimica Acta 1991, 249(2): 381-385. https://doi.org/10.1016/S0003-2670(00)83010-X

Kalecki J, Iskierko Z, Cieplak M, Sharma PS. Oriented immobilization of protein templates: a new trend in surface imprinting. ACS Sens 2020, 5(12): 3710–3720. https://doi.org/10.1021/acssensors.0c01634

Käppel ND, Pröll F, Gauglitz G. Development of a TIRF-based biosensor for sensitive detection of progesterone in bovine milk. Biosens Bioelectron 2007, 22(9–10):2295–2300. https://doi.org/10.1016/j.bios.2006.11.030

Karunakaran C; Pandiaraj M; Santharaman P. Immunosensors. Biosens Bioelectron 2015, 205–245. https://doi.org/10.1016/B978-0-12-803100-1.00004-9

Kerman K, Endo T, Tsukamoto M, Chikae M, Takamura Y, Tamiya E. Quantum dot-based immunosensor for the detection of prostate-specific antigen using fluorescence microscopy. Talanta 2007, 71(4):1494–1499. https://doi.org/10.1016/j.talanta.2006.07.027

Kierny MR, Cunningham TD, Kay BK. Detection of biomarkers using recombinant antibodies coupled to nanostructured platforms. Nano Rev 2012, 3(1):17240. https://doi.org/10.3402/nano.v3i0.17240

Kim YK, Lim SI, Choi S, Cho IS, Park EH, An DJ. A novel assay for detecting canine parvovirus using a quartz crystal microbalance biosensor. J Virol Methods 2015, 219:23–27. https://doi.org/10.1016/j.jviromet.2015.03.015

Kurosawa S, Park JW, Aizawa H, Wakida SI, Tao H, Ishihara K. Quartz crystal microbalance immunosensors for environmental monitoring. Biosens Bioelectron 2006, 22(4):473–481. https://doi.org/10.1016/j.bios.2006.06.030

Lee D, Hwang J, Seo Y, Gilad AA, Choi J. Optical immunosensors for the efficient detection of target biomolecules. Biotechnol Bioprocess Eng 2018, 23(2):123–133. https://doi.org/10.1007/s12257-018-0087-x

Leva-Bueno J, Peyman SA, Millner PA. A review on impedimetric immunosensors for pathogen and biomarker detection. Med Microbiol Immunol 2020, 209(3):343–362. https://doi.org/10.1007/s00430-020-00668-0

Li X, Shen C, Yang M, Rasooly A. Polycytosine DNA electric-current-generated immunosensor for electrochemical detection of human epidermal growth factor receptor 2 (HER2). Anal Chem 2018, 90(7):4764–4769. https://doi.org/10.1021/acs.analchem.8b00023

Li Y. Section 2.3 Biosensors, pp. 52-93, of Chapter 2 Hardware, in CIGR Handbook of Agricultural Engineering Volume VI Information Technology. Edited by CIGR-The International Commission of Agricultural Engineering; Volume Editor, Axel Munack. St. Joseph, Michigan, USA: ASABE, 2006.

Li Y, Schluesener HJ, Xu S, Org WG. Gold nanoparticle-based biosensors. Gold Bulletin 2010, 43:29-41. https://doi.org/10.1007/BF03214964

Lim SA, Ahmed MU. Chapter 1: Introduction to immunosensors. RSC Detection Science 14: 1–20. Royal Society of Chemistry, 2019. https://doi.org/10.1039/9781788016162-00001

Lin PC, Chen SH, Wang KY, Chen ML, Adak AK, Hwu JRR, Chen YJ, Lin CC. Fabrication of oriented antibody-conjugated magnetic nanoprobes and their immunoaffinity application. Anal Chem, 2009, 81(21): 8774–8782. https://doi.org/10.1021/ac9012122

Mradula Raj R, Devi S, Mishra S. Antibody-labeled gold nanoparticles based immunosensor for the detection of thyroxine hormone. Anal Sci 2020, 36(7):799–806. https://doi.org/10.2116/analsci.19P418

Mutlaq S, Albiss B, Al-Nabulsi AA, Jaradat ZW, Olaimat AN, Khalifeh MS, Osaili T, Ayyash MM, Holley RA. Conductometric immunosensor for escherichia coli o157:H7 detection based on polyaniline/zinc oxide (pani/zno) nanocomposite. Polymers (Basel) 2021, 13(19):3288. https://doi.org/10.3390/polym13193288

Park M. Orientation control of the molecular recognition layer for improved sensitivity: a review. Biochip Journal 2019, 13(1)-82-94. https://doi.org/10.1007/s13206-019-3103-0

Pinacho DG, Sánchez-Baeza F, Pividori MI, Marco MP. Electrochemical detection of fluoroquinolone antibiotics in milk using a magneto immunosensor. Sensors (Switzerland) 2014, 14(9): 15965–15980. https://doi.org/10.3390/s140915965

Rahman MA, Shiddiky MJA, Park JS, Shim YB. An impedimetric immunosensor for the label-free detection of bisphenol A. Biosens Bioelectron 2007, 22(11):2464–2470. https://doi.org/10.1016/j.bios.2006.09.010

Ramanathan K, Danielsson B. Principles and applications of thermal biosensors. Biosens Bioelectron 2001, 16(6):417-423. https://doi.org/10.1016/S0956-5663(01)00124-5

Sharma A. Role of biosensors in the field of veterinary practice. Ind J Agric Bus 2016, 2(1).47-50.

Sharma S; Byrne H; O'Kennedy R.J. Antibodies and antibody-derived analytical biosensors. Essays Biochem 2016, 60(1):9–18. https://doi.org/10.1042/EBC20150002

Singh RD, Shandilya R, Bhargava A, Kumar R, Tiwari R, Chaudhury K, Srivastava RK, Goryacheva IY, Mishra PK. Quantum dot based nano-biosensors for detection of circulating cell free miRNAs in lung carcinogenesis: From biology to clinical translation. Front Genet 2018, 9:616. https://doi.org/10.3389/fgene.2018.00616

Singh V, Krishnan S. Voltammetric immunosensor assembled on carbon-pyrenyl nanostructures for clinical diagnosis of type of diabetes. Anal Chem, 2015, 87(5):2648–2654. https://doi.org/10.1021/acs.analchem.5b00016

Su L, Jia W, Hou C, Lei Y. Microbial biosensors: A review. Biosens Bioelectron 2011, 26(5):1788-99. https://doi.org/10.1016/j.bios.2010.09.005

Su XL; Li Y.A. Self-assembled monolayer-based piezoelectric immunosensor for rapid detection of Escherichia coli O157:H7. Biosens Bioelectron 2004, 19(6):563–574. https://doi.org/10.1016/S0956-5663(03)00254-9

Tarasov A, Gray DW, Tsai MY, Shields N, Montrose A, Creedon N, Lovera P, O'Riordan A, Mooney MH, Vogel EM. A potentiometric biosensor for rapid on-site disease diagnostics. Biosens Bioelectron, 2016, 79:669–678. https://doi.org/10.1016/j.bios.2015.12.086

Terry LA, White SF, Tigwell LJ. The application of biosensors to fresh produce and the wider food industry. J Agric Food Chem 2005, 53(5):1309–1316. https://doi.org/10.1021/jf040319t

Toma K, Miki D, Yoshimura N, Arakawa T, Yatsuda H, Mitsubayashi K. A gold nanoparticle-assisted sensitive SAW (surface acoustic wave) immunosensor with a regeneratable surface for monitoring of dust mite allergens. Sens Actuators B Chem 2017, 249:685–690. https://doi.org/10.1016/j.snb.2017.04.073

Tothill I.E. Biosensors developments and potential applications in the agricultural diagnosis sector. Comput Electron Agric 2001, 30:205-218. https://doi.org/10.1016/S0168-1699(00)00165-4

Tothill I.E; Turner A.P.F. Biosensors. New developments and opportunities in the diagnosis of livestock diseases. IAEA, 1998. https://www.osti.gov/etdeweb/biblio/295741

Tschmelak J; Proll G; Gauglitz G. Optical biosensor for pharmaceuticals, antibiotics, hormones, endocrine disrupting chemicals and pesticides in water: Assay optimization process for estrone as example. Talanta, 2005, 65:313–323. https://doi.org/10.1016/j.talanta.2004.07.011

Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of foodborne pathogen detection: In the perspective of biosensors. Biotechnol Adv 2010, 28(2):232–254. https://doi.org/10.1016/j.biotechadv.2009.12.004

Xu N, Bai J, Peng Y, Qie Z, Liu Z, Tang H, Liu C, Gao Z, Ning B. Pretreatment-free detection of diazepam in beverages based on a thermometric biosensor. Sens Actuators B Chem 2017, 241:504–512. https://doi.org/10.1016/j.snb.2016.10.081

Yang Y, Zhao R, Wang Y, Song D, Jiang B, Guo X, Liu W, Long F, Song H, Hao R. Rapid and universal detection of SARS-CoV-2 and influenza A virus using a reusable dual-channel optic fiber immunosensor. J Med Virol 2022, 94(11):5325-5335. https://doi.org/10.1002/jmv.28015

Glycosylation and Inhibition of Proteins

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Introduction

The term glycosylation refers to the incorporation of various carbohydrates into the structures of biologically important molecules such as lipids and nucleic acids, especially proteins. Incorporation into this structure can occur in two different ways, enzymatic and non-enzymatic. If the binding of carbohydrates to proteins occurs in the catalysis of an enzyme and by glycosidic bond, it is known as **enzymatic glycosylation (Glycolization).** However, if this binding occurs as a result of the reaction of reducing sugars or metabolic derivatives with proteins without enzyme catalysis, it is called **non-enzymatic glycosylation (Glycation)** (Kılınç, 2011; Kayılı, 2016; Aydınhan, 2018). This review will focus more on glycation events and products.

Enzymatic glycosylation reactions (glycosylation)

It is known that in order for proteins to become functional, they must undergo post-translational modifications, including permanent or temporary enzymatic processes, during or after the translation process. One of the most important post-translational modifications that occur frequently during the synthesis of proteins is protein glycosylation. It is known that after translation of many proteins, especially membrane proteins and a significant part of the proteins secreted out of the cell, various carbohydrate units are attached to their structures and these modifications are important for the structure and functions of these proteins. It has also been demonstrated by studies that glycosylation directly or indirectly affects the function of proteins in various ways and gives glycoproteins various functions in vital processes such as cell stimulation, cell-cell interaction and signal transmission (Kılınç, 2011; Kayılı, 2016).

Structural modification events, which occur by the addition of carbohydrate units to proteins, are usually catalyzed by glycosyl transferase enzymes located in the membranes of the endoplasmic reticulum and Golgi organelles inside the cell. In these highly specific enzymatic glycosylation events, glucose in particular, galactose, mannose, fructose, sialic acids, fucose, N-acetyl mannosamine, N-acetyl glucosamine (GlcNAc), N-acetyl galactosamine (GalNAc) and N-acetyl neuraminic acid (NeuAc) such as monosaccharide units participate in the structure of proteins. During these reactions, carbohydrate units are attached to the side chains of asparagine, serine, threonine or hydroxylysine amino acids in proteins with their anomeric carbons by glycosidic bonds. These bonds can occur in 2 different ways as **O-glycosidic (O-linked)** to the hydroxyl groups in the side chains or **N-glycosidic (N-linked)** glycosylation attached to the amide nitrogen (Kılınç, 2011; Yalçın, 2012; Kayılı, 2016; Aydınhan, 2018).

Since glycosylation is part of the natural functioning of the cell, it takes place in a controlled manner. For this reason, its importance can be better understood when the diseases that occur as a result of abnormal glycosylation of proteins are examined. İt has been reported Glycosylation plays a role in various biological events, especially in antigen recognition, infection and immunity, and therefore, the relationship of abnormal glycosylation in proteins with various pathological

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conditions, especially congenital glycosylation disorders (CDG), cancer and immune system diseases (Özaydın & et al., 2012; Yalçın, 2012; Kayılı, 2016).

Non-enzymatic glycosylation reactions (glycation)

Non-enzymatic glycosylation of compounds carrying free carbonyl groups; It refers to the reaction of free amino groups of proteins, nucleic acids and lipids with reducing sugars or their metabolic derivatives without enzyme catalysis. Although this spontaneous reaction is more commonly referred to as protein glycation, it is in today known as the "**Maillard reaction**" (Kılınç, 2011; Toprak, 2014; Demirel & Yıldıran, 2018; Yalçın & Rakıcıoğlu, 2022).

It was first reported by Louis Camille Maillard in 1912 that reducing sugars in a solution cause the formation of dark colored products (melanoidins) as a result of the reaction of amino acids. The Maillard reaction, which consists of a series of complex reactions that proceed sequentially and simultaneously, consists of three main stages: early, intermediate and late. The Maillard reaction leads to the formation of products with different chemical properties, such as heterocyclic amines, acrylamide, and 5-hydroxymethylfurfural. In the early stage of the reaction; The carbonyl group of reducing sugars such as glucose, galactose, fructose, mannose, ascorbate, pentoses undergoes condensation with the ε-amino group, which is the side chain of lysine amino acids in proteins, with the release of water. In this reaction, which takes place non-enzymatically, the "**Schiff base**" intermediate product, which is an unstable and ketimine intermediate, first appears. Although the Schiff base formation that occurs at this stage of the reaction is reversible, it can also undergo an irreversible Amadori regulation, usually after the reaction progresses to the intermediate stage. The proteins formed at this stage are known as Amadori products. In other words, since the formed Schiff base is an unstable product; By adding water back to the structure, the reaction may be reversed or the reaction may spontaneously convert irreversibly to the stable product 1-amino-1-deoxy-2-ketose (ketoamine or fructosamine). The formation reaction of this stable form of ketoamine is called "**Amadori regulation**". When glycation occurs between glucose and lysine residues in proteins, the resulting Amadori product is called fructoselysine. It has been reported that various Amadori products such as fructosamine and fructoselysine, which are formed as a result of the reaction, have circular and flat forms and these forms are in equilibrium with each other in solution. Under *in vivo* conditions, Amadori products have been found to accumulate by irreversibly binding to both short- and long-lived proteins, reaching equilibrium over a period of 15 to 20 days (Figures 1 and 2) (Singh & et al., 2001; Vlassara & Palace, 2002; Kılınç, 2011; Toprak, 2014; Yılmaz & Karabudak, 2016; Aydınhan, 2018; Kerimoğlu & Türk, 2018; Arslan & et al., 2021; Erim, Ergene & Hecer, 2022; Yalçın & Rakıcıoğlu, 2022).

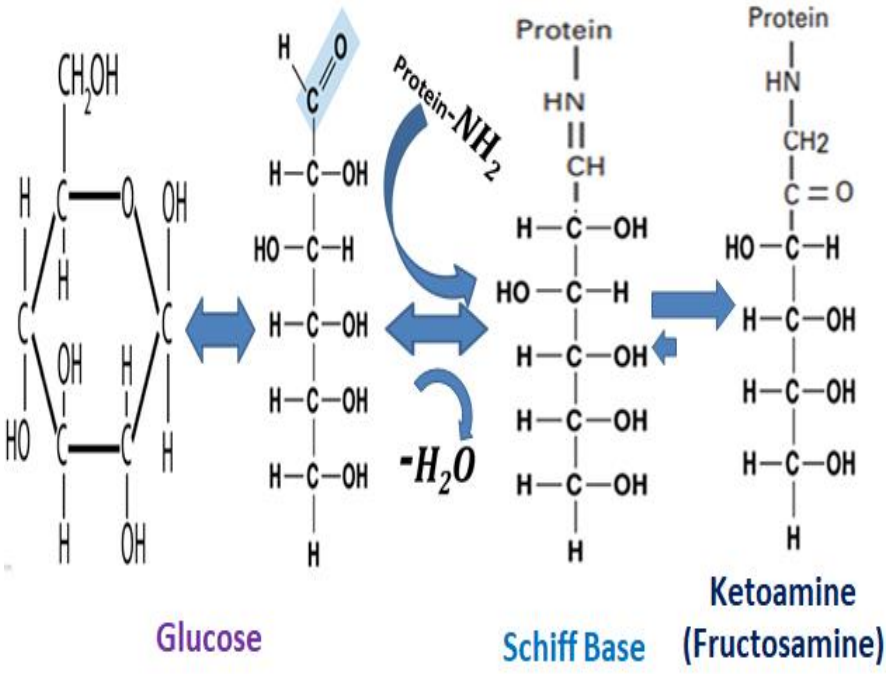

Figure 1. Non-enzymatic reaction of glucose with proteins. The reaction mechanism of other aldehydes and ketones with the amino group is the same. Adapted from Kılınç, 2011.

In the intermediate stage, it is observed that Amadori products undergo oxidation and dehydration reactions and are transformed into various carbonyl derivatives such as glyoxal, methylglyoxal and deoxyglucosons, which are more reactive than the sugars from which they are produced (Figure 2). Studies have shown that dicarbonyl derivatives can arise from the degradation of highly glycated proteins, from glycolysis intermediates, and from peroxidation of lipids (Kılınç, 2011; Yılmaz & Karabudak, 2016; Aydınhan, 2018; Yalçın & Rakıcıoğlu, 2022).

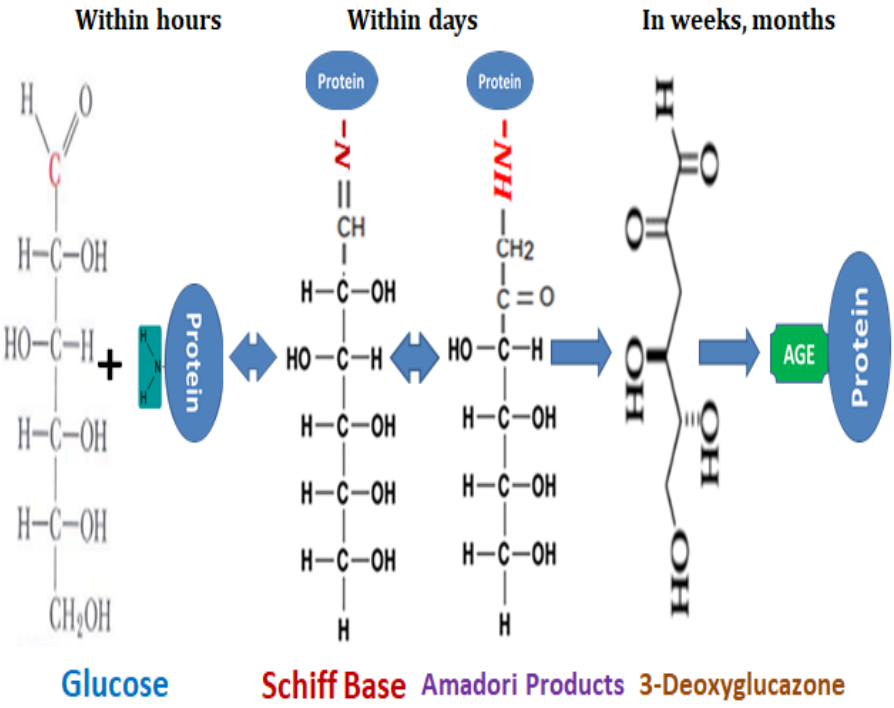

Figure 2. Formation of reactive carbonyl derivatives from Amadori products. Adapted from Toprak, 2014.

In the late stage of the reaction, Amadori products are seen to undergo rearrangements following reactions such as cyclization, oxidation and dehydration to form more stable products. After rearrangements, they form compounds called **Advanced Glycation End Products (AGE's)**, which are generally yellow-brown in color, which can also fluoresce and accumulate in long-lived proteins and cause damage, in an insoluble form with an irreversible reaction (Kılınç, 2011; Aydınhan, 2018; Yalçın & Rakıcıoğlu, 2022).

Although the vast majority of these compounds are known, efforts to understand their chemical properties continue. Recent research shows that Amadori products undergo oxidative and non-oxidative modifications or decompose to form glycation intermediates and AGEs, mainly including furfurals, reductones, dicarbonyls and compounds from the hydroxycarbonyl class (Kılınç, 2011; Aydınhan, 2018; Kerimoğlu & Türk, 2018).

Glycosylated hemoglobin (HbA1c)

Although studies on non-enzymatic glycosylation reactions go back a long time, research on proteins gained momentum with the discovery of hemoglobin forms and elucidation of their structures towards the end of the 1970s. Hemoglobin was the first protein determined to undergo changes as a result of non-enzymatic glycosylation. Studies have focused on HbA, which usually constitutes a large portion (~97%) of total hemoglobin in adults, in which a wide variety of monosaccharide units are bound, such as glucose and fructose. Among the identified forms, HbA1a1, HbA1a2 and HbA1b are respectively attached to the amino groups of the N-terminal valine of the ß-chains; They are small amounts of products formed as a result of adding fructose 1,6-bisphosphate, glucose 6-phosphate and unknown carbohydrates to HbA0. One of the most important and most studied forms of HbA1 is HbA1c (Kılınç, 2011; Toprak, 2014; Aydınhan, 2018).

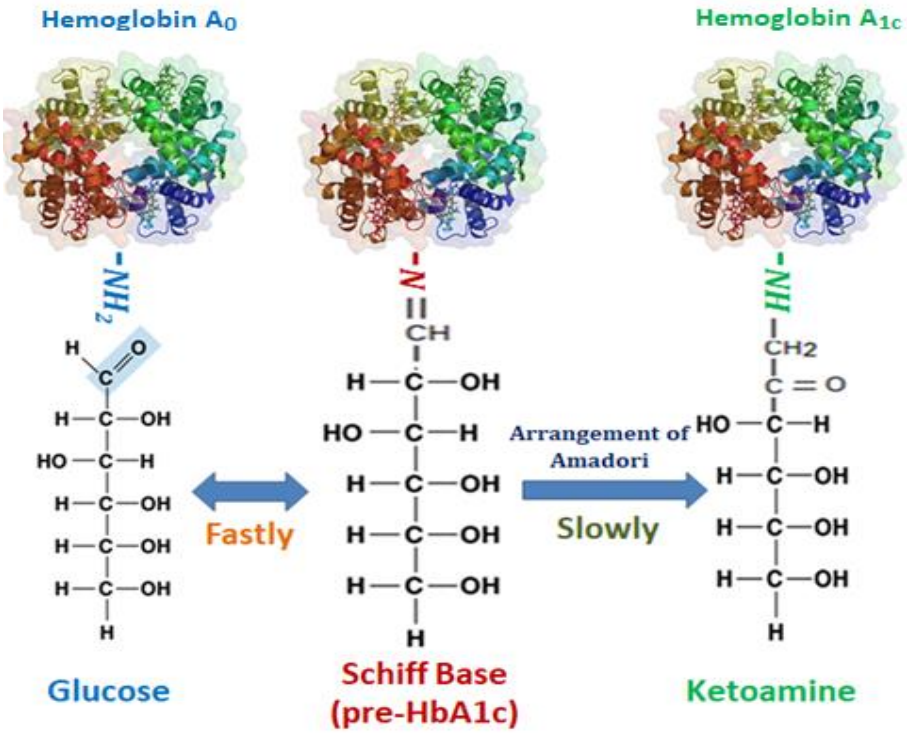

Figure 3. Amadori regulation of hemoglobin and glucose. Adapted from Yılmaz, 2022.

Hemoglobin A1c is the main glycosylated hemoglobin in the blood, accounting for the majority (~80%) of HbA forms. HbA1c is defined as a durable structure formed as a result of non-enzymatic binding of the glucose molecule to the amino group of the N-terminal governor of the β chains of hemoglobin. At the very beginning of HbA1c formation, the structure called pre-

HbA1c occurs as a result of the formation of an aldimine bond (Schiff base) between unstable amino and aldehyde groups. At this stage, the reaction takes place rapidly and bidirectionally; The pre-HbA1c structure is unstable at this time. At this point, the Schiff base can separate or undergo Amadori rearrangement to slowly and irreversibly form the stable ketoamine structure. These stable ketoamines formed by Amadori regulation are called advanced glycation end products (AGEs) (Figure 3) (Kılınç, 2011; Toprak, 2014; Aydınhan, 2018; Yılmaz, 2022).

Glycation of lipids

Since glycation is a reaction between carbonyl groups and amino groups, it was thought for years that only phosphatidylethanolamine (PE) and phosphatidylserine (PS) lipids could participate in glycation reactions. However, contrary to this idea, many *in vivo* and *in vitro* studies have shown that only PE can undergo glycation reactions. As with protein glycation, the level of lipid glycation can also vary depending on the glucose concentration in the medium. Studies have shown that the plasma glycemic PE concentration of diabetics can increase up to 2-3 times compared to individuals with a normal glycemic index. In addition, experimental animal studies have shown that PE glycation occurs in foods that have been processed and stored for a long time. Amadori products, which are formed as a result of glycation of phosphatidylethanolamine, can cause the formation of reactive oxygen species (ROS) by undergoing oxidation and destruction since they are not stable compounds. It has been reported that reactive oxygen species also cause peroxidation in membrane lipids and contribute to the abnormal changes that occur in membrane lipids in various pathologies, especially diabetes and aging (Kılınç, 2011).

Glycation of nucleic acids

Over the years it has been discovered that nucleic acids, like proteins, are non-enzymatically glycosylated. It has been understood that glycation in nucleic acids occurs with reducing sugars over bases containing free amino groups in their structures such as adenine, guanine and cytosine. Glycation reactions have been studied in detail *in vivo* and *in vitro*, focusing more on proteins. Studies involving the glycation of nucleobases have been highly limited to *in vitro* studies, and it has been proven that glycation occurs again with the Amadori reaction. On the other hand, the difference with proteins was observed not in the formation of glycation products, but in the degree of accumulation of the formed products. Accordingly, glycation products formed as a result of glycation of nucleobases show a lower level of accumulation in the cell than products formed by glycation of proteins. The reason for this situation is that the free nucleotides that undergo glycation can be rapidly excreted from the body. In addition, it is thought that the short-lived RNA reduces the glycation rate, and in the longer-lived DNA, the interaction of the bases forming the helix structure through hydrogen bonds is important in protecting nucleobases against glycation. This restriction of glycation of nucleobases has led to the fact that the glycation of these molecules can only be investigated *in vitro*. Studies have shown that guanine bases in DNA are prone to glycation, but they can be separated by hydrolysis due to the instability of the N-glycosidic bond in the formed glycation products. It is thought that the apurinic DNA regions formed as a result of hydrolysis are repaired by the normal DNA repair mechanism and therefore, although the DNA is very long-lived, the accumulation of glycation advanced products on DNA at significant levels cannot occur (Kılınç, 2011).

Factors affecting glycation reactions

Since glycation reactions occur independently of enzymes; The reaction rate is affected by *in vivo* variables such as cellular glucose permeability, the concentration, half-life and reactivity of the reducing sugar and protein to be reacted in terms of free amino groups. Advanced glycation end products can occur endogenously as a part of metabolism, or they can be taken exogenously through food and smoking. It is known that advanced glycation end products occur naturally in

unprocessed raw foods of animal origin, and the cooking process increases the reaction rate by providing more AGEs in foods. It has been reported that cooking methods applied with high temperature for a long time, especially grilling, frying and roasting, increase the formation of AGEs in foods. The rate and amount of AGE formation in foods *in vitro* conditions; It has been proven that many factors such as the level and duration of heat applied to the food, pH in the environment, humidity, composition of the food, food additives and whether the marination process is done or not. In addition to the fact that protein glycation is a slowly progressing reaction, it has been reported that the glycation rates of different amino groups on the same protein molecule may differ from each other. For this reason, since glycation occurs at low levels in proteins with short half-lives, it naturally becomes difficult to detect in these proteins. On the contrary, in proteins with a long half-life, the amount of reducing sugar accumulated on the protein also increases over time, making it detectable. For this reason, proteins with long half-lives such as alkaline phosphatase, elastin, collagen, lysozyme and hemoglobin are generally preferred in *in vivo* protein glycation studies. It has been reported that *in vivo*, protein glycation rate and level vary depending on the half-life of the protein and the concentration of the reducing sugar and free amino group to which the protein is exposed, regardless of the age of the individual (Kılınç, 2011; Yılmaz & Karabudak, 2016; Aydınhan, 2018; Demirel & Yıldıran, 2018; Döner, 2021; Demirer & Yardımcı, 2022; Erim, Ergene & Hecer, 2022).

Advanced glycation end products (AGEs)

Advanced glycation end products are compounds formed as a result of non-enzymatic reactions between reducing sugars and amino groups in the structure of proteins, lipids and nucleic acids. Studies have also revealed different mechanisms of AGE formation. Formation of advanced glycation end products; It has been reported that it occurs by three different mechanisms: Maillard reaction, polyol pathway and increased oxidative stress, and α-dicarbonyl compounds such as glyoxal, glycolaldehyde, glyceraldehyde, methylglyoxal, 3-deoxyglucazone occur in all of them. The polyol pathway is known as a pathway that is activated in the presence of high concentrations of glucose, resulting in fructose. In the polyol pathway, sorbitol, formed by the reduction of glucose by aldose reductase, is converted to fructose by sorbitol dehydrogenase. As a result of overstimulation of the polyol pathway, metabolites such as fructose and triose phosphates accumulate and the intracellular Nicotinamide adenine dinucleotide (NAD+) concentration decreases. It has been discovered that the resulting fructose is then converted into products such as fructose-1-phosphate and fructose-6-phosphate, leading to the formation of α-dicarbonyl compounds and contributing to the *in vivo* AGE pool. On the other hand, another mechanism leading to AGE formation is auto-oxidation triggered by an increase in oxidative stress level in the cell. In this mechanism, it has been reported that the formation of α-dicarbonyl compounds occurs as a result of the oxidation of monosaccharides such as glucose, ribose, galactose and fructose by catalytic metals and oxygen. (Döner, 2021; Yalçın & Rakıcıoğlu, 2022; Yılmaz, 2022).

Since Amadori products resulting from glycation are unstable products, they are converted into glycation end products with a large number of glycation intermediates by changing or breaking down by various mechanisms. Glycation can occur by non-oxidative mechanisms, or it can occur with oxidation, and in this case, glycoxidation products are formed. As a result of these mechanisms, it is observed that various compounds, primarily furfurals, reductones, dicarbonyls and hydroxycarbonyl derivative products, are formed. Studies show that these compounds can also cause radical formation by entering redox reactions in the presence of transition elements such as iron and copper. On the other hand, since the reactive products produced by oxidative destruction can react with amino groups in proteins, they form brown macromolecule complexes known as melanoidins by forming covalent cross-links between proteins. In addition, with the oxidative degradation of Amadori products bound to proteins, products such as glyoxal, methylglyoxal, glycolaldehyde, 3-deoxyglucosone, 1-deoxyglucosone, 4,5-dioxopentose and 5,6-dioxohexose can

be formed either free or bound to proteins in the glycation medium (Kılınç, 2011; Toprak, 2014; Muthyalaiah & et al., 2021).

As 4,5-dioxopentose and 5,6-dioxohexane products formed by the oxidative degradation of Amadori products are involved in covalent cross-linking between proteins, they are generally found bound to proteins; They are the precursors of the two AGE molecules pentosidin and glucospan. The resulting products, glyoxal and methylglyoxal, are imidazole derivatives that cause an increase in cross-linking between proteins in cells and tissues. These compounds formed; It can also occur during the breakdown of amino acids such as threonine and serine, and triosephosphates, especially in the breakdown of Amadori products and lipid peroxidation intermediates. Carbonyls and dicarbonyls, which are among the reactive intermediates formed, cause covalent cross-linking as a result of reaction with lysine/arginine side groups and N-terminal amino groups in proteins. Covalent cross-linking can occur on the same protein or between different proteins. Although it is not fully understood yet, it is thought that the formation of cross-links takes place in a very complex way and lysine residues are of great importance in this formation (Kılınç, 2011; Toprak, 2014; Muthyalaiah & et al., 2021).

Today, nearly 40 types of AGEs have been defined in free and bound form. Various advanced glycation end products that have been shown to accumulate in tissues have been identified by studies, and as the number of AGEs discovered over time has increased, it has been necessary to classify them. When classifying advanced glycation end products, they are divided into four different groups according to their ability to reflect fluorescent light and their biochemical properties (Döner, 2021; Yalçın & Rakıcıoğlu, 2022):

1. Fluorescent and cross-linking molecules
2. Non-fluorescent and non-crosslinking molecules
3. Non-fluorescent and cross-linking molecules
4. Molecules that fluoresce and do not crosslink

For example; Pentosidine is a fluorescent glycoxidation product formed by cross-linking of arginine and lysine residues with ascorbic acid or monosaccharides. Apart from pentosidine, pentodilysin, AGE-IX, vesperlysin A and C are other AGE molecules known to be cross-linking and fluorescent. The methyl glyoxal-lysine dimer (MOLD) and glyoxal-lysine dimer (GOLD) formations, which are formed as a result of the reaction of methyl glyoxal and glyoxal with lysine amino groups, are cross-linking but non-fluorescent AGE molecules (Figure 4). Although it has been reported that imidazolium cross-links are formed with lysine groups during the formation of these two AGE types, it has been reported that they can also be cross-linked with arginine groups (Two AGEs: GODIC and MODIC). Carboxy methyl lysine (CML), carboxy ethyl lysine (CEL), imidazolone and pyralin are advanced glycation end products that are determined to have no cross-linking and fluorescent properties. Carboxy methyl lysine is widely used in studies to evaluate the level of non-fluorescent AGEs in plasma and bone tissue. Although it can occur as a result of oxidation in various mechanisms, CML can also occur directly as a result of the reaction between the amino groups of glyoxal and lysine. Pyraline, on the other hand, is an important AGE molecule, which has a ring structure and is formed as a result of the reaction between lysine and glucose. The most important AGE molecule, which is not cross-linked but has fluorescent properties, is Argypyrimidine; It has been reported that it may occur as a result of the reaction between methyl glyoxal and arginine, and its serum levels are detectable in individuals with diabetes (Kılınç, 2011; Toprak, 2014; Demirel & Yıldıran, 2018; Döner, 2021; Muthyalaiah & et al., 2021; Yalçın & Rakıcıoğlu, 2022).

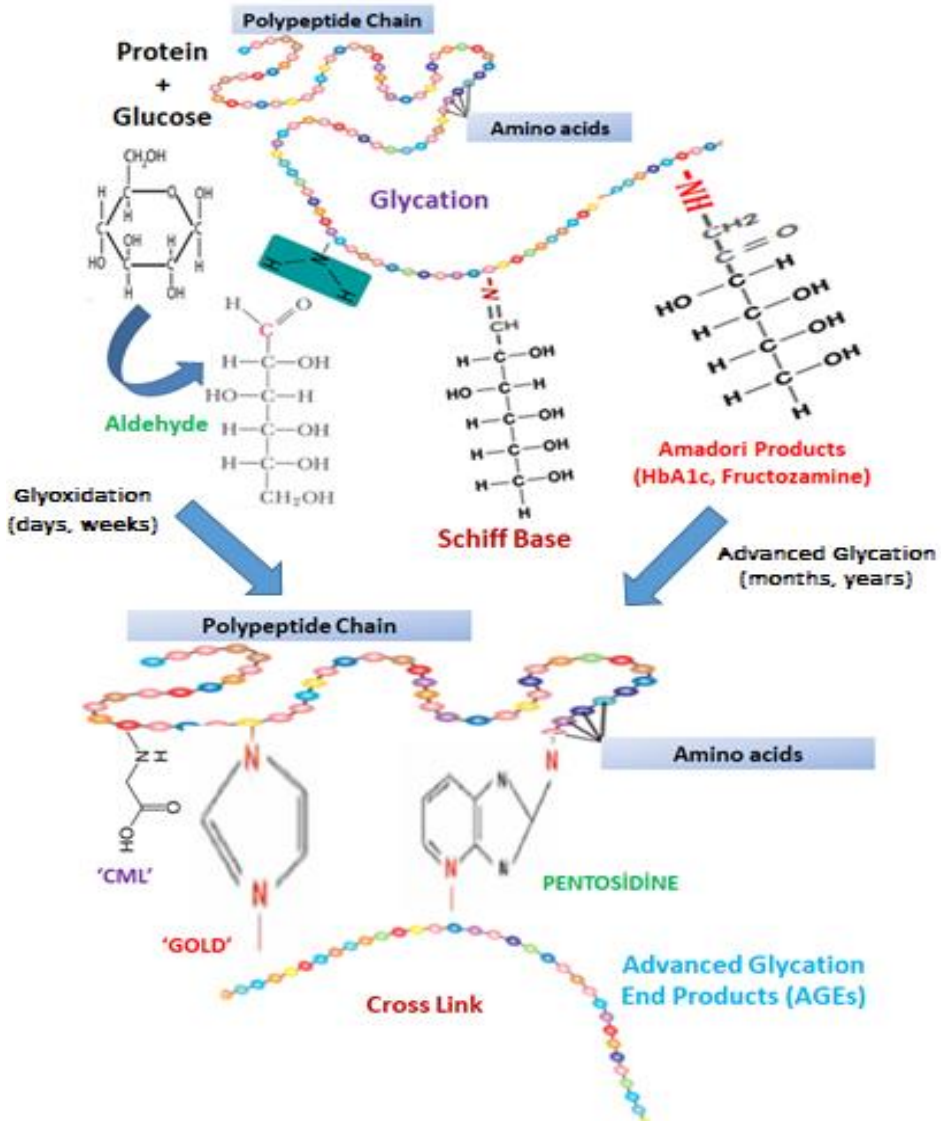

Figure 4. Formation of advanced glycation end products (AGEs). Adapted from Kerimoğlu & Türk, 2018.

RAGE (Receptor for AGEs)a

The undesirable *in vivo* effects of advanced glycation end products are thought to occur in two different ways. It is thought that the first of the exit mechanisms is the change of tissue or vascular structure after the formation of cross-linked structures, and the other mechanism arises through AGE-sensitive receptors. Different receptors with which advanced glycation end products interact in many cell types such as leukocytes, macrophages, neuronal cells and muscle cells have been identified. Among these receptors; **receptors for advanced glycation end products (RAGE)**, scavenger receptors (Class A, CD36, Class B tip 1, LOX-1, FEEL-1, FEEL-2), oligosaccharyl transferase-48 (AGE-R1/OST-48), AGE-R2/80K-H and Galectin-3 (AGE-R3) are reported to be involved. Among these receptors, the most studied is RAGE, a member of the immunoglobulin G superfamily. Advanced glycation end-product receptors (RAGE) are defined as a transmembrane pattern recognition receptor that can be stimulated by many ligands, belongs to the immunoglobulin superfamily, is located on chromosome 6, has a molecular weight of 45 kDa, and acts as a cell adhesion molecule. It was detected and purified for the first time in bovine lung endothelial cells. It is thought that the accumulation of advanced glycation end products in tissues and organs has important effects on the organism, causing glycative stress mediated by RAGE, and binding of RAGE with its ligands generally increases oxidation and inflammation. Advanced

glycation end products, via RAGE; It has been shown that it has a role in important biological events such as autophagy, endoplasmic reticulum stress, mitochondrial dysfunction, hypoxia, epigenetic modification and cancer, and contributes to the development of various pathologies by inducing or inhibiting various intrinsic cell signaling pathways. It is known that important ligands such as β-amyloid, advanced oxidation protein products (AOPPs), calcium-binding S100 proteins and high mobility group box protein 1 (HMGB-1) can also bind to advanced glycation end-product receptors (Singh & et al., 2001; Demirel & Yıldıran, 2018; Yılmaz & Karabudak 2018; Döner, 2021; Muthyalaiah & et al., 2021; Oshima & et al., 2022; Yalçın & Rakıcıoğlu, 2022).

Genome analyzes including other vertebrates such as birds, amphibians, fish and reptiles have proven that RAGE homologs are only found in mammals. From an evolutionary perspective, this information highlights the importance of understanding why mammals need RAGE and what its physiological roles are. Studies show that RAGE has 2 isoforms known as **soluble RAGE (sRAGE)**. These two isoforms of sRAGE; endogenous secretory RAGE (esRAGE) and cleaved RAGE (cRAGE). Endogenous secretory RAGE (esRAGE) form; It occurs as a result of alternative splicing of RAGE mRNA. Fragmented RAGE (cRAGE); It is a non-membrane-bound extracellular form of RAGE as a result of degradation of full-length RAGE by metalloproteases. It is thought that soluble RAGEs (sRAGEs) act as traps by preventing membrane-bound RAGEs from binding with their ligands and removing AGEs from the circulation (Döner, 2021; Muthyalaiah & et al., 2021; Oshima & et al., 2022).

Glycation inhibitors

With the understanding of the biological effects of advanced glycation end products, different approaches have begun to be developed within the methods of eliminating the harmful effects they create. One of the most studied approaches among these approaches is to reduce or prevent the formation of AGEs. For the inhibition of the production of advanced glycation end products, compounds that reduce the amount of AGE formation by affecting the formation steps were used; For this, Aminoguanidine, which is a nucleophilic hydrazine compound, and pyridoxamine, which can bind to carbonyl compounds during their formation, have been frequently used molecules over the years (Vlassara & Palace, 2002; Toprak, 2014).

Another method that is the subject of the studies is the breaking of the formed AGE cross-links. Agents known as AGE 'bond' breakers used in studies are defined as compounds that break down AGEs and allow them to be removed from the kidney and liver. Phenyl-thiazolium-bromide (PTB), which can break the carbon-carbon bond between dicarbonyls, is a compound in this group. In addition, ALT-711, now known as alegebrium chloride, has enzymatic properties and is reported to be able to break covalent bonds repeatedly, is another important compound in this group (Vlassara & Palace, 2002; Toprak, 2014).

AGE inhibitors and cross-link breakers act on AGE-related pathologies in different ways. Inhibitors are compounds known to reduce the rate of AGE formation but are unable to break newly formed AGE cross-links on long-lived tissue proteins. It is known that crosslink breakers can break AGE crosslinks but cannot prevent new AGE formations. For this reason, it is thought that the most effective approach in pathological conditions due to advanced glycation and its products may be a combined treatment in which inhibitor and bond-breaking agents are used together (Toprak, 2014).

One of the methods that are the subject of research is the RAGE blockade. In this method, sRAGE and RAGE antibodies are used in studies to prevent AGE-RAGE interaction. The main purpose of this method is to bind AGEs in tissues and prevent them from binding to their receptors, thus preventing RAGE-mediated cell activation (Toprak, 2014).

In addition to *in vivo* AGE production, it is known that AGEs are an important exogenous source and contribute to the *in vivo* AGE pool, especially in heat-treated foods. Therefore, another

research topic has been the focus on reducing exogenous AGE exposure. It did not take long to realize that the reducing sugars found in foods, apart from being able to react with proteins and molecules containing amino groups, also form aldehydes and ketones by spontaneous oxidation without reacting with proteins or by breaking down especially during cooking. It has been understood that the aldehydes and ketones formed can form glycation intermediates and glycation end products by reacting with proteins. Understanding that they are formed in high amounts especially in foods cooked/fried at high temperatures in the studies carried out, has led to a rapid increase in research on this subject. It has been reported that approximately 10-30% of dietary AGEs are absorbed through the gastrointestinal tract after oral intake and enter the circulation. It is stated that approximately 1/3 of the AGEs that enter the circulation are excreted in the urine within 48 hours, and the remainder accumulates in the body.

In studies, the effects of making changes in eating habits or using some agents that inhibit the absorption of AGEs taken with the diet were investigated. Among these agents, the most studied is AST-120, an oral adsorbent. Studies have shown that regular use of AST-120 has positive effects in individuals with chronic renal failure and slows the progression of the disease. It was also stated that after the use of AST-120 in non-diabetic individuals with chronic renal failure, this compound binds to CML and causes a decrease in AGE serum levels (Toprak, 2014; Demirer & Yardımcı, 2022; Oshima & et al., 2022; Yalçın & Rakıcıoğlu, 2022).

Over the years, compounds with antioxidant activity and/or metal chelation have also been tried in studies and their effects have been investigated. Many *in vitro* studies have shown that AGE formation is inhibited by the use of aspirin, ibuprofen, indomethacin, D-penicillamine, desferoxamine and flavonoids. The reduction of oxidative stress of these molecules has been shown as a possible mechanism. In addition, *in vitro* studies have shown that Diclofenac can also inhibit early glycation products, while piaglitazone, a thiazolidinedione derivative, acts on metal chelation by keeping dicarbonyl compounds and has effects on reducing AGE formation (Toprak, 2014; Yalçın & Rakıcıoğlu, 2022).

In addition, it has been reported that positive results have been obtained with the use of agents such as Aldose reductase inhibitors, angiotensin receptor blockers, metformin, thiamine and benfotiamine in studies over the years (Toprak, 2014).

Although it has been accepted for many years that repair activities occur only on DNA, recent studies have revealed that proteins that have undergone different chemical changes, especially glycation, can be repaired by various mechanisms (Kılınç, 2011).

After the discovery that isoaspartate is formed by the reduction of methionine sulfoxide, which is first produced as a result of the oxidation of methionine in biological systems, by the related enzyme; It did not take long to realize that ketoamines, which are formed by the glycation of proteins, can also be repaired by isomerases, oxidases and kinases. Studies show that these enzymes phosphorylate the carbon-nitrogen bond, thereby destabilizing monosaccharides and separating them from proteins. This mechanism, which has been shown to be able to reverse glycation, has been called **"deglycation"**. The first enzyme found to be involved in deglycation was fructosamine-3-kinase (FN3K). In this process, fructosamine becomes unstable by being phosphorylated from carbon 3 by FN3K and is separated from the protein. Studies show that FN3K can mediate not only protein deglycation but also the deglycation of free fructoselysine. After the discovery of fructosamine-3-kinase, the existence of other enzymes that lead to the separation of monosaccharides from proteins, resulting in decreased stability of various amadori products, has also been reported (Kılınç, 2011).

It has been reported that glycation causes various pathologies through direct or indirect changes in biochemical, functional and structural properties of proteins, and inhibition of glycation

has positive effects on these pathological conditions and related complications (Kılınç, 2011; Toprak, 2014).

Today, it has been revealed that the causes of complications in metabolic disorders in which hyperglycemia is seen, especially Diabetes Mellitus, are related to glycation and/or advanced glycation end products. Studies continue to reactivate inactive molecules (proteins, lipids, nucleic acids, etc.) resulting from glycation and/or to eliminate glycation end products.

KAYNAKÇA

Arslan, S., Kırağı, D., Kadayıfçılar, S. & Samur, G. (2021). Diyabetik Makula Ödemi ile Diyet İleri Glikasyon Son Ürünleri (AGEs) ve Oksidatif Stres Arasındaki İlişkinin Değerlendirilmesi. *Sağlık Akademisi Kastamonu, 6* (1), 1-22. DOI: 10.25279/sak.757689

Aydınhan, M. (2018). *Glikozillenmiş Tırnak Proteinlerinin Biyolojik Varyasyon ve Referans Değişim Değerlerinin Belirlenmesi*. Giresun: Giresun Üniversitesi, Sağlık Bilimleri Enstitüsü. Erişim linki: https://acikbilim.yok.gov.tr/handle/20.500.12812/87399

Demirel, Y. & Yıldıran, H. (2018). İleri Glikasyon Son Ürünleri ve Böbrek Hastalıkları. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi, 7* (1), 210-217. Erişim linki: https://dergipark.org.tr/tr/pub/gumussagbil/issue/36260/399677

Demirer, B. & Yardımcı, H. (2022). İleri Glikasyon Son Ürünlerinin Diyabet Komplikasyonları Üzerine Etkileri: Bir Derleme. *Beslenme Ve Diyet Dergisi, 50* (1), 101-108. https://doi.org/10.33076/2022.BDD.1516

Döner, H. C. (2021). *Tip 1 diabetli çocuk ve adölesanlarda galektin 3, ileriglikasyon son ürünleri (AGE), ve ileri glikasyon son ürünleri reseptörü (RAGE) seviyelerinin araştırılması*. Konya: Necmettin Erbakan Üniversitesi, Meram Tıp Fakültesi. Erişim linki: https://hdl.handle.net/20.500.12452/8154

Erim, B., Ergene, E. & Hecer, C. (2022). Besin Hazırlama Ve Pişirme Yöntemlerinin İleri Glikasyon Son Ürünleri Üzerine Etkisi. *Aydın Gastronomy, 6* (2), 275-281. Doi: https://dergipark.org.tr/en/pub/aydingas/issue/70799/1023704

Kayılı, H. M. (2016). *Glikopeptitlerin Kütle Spektrometrik Analizleri İçin Yeni Bir Biyoanalitik Yöntem Geliştirilmesi ve İnsan C1-İnhibitör Proteininin Ayrıntılı Bölgeye Özgü Glikozilasyon Analizinin Gerçekleştirilmesi*. Ankara: Hacettepe Üniversitesi, Fen Bilimleri Enstitüsü.

Kerimoğlu, H. & Türk, H. B. (2018). Diyabetik Retinopati ve Diyabetik Maküla Ödeminde Patogenez. *Güncel Retina, 2* (2), 94-101.

Kılınç, K. (2011). Protein Glikasyonu. *Hacettepe Tıp Dergisi, 42* (2), 95-104.

Muthyalaiah, Y. S., Jonnalagadda, B., John, C. M. & Arockiasamy S. (2021). Impact of Advanced Glycation End products (AGEs) and its receptor (RAGE) on cancer metabolic signaling pathways and its progression. *Glycoconj Journal, 38*, 717-734. https://doi.org/10.1007/s10719-021-10031-x

Oshima, Y., Harashima, A., Munesue, S., Kimura, K., Leerach, N., Goto, H., Tanaka, M., Niimura, A., Hayashi, K., Yamamoto, H., Higashida, H. & Yamamoto, Y. (2022). "Dual Nature of RAGE in Host Reaction and Nurturing the Mother–Infant Bond. *International Journal of Molecular Sciences, 23* (4), 2086. https://doi.org/10.3390/ijms23042086

Özaydın, E., Yalçın, F., Gündüz, M. & Köse, G. (2012). "Konjenital Glikozilasyon Bozukluğu Tip II". *Türkiye Çocuk Hastalıkları Dergisi, 6*(1), 47-53.

Singh, R., Barden, A., Mori, T. & Beilin, L. (2001). Advanced glycation end-products: a review. *Diabetologia, 44*, 129-146. https://doi.org/10.1007/s001250051591

Toprak, Ç. (2014). *Alegebrium (ALT-711)'un İzole Karotis Arter Preparatlarında Fonksiyonel Etkisi*. Eskişehir: Eskişehir Osmangazi Üniversitesi, Sağlık Bilimleri Enstitüsü.

Vlassara, H. & Palace, M. R. (2002). Diabetes and advanced glycation endproducts. *Journal of Internal Medicine, 251* (2), 87-101. doi:10.1046/j.1365-2796.2002.00932.x

Yalçın, A. (2012). Posttranslasyonel Modifikasyon ve Protein Fonksiyonu. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi, 31* (1), 29-37.

Yalçın, E. & Rakıcıoğlu, N. (2022). Besinlerde Oluşan İleri Glikasyon Son Ürünlerine Polifenollerin Etkisi. *Beslenme Ve Diyet Dergisi*, *50* (2), 66-75. https://doi.org/10.33076/2022.BDD.1554

Yılmaz, B. & Karabudak, E. (2016). Besinlerdeki İleri Glikasyon Son Ürünleri ve Azaltma Yöntemleri. *Beslenme ve Diyetetik Dergisi, 44* (3), 280-288.

Yılmaz, B. & Karabudak, E. (2018). Diyet Kaynaklı İleri Glikasyon Son Ürünleri ve Sağlık Üzerine Etkileri. *Acıbadem Üniversitesi Sağlık Bilimleri Dergisi, 9* (4), 349-356. https://doi.org/10.31067/0.2018.55

Yılmaz, Y. (2022). *HbA1C Ölçümünde Yöntem Karşılaştırması*. Denizli: Pamukkale Üniversitesi, Sağlık Bilimleri Enstitüsü. http://hdl.handle.net/11499/45554

Pathophysiological Effects of Advanced Glycation end Products

Pınar COŞKUN[1]
Soner YILDIZ

Introduction

Advanced glycation end products (AGEs) can be formed as a result of endogenous or exogenous non-enzymatic glycation, by reactions between the reactive carbonyl group of a reducing sugar and nucleic acids, lipids or proteins. For years it was assumed that AGEs were only more stable structures formed by the non-enzymatically binding of a reducing sugar to lysine residues of proteins. However, in recent years, it has been shown that Methylglyoxal, one of the AGEs, may occur as a result of the enzymatic reaction of glyceraldehyde 3-phosphate with cysteine, lysine and arginine residues of proteins. AGEs that can be produced endogenously or exogenously; It has been reported to have undesirable effects on cellular functions in various ways such as the production of free radicals, oxidation of lipids and nucleic acids, changes in enzyme activities, hydrolysis of lipids and proteins, carbonyl stress and interaction with AGE receptors. Two mechanisms are at the root of the pathological conditions and associated complications caused by advanced glycation end products at tissue and cell level in vivo: The first of these mechanisms is that AGEs cause the deterioration of matrix structure and functions by forming cross-links to the proteins in the structure of the extracellular matrix (ECM). The other mechanism emerges with metabolic changes resulting from the synthesis and release of some proteins and cytokines by activating or inhibiting various signaling pathways via AGE-sensitive receptors (RAGE). It has been reported that these receptors can also induce inflammation by being stimulated by inflammatory cytokines, amyloid-β and other fibrillar proteins besides AGEs (Çetiner & Rakıcıoğlu, 2020; Vıcıl & Ulutaş, 2020; Arslan & et al., 2021; Döner, 2021; Rungratanawanich & et al., 2021; Demirer & Yardımcı, 2022).

It is thought that the pathological effects of advanced glycation end products may be due to their ability to directly trigger oxidative stress and inflammation, as well as providing the generation of reactive oxygen (ROS) or nitrogen (RNS) species. It has been reported that these properties are directly or indirectly related to changes in structural and functional properties in proteins, cellular dysfunction, apoptosis, and ultimately disruption of integrity in tissues and organs. In addition, the binding resulting from the interaction of advanced glycation end products with RAGE receptors; It has been discovered that it is an important factor in the development and progression of diabetes, cancer, neurodegenerative diseases, cardiovascular complications, osteoporosis, kidney and liver dysfunctions, and various diseases related to aging (Rungratanawanich & et al., 2021; Oshima & et al., 2022).

The effect of crosslinking

Considering the normal tissue architecture, it is seen that the cross-linking of the proteins in the extracellular matrix (ECM) with each other is of great importance in ensuring and maintaining tissue integrity. However, the formation of this structure consisting of cross-linked proteins is not sufficient alone, and it is an important condition for this structure to have a certain level of flexibility for its functionality and sustainability. It is known that AGE accumulation in cells and tissues can cause various toxic effects. With the accumulation of AGEs directly in ECM proteins, the formation of new cross-links in addition to the existing cross-links occurs, thus reducing the

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flexibility of the proteins. It has been shown that the decrease in flexibility, which occurs due to the accumulation of advanced glycation end products, especially in ECM proteins such as collagen, elastin and laminin, has a role in various pathologies (Demirel & Yıldıran, 2018; Bijnen & et al., 2019; Arslan & et al., 2021; Döner, 2021).

The heart is one of the organs most affected by the decrease in flexibility caused by these reasons. Decreased flexibility has been shown to mediate various heart conditions by causing diastolic dysfunction in the heart. It is known that the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and the ryanodine receptor (RyR) are of critical importance for the cardiac muscle to be adequately contracted and relaxed. It has been clarified by various studies that the glycation of these molecules in the aforementioned system and their cross-linking with AGEs inhibit their functions and may lead to deterioration in diastolic functions by reducing cardiac contractility. (Döner, 2021).

Since the formation of advanced glycation end products is usually due to the high amount of glucose in the blood, intermolecular cross-linking that leads to a decrease in the elasticity of ECM proteins such as collagen also occurs at a high rate in hyperglycemic diseases. It has been reported by various studies that the accumulation of AGEs on proteins prevents the proteolytic degradation of proteins and especially the cross-linked collagen becomes resistant to matrix metalloproteinases and causes disruption of the collagen cycle. It has also been shown that inhibiting proteolytic degradation may cause thickening of the capillary basement membrane, resulting in narrowing of the vessel diameter. It has also been reported that the cross-links between type IV collagen and laminin under the influence of AGEs disrupt the spread and adhesion of endothelial cells to the basement membrane. As a result of glycation of the NC1 chain (non-collagenous), which is expected to be attached to the helix-rich chain in the type IV collagen structure under normal conditions, it is not possible to bind to Type IV collagen. It has also been reported that AGE accumulation can inhibit the binding of collagen and heparan sulfate to vitronectin, an adhesive matrix molecule, and inhibit intramolecular interactions and polymerization by disrupting the cross-shaped structure of laminin. These events may lead to the formation of atherosclerosis by disrupting the ECM structure (Döner, 2021; Selvakumar & et al., 2022).

It is reported that one of the proteins most affected by glycation with the formation of cross-links is LDL. It has been reported that as a result of the formation of intramolecular cross-links on LDL by the effect of AGEs, modified clearance of LDL and its uptake into the cell by receptor-mediated endocytosis have been reported. At this point, it has been determined that AGE-modified LDL becomes more prone to foam cell formation and increases the likelihood of atherosclerosis. It was also understood that the rate of degradation and internalization of glycated LDL decreased and became more open to oxidation. It has also been reported that AGE accumulation on LDL can also reduce nitric oxide (NO) formation by inhibiting the reuptake of L-arginine, which is activated due to blood flow tension in endothelial cells (Döner, 2021).

Glycation reactions are reactions that can cause changes at the molecular level by progressing at different rates in different periods of life. Accordingly, glycation products can be defined as products that are formed under physiological conditions, but whose formation is observed to increase with age. For years, aging has been recognized as a major risk factor for the occurrence of osteoarthritis. However, this idea has begun to be better understood with the examination of the role of AGEs, which are caused by the glycosylation of macromolecules, in aging. It is now accepted that osteoarthritis occurs as a clinical and pathological result of a series of disorders that cause limitation of movement and structural and functional disorders of the synovial joints, which are primarily seen in elderly individuals. At this point, it was also effective to elucidate the product accumulation caused by the low level of AGE inhibition in the aging process and the deterioration in ECM metabolism (Demirel & Yıldıran, 2018; Karataş, Şentürk, Efe Arslan & Korkmaz, 2021; Yılmaz & Polat, 2022; Zhou & et al., 2022).

Receptor-mediated effects of advanced glycation products

The most important known mediators of the effects of advanced glycation end products on tissue and cells are AGE-sensitive receptors (RAGE). Some metabolic changes occur as a result of the release of certain proteins and cytokines by activating or inhibiting various signaling pathways through these receptors. In addition, it has been reported that RAGE receptors can also be stimulated by inflammatory cytokines, amyloid-β and other fibrillar proteins other than AGEs. It has also been demonstrated that various cell signaling pathways such as p38, MAPKs, ERK, p21RAS, Pi3K and janus kinase (JAK) can be activated as a result of the interaction of advanced glycation end products with RAGE. Finally, various studies show that AGEs have a role in important biological events such as RAGE-mediated angiogenesis, proliferation, inflammation, fibrosis, and thrombosis (Arslan & et al., 2021; Çetiner & Rakıcıoğlu, 2020; Döner, 2021; Rungratanawanich & et al., 2021).

One of the signaling pathways induced by the binding of RAGE with advanced glycation end products is the NF-ϰB (nuclear factor ϰB) signaling pathway. The NF-ϰB family of transcription factors is known as a signaling pathway that plays a role in cells' response to various stimuli. It is also a signal regulator that is associated with many signaling pathways. It has been understood that advanced glycation end products may play a role in collagen degradation by inducing phosphorylation of the NF-ϰB and MAPK families. Therefore, it is thought that controlled inhibition of NF-ϰB/MAPK phosphorylation may be an important therapeutic approach in the prevention of age-related chondrocyte inflammation and extracellular matrix degradation. In addition, it has been shown that AGE-RAGE interaction can stimulate the synthesis of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule (VCAM) by inducing NF-ϰB, thus impairing endothelial cell function and increasing vascular permeability. . These molecular adhesives, known as "Cell Adhesion Molecules (CAM)" and providing the coexistence of cell clusters in biological structures; They are protein molecules found on the cell surface and involved in cell-cell and cell-matrix interactions. However, the most important feature of the NF-ϰB signaling pathway is its ability to induce inflammatory gene expression. It has been understood that NF-kB stimulation, which occurs as a result of the interaction of advanced glycation end products and RAGE, stimulates the synthesis of many proinflammatory cytokines and the formation of free oxygen radicals as a result. It was understood that these ROSs increased the expression of monocyte chemoattractant protein 1 (MCP-1) and accelerated the migration of monocytes to the subendothelial region by inducing chemotaxis. Apart from the mentioned mechanism, free oxygen radicals can also occur with the activation of NADPH oxidase directly as a result of the AGE-RAGE interaction. Advanced glycation end products-RAGE interaction can stimulate granulocyte-monocyte colony stimulating factor (GM-CSF) synthesis from macrophages. It is also reported that this interaction can induce the synthesis of tumor necrosis factor α (TNF-α), platelet-derived growth factor (PDGF), IL-1β and insulin growth factor-1 (IGF-1). One of the signaling pathways indicated to be induced by the interaction of advanced glycation end products and RAGE is the Janus kinase/signal converters and activators of transcription (JAK/STAT) signaling pathway. This pathway is one of the leading pathways used to convert a large number of signals for development and homeostasis in animals from humans to flies. In mammals, the JAK/STAT pathway also serves as the main signaling mechanism for a wide variety of cytokines and growth factors. It has been reported that the JAK-STAT signaling pathway activated as a result of the said AGE-RAGE interaction mediates the proliferation of smooth muscle cells in the damaged vessel wall (Rawlings, 2004; Terekeci, Şahan & Top, 2008; Şensoy & Öznurlu, 2009; Ledoux & Perkins, 2014; Döner, 2021; Zhou & et al., 2022).

Anti-aging treatment studies; Focusing on anti-glycation to reduce morbidity, stimulate healthier aging, longevity and faster recovery, it has received and continues to receive a rapidly increasing interest in recent years. At this point, studies; Targeting of RAGE continues with a focus on developing a preventive and therapeutic strategy against numerous RAGE-related diseases,

including diabetes mellitus and its complications, inflammatory disorders, aging-related diseases, neurodegenerative disorders, cancer (Oshima & et al., 2022).

Effects of advanced glycation end products on tissues and cells

In addition to being exogenously taken from the diet, carbonyl compounds can be formed endogenously in the body as a result of protein glycation and lipid peroxidation; Since they have reactive properties, they can react with proteins and cause unexpected changes in the existing functional, biochemical and structural properties of proteins. These changes that occur with the reaction of carbonyl compounds with proteins; As they may arise independently of AGE receptors, these proteins, whose structure changes with these reactions, also have various effects, especially triggering oxidative stress in cells and tissues by binding to AGE receptors (Kılınç, 2011).

The main pathologies determined to be caused by the effects of glycation on proteins at the cell and tissue level directly or through AGE receptors can be generally classified as follows (Kılınç, 2011):

Increased lipid peroxidation: In case of an increase or a decrease in the inactivation of reactive oxygen derivatives (ROS) in cells and tissues, the balance in the body is disrupted and this is known as oxidative stress. Oxygen free radicals are highly reactive because they are molecules containing one or more unpaired electrons in their outer orbitals. The increase in oxygen radicals resulting from oxidative stress is a mechanism that can be triggered in various ways within the cell. Advanced glycation products can cause oxidative stress by forming pro-oxidant molecules in the body. In particular, diabetes is also defined as a state of increased oxidative stress. AGEs, which may occur as a result of hyperglycemia-induced glycotoxicity, may mediate the formation of superoxide by causing activation of NADPH oxidase in the cell. In this case, increasing reactive oxygen derivatives; It can interact with biomolecules such as proteins, lipids and nucleic acids, causing structural or functional changes in proteins, loss of membrane integrity of cells and genetic-based disorders. It is known that cells have some enzymatic and non-enzymatic antioxidant defense systems that can eliminate these radicals in order to cope with the harmful effects of ROS. One of the most important antioxidant enzymes used by cells to resist oxidative stress is the enzyme superoxide dismutase (SOD). However, it has also been discovered that this enzyme can be inhibited as a result of glycation. It has been understood that the induction of oxidative stress by oxidative destruction of glycation products or various activation of AGE receptors reduces nitric oxide (NO) levels, while inducing endothelin-1 synthesis, leading to lipid peroxidation and increased vasoconstriction at the cell and tissue level. It has also been reported in various studies that some AGE inhibitors have positive effects on reducing oxidative stress (Kılınç, 2011; Demirel & Yıldıran, 2018; Çetiner & Rakıcıoğlu, 2020; Arslan & et al., 2021).

Induction of various cellular activities: Various studies have shown that synthesis and release of many proteins and cytokines occur by activating or inhibiting various signaling pathways via AGE-sensitive receptors (RAGE). It has been reported that many metabolic changes occur as a result of this synthesis and release. It has been revealed by various studies that these receptors can also induce inflammation by stimulating with inflammatory cytokines, amyloid-β and other fibrillar proteins besides AGEs. Stimulation of glycated and glycated proteins and AGE receptors can trigger the synthesis and secretion of cytokines, primarily TNF-α, IGF-1A, VCAM-1 and IL-1. In parallel with the induction of the synthesis of inflammatory cytokines, chemotaxis of mononuclear cells, gamma-interferon synthesis, T-cell activation and mitogenesis can be observed. In this way, it can be seen that as a result of uncontrolled cellular activities, in addition to thickening of the basement membrane, reorganization may occur in the tissues (Kılınç, 2011; Vıcıl & Ulutaş, 2020; Döner, 2021).

Structural and functional changes of proteins: It is known that AGE accumulation in cells and tissues can cause various toxic effects. The formation of cross-links occurs with the

accumulation of AGEs directly in the proteins, and thus, changes in the structure and functions of the proteins are observed. It is known that glycation can change the net charges and three-dimensional conformations of proteins and lead to the formation of protein aggregates resistant to proteases by cross-linking. The best example that can be given to this subject is the advanced glycation of the crystalline protein in the lens tissue. While the formed glycated crystal causes opacification in the eye and vision problems; It is observed that collagens undergoing rapid glycation in individuals with diabetes age prematurely and lose their elastic properties faster. It has been understood that the cell-matrix interactions are changed as a result of glycation of the proteins in the cell membrane and the extracellular matrix, and the adhesion and spreading properties of the cells are also directly and indirectly affected by this process (Kılınç, 2011; Demirel & Yıldıran, 2018; Bijnen & et al., 2019; Arslan & et al., 2021; Döner, 2021).

Effects on thrombosis and fibrinolysis: Thrombosis can also be defined as an abnormal plug formed by blood elements in the vessel. It appears to occur as a result of the disruption of the delicate balance between coagulation and the fibrinolytic system. It has been reported that glycation, in addition to increasing tissue-related thrombosis factors, also reduces thrombomodulin (TM) synthesis. Thrombomodulin is a glycosylated thrombin receptor with a proteoglycan structure, mostly found in endothelial cells. It is so named because it has a regulatory role on thrombin functions. In addition to being an important factor on the delicate balance between coagulation and fibrinolytic processes, it is also known to play an active role in inflammation, embryogenesis, cancer, diabetes and collagen vascular diseases. It has also been shown that glycation plays a role in stabilizing fibrin by increasing platelet aggregation and the synthesis of plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor. As a result, the life span of platelets is shortened and their tendency to form aggregates increases. In parallel, it is seen that the sensitivity of fibrin and fibrinogen to plasmin decreases and the functions of antithrombin III are suppressed. As a result, all these chain events result in the disruption of the delicate balance between coagulation and the fibrinolytic system. Disruption of this delicate balance in the direction of fibrinolysis can lead to bleeding; Increased coagulation can cause thrombus formation (Ermiş, Turgan & Ersöz, 2006; Kılınç, 2011; Celkan & Dikme, 2018).

Advanced glycation end products and glucotoxicity

It is seen that the formation of advanced glycation end products generally occurs due to the high amount of glucose in the blood. If this rise in glucose levels above normal physiological concentrations is prolonged, the amount and function of insulin produced by the beta-cells of the pancreas is affected, and thus beta-cell function is gradually deteriorated. This pathophysiological condition, which is frequently seen in diabetic individuals, is generally known as "beta-cell glucose toxicity". AGEs, which occur as a result of hyperglycemia-induced glycotoxicity and are also defined as glycotoxins; It has a series of chemical effects on cells and tissues, and it has been demonstrated by various studies that it has a role in many pathologies, including diabetes-related complications, atherosclerosis, kidney and neurodegenerative diseases (Vlassara & Palace, 2002; Yılmaz & Karabudak, 2016; Çetiner & Rakıcıoğlu, 2020; Demirer & Yardımcı, 2022; Küçükkatırcı, Caferoğlu, & Hatipoğlu, 2022).

Since the formation of advanced glycation end products is generally due to the high amount of glucose in the medium, studies in this field have largely focused on diabetes and its complications. Although the general idea on this subject is that AGEs occur as a result of diabetes, studies show that AGEs also have diabetic effects and there are basically two mechanisms involved in the emergence of this effect. Firstly; It has been shown that AGEs can directly cause pancreatic beta-cell damage, leading to disruptions in insulin secretion. The second mechanism occurs when the accumulation of AGE on the respiratory proteins in the mitochondria induces the formation of reactive oxygen species (ROS), thus triggering a vicious inflammatory cycle (Yılmaz & Karabudak, 2016; Demirer & Yardımcı, 2022).

The role of AGEs in metabolic memory

The concept of metabolic memory; It is defined as metabolic disorders seen in various diseases, especially diabetes, and hyperglycemia that occurs even for a temporary period, mediating cardiovascular complications in the future with the permanent effect of epigenetic modifications, even if blood sugar control is achieved later on. This concept fully emphasizes the effects and complication risks of metabolic changes that occur in the early stages of pathological disorders, especially diabetes. Many researchers, who have focused on the pathogenesis of metabolic memory for years, are trying to find an answer to the question of why diabetes-related complications continue to occur in the later periods, although early glycemic control can be achieved in diabetic individuals (Küçükkatırcı, Caferoğlu, & Hatipoğlu, 2022).

One of the key factors in the emergence of diabetic complications is the production of high reactive oxygen species (ROS), which occurs in mitochondria and endothelial cells, which leads to increased oxidative stress. High amounts of glucose metabolized in mitochondria and endothelial cells appear to lead to even more free radical production. It is known that the high production of ROS in mitochondria is the first step of the chain reactions that play a role in the emergence of chronic complications of diabetes. In particular, this situation has also been described as the vicious circle of metabolic memory. In this case, free radicals released at high levels in the respiratory chain in the mitochondria; It has also been reported to trigger various intracellular pathways such as polyol and hexoamine pathway activation, protein kinase C induction and AGE formation. In addition to this situation, free radicals can also affect the expression levels of many genes that play a role in the pathogenesis of chronic diabetic complications. Protein kinase C induction; It can increase the expression levels of pro-inflammatory cytokines, various adhesion molecules and growth factors. In addition to this situation; It has been discovered that by stimulating NF-kB, it can induce approximately 150 pro-inflammatory genes, including cyclooxygenase-2, interleukin (IL)-1, IL-6, IL-8, TNF-β and NO synthetase in vascular cells (Küçükkatırcı, Caferoğlu, & Hatipoğlu, 2022).

It is known that hyperglycemia in endothelial cells inhibits the production of NO, leading to abnormal vascular reactivation, which impairs the perfusion of tissues and organs. On the other hand, there are studies that determined that oxidative stress and high glucose levels increase inducible nitric oxide synthase (iNOS) activity and NO synthesis. This causes damage to proteins involved in mitochondrial electron transfer, decreased peroxide detoxification, and ultimately a shift of glucose metabolism to polyol and hexokinase pathways. It is observed that protein and lipid glycation, which occurs in parallel with this trend in glucose metabolism, leads to deterioration in vascular reactivation. The toxic effects on tissues and organs that develop due to glucose levels are also directly related to the increase in AGE production. Intracellular protein glycation and the resulting AGE formation affect the regulation of many genes that increase ROS production in mitochondria, as in hyperglycemia. Binding of advanced glycation end products to their receptors in various cells also leads to the induction of NF-kB, which affects the regulation of genes associated with diabetes and its complications. It appears that increased glucose concentration in mitochondria and consequent increased AGE formation in mitochondria lead to increased ROS production and induction. In short, it is seen that the glycation of the proteins involved in respiration in mitochondria has a great importance in the concept of metabolic memory (Küçükkatırcı, Caferoğlu, & Hatipoğlu, 2022).

Retinopathy and eye complications

It has been observed that advanced glycation end products can accumulate in different parts of the eye such as the lens, cornea, Bruch's membrane, retina and optic nerve. It has been shown that this situation has a role in various complications, especially diabetic retinopathy, cataract and diabetic keratopathy in individuals with diabetes. In a study conducted on diabetic rats, compared to the control group, in addition to higher AGE accumulation in the experimental groups, there

was a higher expression of 8-hydroxyguanosine and NF-kB in the corneal tissues, and this revealed that AGE accumulation may have a role in the pathogenesis of diabetic keratopathy. The accumulation of advanced glycation end products may also contribute to the formation of cataracts by causing accumulation in structural proteins such as tubulin, myelin and lens crystals in the eye and lens epithelial cells. In a study of individuals with type 2 diabetes, it was found that the degree of retinopathy was correlated with the levels of AGEs in samples taken from retinal blood vessels. This suggested that AGEs may have a role in the pathophysiology of retinopathy. Various studies of diabetic retinopathy; showed that it is characterized by proliferation or occlusion of intraocular blood vessels, increased permeability of capillaries, proliferative loss of endothelial cells and surrounding retinal pericytes. It has been reported that extracellular proteins modified as a result of glycation can cause retinal damage by binding to RAGE. It has been demonstrated that binding to the aforementioned RAGE receptor can induce various signaling pathways as well as increase oxidative stress. It has been observed that the interaction of advanced glycation end products with RAGE leads to an increase in ROS and induction of apoptosis in pericytes through the activation of NF-ϰB and NADPH oxidase in endothelial cells. It has been determined that these events cause disruptions in the blood retinal barrier and cause mitochondrial damage to retinal neurons through ROS. In addition, various studies have shown that the interaction of AGEs with RAGE, which is formed not only in the cell but also in other parts of the eye, can potentially stimulate angiogenesis (Vlassara & Palace, 2002; Kerimoğlu & Türk, 2018; Çetiner & Rakıcıoğlu, 2020; Demirer & Yardımcı, 2022).

Effect of AGEs on renal functions

One of the most important causes of chronic kidney diseases in the world is known as diabetes; The incidence of diabetic nephropathy is approximately 30-40%. It is known that the kidneys, which are known to have a direct or indirect role in all metabolic events in the body, also have importance on AGE metabolism. It has also been shown that advanced glycation end products are absorbed through the glomerular filtrate through the proximal tubule cells and catabolized by these cells. Since the kidneys are important in AGE metabolism; In case of any damage to the kidneys, serum AGE levels are also directly increased. Elevated serum AGE levels also cause damage to the kidneys and this becomes a vicious cycle. Studies have revealed that the renal and vascular accumulation of AGEs triggers the accumulation of AGEs in most of the systems in the human body and mediates the complications that occur due to this accumulation. Nephropathy due to hyperglycemia; It is reported to be characterized by excessive ECM deposition, glomerular hypertrophy, glomerulosclerosis and thickening of the basement membrane (Vlassara & Palace, 2002; Ergenç & et al., 2017; Demirel & Yıldıran, 2018; Çetiner & Rakıcıoğlu, 2020).

It has been suggested that advanced glycation end products interact with RAGE to induce some signaling pathways and thus play a role in the development of nephropathy in diabetes. It has also been demonstrated that AGEs have a role in podocyte DNA damage and segregation, in part by stimulating the angiotensin II type 1 receptor (AT1R). Since nephropathy is characterized by the accumulation of ECM proteins in the glomerular mesengium and tubulointerstitium; It has been understood that AGEs lead to the deterioration of the balance between the synthesis and degradation of components in the ECM structure, leading to the accumulation of collagen, fibronectin and laminins, and thus to various pathologies. It has been shown that advanced glycation end products increase the formation of ECM proteins such as fibronectin and type I-IV collagen depending on dose and time. This increase was thought to be mediated directly by RAGE receptors, which involve induction of the Janus kinase (JAK)/signal converter and activator of transcription (STAT) pathways. It has been understood that these signaling pathways mediate the emergence of these effects by causing an increase in the expression of growth factors and profibrotic cytokines, including platelet-derived growth factor (TGF) and connective tissue growth factor (CTGF) (Demirer & Yardımcı, 2022).

Relationship between neuropathy and AGEs

One of the most common complications in hyperglycemia is neuropathy, and it is generally one of the most common causes of non-traumatic lower extremity amputations in individuals with diabetes. Although the pathogenesis of diabetic neuropathy is not fully understood, AGEs caused by high glucose levels are thought to have a key role in this regard. It is known that the effects of advanced glycation end products such as the polyol pathway, increase in oxidative stress and induction of the protein kinase C pathway, and that these pathways cause an increase in oxidative stress levels in general. It has been reported that oxidative stress resulting from these reasons also contributes to the accumulation of glycoxidation products such as carboxymethyllysine (CML) and pentosidine. Modification of proteins in peripheral nerves with AGEs leads to structural and functional changes. In vitro studies have shown that incubation of Schwann cells and neurons with AGEs decreases the survival rate of the cells. It has been reported that one of the main factors in peripheral nerve fiber loss seen in diabetics is AGE accumulation. The basis of this loss is thought to be the inhibition of axonal transport as a result of AGE accumulation in tubulin and neurofilaments. This inhibition of axonal transport leads to degeneration and atrophy of nerve fibers, and ultimately contributes to neuropathy. Since advanced glycation end products receptors are also found in the peripheral nervous system, previous studies have revealed that the interaction between AGEs and RAGE has various effects on the nervous system. In a study, it was shown that the AGE-RAGE interaction can induce caspase-3 activation, production of ROS, and nuclear DNA damage in dorsal root ganglion neurons. In other studies, it has been revealed that the loss of sensation in diabetic individuals and the increase in NF-kB and NF-kB-dependent pro-inflammatory gene expression levels in peripheral nerves are due to the interaction of AGE-RAGE. It is reported that all these pathological processes can affect every component in the peripheral nervous system cells (Demirer & Yardımcı, 2022).

Therapeutic approaches and prospects

The data obtained as a result of the current and ongoing observations obtained over the years have shown that the accumulation of AGEs in cells and tissues can cause various pathologies. In particular, studies confirm that AGEs are an important factor in the long-term complications of hyperglycemia. Therefore, it has been understood that the success of treatment options in diabetics can be achieved with early and intensive hyperglycemic control. One of the possible strategies is to try methods to reduce AGE formation, AGE receptor expression and oxidative stress, together with early and intense hyperglycemic control. One of the methods studied in this regard is the use of bioactive components such as polyphenols. These compounds; They are secondary metabolites found in the structure of plants, containing at least one carboxyl group and reported to have antiglycation activity. In various studies, it has been shown that thymoquinone, one of this group of compounds, has stronger antiglycation activity than aminoguanidine, which is an artificial antiglycation agent. It is also known that drugs that are widely used in the treatment of type 2 diabetes have an inhibitory effect on the formation of AGEs. In vitro studies with pioglitazone and metformin, which are among these drugs, have been shown to prevent AGE formation. Angiotensin converting enzyme (ACE) inhibitors and angiotensin II subtype 1 receptor (AT-1) blockers have been found to be able to reduce AGE formation while providing blood pressure control. Telmisartan, on the other hand, is a compound that has been shown to be able to gradually inhibit the formation of superoxide over the years, as well as reduce the levels of AGE receptor messenger RNA (mRNA). In addition, these drugs also have antioxidant effects. There is evidence that especially AT-1 blockers are directly effective against oxidative stress resulting from hyperglycemia and have a role in metabolic memory. Considering all these data together, it is clear that mixed therapies should be preferred, which includes methods that prevent AGE formation while treating hyperglycemia in treatment approaches. Preferring such combined methods in treatment; It is quite clear that it will make significant contributions to the prevention of many diabetes-related microvascular and peripheral - cerebrovascular diseases such as nephropathy,

retinopathy and neuropathy, and macrovascular complications related to diabetes such as ischemic heart disease and hypertension (Küçükkatırcı, Caferoğlu, & Hatipoğlu, 2022; Yalçın & Rakıcıoğlu, 2022).

REFERENCES

Arslan, S., Kırağı, D., Kadayıfçılar, S. & Samur, G. (2021). Diyabetik Makula Ödemi ile Diyet İleri Glikasyon Son Ürünleri (AGEs) ve Oksidatif Stres Arasındaki İlişkinin Değerlendirilmesi. *Sağlık Akademisi Kastamonu (SAK), 6* (3), 1-21. DOİ: 10.25279/sak.757689

Bijnen, M., van Greevenbroek, M.M.J., van der Kallen, C. J. H., Scheijen, J. L., van de Waarenburg, M. P. H., Stehouwer, C. D. A., Wouters, K. & Schalkwijk, C. G. (2019). Hepatic Fat Content and Liver Enzymes Are Associated with Circulating Free and Protein-Bound Advanced Glycation End Products, Which Are Associated with Low-Grade Inflammation: The CODAM Study. *Journal of Diabetes Research, 2019,* 1-10. Doi: 10.1155/2019/6289831

Celkan, T. & Dikme, G. (2018). Çocukta tromboz: Kime, hangi test, ne zaman, ne kadar gerekli?. *Turkish Archives of Pediatrics*, *53* (1).

Çetiner, Ö. & Rakıcıoğlu, N. (2020). Hiperglisemi, Oksidatif Stres ve Tip 2 Diyabette Oksidatif Stres Belirteçlerinin Tanımlanması. *Türkiye Diyabet ve Obezite Dergisi, 4* (1), 60-68. DOI: 10.25048/tudod.638744

Demirel, Y. & Yıldıran, H. (2018). İleri Glikasyon Son Ürünleri ve Böbrek Hastalıkları. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi, 7* (1), 210-217. Erişim linki: https://dergipark.org.tr/tr/pub/gumussagbil/issue/36260/399677

Demirer, B. & Yardımcı, H. (2022). İleri Glikasyon Son Ürünlerinin Diyabet Komplikasyonları Üzerine Etkileri: Bir Derleme. *Beslenme Ve Diyet Dergisi, 50* (1), 101-108. https://doi.org/10.33076/2022.BDD.1516

Döner, H. C. (2021). *Tip 1 diabetli çocuk ve adölesanlarda galektin 3, ileriglikasyon son ürünleri (AGE), ve ileri glikasyon son ürünleri reseptörü (RAGE) seviyelerinin araştırılması*. Konya: Necmettin Erbakan Üniversitesi, Meram Tıp Fakültesi. Erişim linki: https://hdl.handle.net/20.500.12452/8154

Ergenç, M., Özenoğlu, S., Turan, İ., Özaçmak, V. H. & Sayan Özaçmak, H. (2017). Diyabetik Sıçanlarda Melatonin Uygulamasının Karaciğer, Böbrek, Mide, Pankreas ve Göz Dokularında Oksidatif Stres Üzerine Etkisi. *Türk Diyabet ve Obezite Dergisi, 3*, 117-123. DOI: 10.25048/tjdo.2017.19

Ermiş, T., Turgan, N. & Ersöz, B. (2006). Trombomodulin. *Türk Klinik Biyokimya Dergisi, 4* (1), 39-48.

Karataş, T., Yılmaz, E. & Polat, Ü. (2022). Osteoartrit Yönetimi, Yaşam Kalitesi Ve Hemşirenin Destekleyici Rolü. *Süleyman Demirel Üniversitesi Tıp Fakültesi Dergisi, 29* (2), 265-271. Doi: 10.17343/sdutfd.1017250

Kerimoğlu, H. & Türk, H.B. (2018). Diyabetik Retinopati ve Diyabetik Maküla Ödeminde Patogenez. *Güncel Retina, 2* (2), 94-101.

Kılınç, K. (2011). Protein Glikasyonu. *Hacettepe Tıp Dergisi, 42* (2), 95-104.

Küçükkatırcı, H., Caferoğlu, Z. & Hatipoğlu, N. (2022). Diabetes Mellitusta Metabolik Hafızanın Rolü. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi, 11* (3), 1257-1264. DOI: 10.37989/gumussagbil.9752093

Ledoux, A. C. & Perkins, N. D. (2014). NF-κB and the cell cycle. *Biochemical Society Transactions, 42* (1), 76-81. Doi: 10.1042/bst20130156

Oshima, Y., Harashima, A., Munesue, S., Kimura, K., Leerach, N., Goto, H., Tanaka, M., Niimura, A., Hayashi, K., Yamamoto, H., Higashida, H. & Yamamoto, Y. (2022). Dual Nature of

RAGE in Host Reaction and Nurturing the Mother–Infant Bond. *International Journal of Molecular Sciences, 23* (4), 2086. Doi: https://doi.org/10.3390/ijms23042086

Rawlings, J. S. (2004). The JAK/STAT signaling pathway. *Journal of Cell Science, 117* (8), 1281-1283. Doi:10.1242/jcs.00963

Rungratanawanich, W., Qu, Y., Wang, X., Essa, M. M., & Song, B.-J. (2021). Advanced glycation end products (AGEs) and other adducts in aging-related diseases and alcohol-mediated tissue injury. *Experimental & Molecular Medicine, 53* (2), 168–188. Doi: 10.1038/s12276-021-00561-7

Selvakumar, G., Venu, D., Kuttalam, I., & Lonchin, S. (2022). Inhibition of Advanced Glycation End Product Formation in Rat Tail Tendons by Polydatin and p-Coumaric acid: an *In Vitro* Study. *Applied biochemistry and biotechnology*, *194* (1), 339-353. Doi: https://doi.org/10.1007/s12010-021-03762-y

Şensoy, E. & Öznurlu, Y. (2019). Hücre Adezyon Molekülleri. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 4* (1) , 57-68. Doi: https://dergipark.org.tr/en/pub/ataunivbd/issue/2906/40216

Şentürk, S., Efe Arslan, D. & Korkmaz, M. (2021). Osteoartritli Hastalarda Metabolik Sendrom Sıklığı Ve Etkileyen Faktörler. *İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Dergisi, 9* (1), 105-116. DOI: 10.33715/inonusaglik.812103

Terekeci, M. H., Şahan, B., & Top, C. (2008). Hücre adezyon molekülleri. *Nobel Medicine, 4* (1), 04-10.

Vıcıl, S. & Ulutaş, E. (2020). Metilglioksal ve İleri Glikasyon Son Ürünleri. *Bozok Veterinary Sciences, 1* (1), 74-79. https://vetdergi.bozok.edu.tr/vetdergi

Vlassara, H. & Palace, M. R. (2002). Diabetes and advanced glycation endproducts. *Journal of Internal Medicine, 251* (2), 87-101. Doi: 10.1046/j.1365-2796.2002.00932.x

Yalçın, E., & Rakıcıoğlu, N. (2022). Besinlerde Oluşan İleri Glikasyon Son Ürünlerine Polifenollerin Etkisi. *Beslenme Ve Diyet Dergisi*, *50* (2), 66-75. https://doi.org/10.33076/2022.BDD.1554

Yılmaz, B. & Karabudak, E. (2016). Besinlerdeki İleri Glikasyon Son Ürünleri ve Azaltma Yöntemleri. *Beslenme ve Diyetetik Dergisi, 44* (3), 280-288.

Zhou, Y., Li, J., Wang, C. & Pan, Z. (2022). Fumitremorgin C alleviates advanced glycation end products (AGE)-induced chondrocyte inflammation and collagen II and aggrecan degradation through sirtuin-1 (SIRT1)/nuclear factor (NF)-κB/mitogen-activated protein kinase (MAPK). *Bioengineered, 13* (2), 3867-3876. DOI: 10.1080/21655979.2021.2024387

Non-Arteritic Anterior Ischemic Optic Neuropathy Developing after COVID-19 mRNA Vaccine

Ramazan Birgül[1]

Introduction

The COVID-19 virus, which emerged in China in 2019, is affecting the entire world, and mankind has mobilized all its means for such a mortal virus. Vaccination, which is the most effective way, is among them. One of the most widely used vaccine types in the world is the BioNTech mRNA COVID-19 vaccine. However, the vaccine, which is widely used all over the world, is likely to have side effects.

Non-arteritic anterior ischemic optic neuropathy (NAAION) occurs with a sudden and dramatic decrease in vision. Its clinical manifestation takes the form of a swollen and edematous optic disc. There are various diseases, such as diabetes mellitus (DM), hypertension, anemia and hyperlipidemia, which are among the most common causes of NAAION. [1, 2]

Radiological, biochemical and microbiological examinations were carried out for the patient in this case. No significant evidence was detected for the development of NAAION for the aforementioned reasons. The patient specifically stated that the clinical manifestation of the patient developed in a very short time after receiving vaccine (BioNTech, Pfizer). Despite the frequent administration of the COVID-19 mRNA vaccine (BioNTech, Pfizer), no cases with optic neuropathy involvement were reported in the literature after receiving the COVID-19 mRNA vaccine (BioNTech, Pfizer). This case is the first to develop NAAION following the COVID-19 mRNA vaccine (BioNTech, Pfizer).

The Case

A 53-year-old female patient was admitted to our clinic with sudden vision loss in the left eye. The patient suddenly noticed a narrowing in the lower-left visual field three days after the second dose of the BioNTech mRNA COVID-19 vaccine. She also experienced fatigue and muscle and joint pain, which are common side effects after the vaccination. It was recorded that the patient had diabetes as a comorbidity and was taking metformin. The patient stated that her diabetes was under control. No smoking or alcohol intake was present.

The patient's vision was 1.0 with correction on the right and at hand movement level on the left. The anterior segment was normal bilaterally. Intraocular pressure was 14 mmHg on the right and 16 mmHg on the left. There was a relative afferent pupillary defect on the left. There was no pain with eye movements. After the patient's pupil was dilated, the right fundus was normal, and the optic disc was swollen and edematous in the left fundus. Optical coherence tomography (OCT), fundus fluorescein angiography (FFA), and cranial and orbital magnetic resonance (MR) were performed for the patient, who was hospitalized and received pulse steroid therapy at 1 g a day for three days. The steroid treatment was continued orally for 14 days and was terminated by

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The present study was conducted in the Department of Ophthalmology, Uşak Training and Research Hospital, Uşak, Turkey.

lowering the dose. The sedimentation, CRP and whole blood levels were checked before the steroids, and they were found to be within normal values. Biochemical and microbiological parameters, such as fibrinogen, homocysteine, the Epstein Barr Virus, c-ANCA, p-ANCA, TORCH panel and adenosine deaminase levels were checked to rule out the causes of NAAION. Neurology and internal medicine department consultations were requested from the patient. No systemic condition was detected in the patient's blood parameters, and MRIs were taken to explain this during all this time. The OCT, fundus images, FFA, visual field, MRI examination results and blood parameters of the patient are shown below (Figure 1a, 1b, 1c , 1d, 1e, 1f).

Figure 1a. Fundus images of the patient

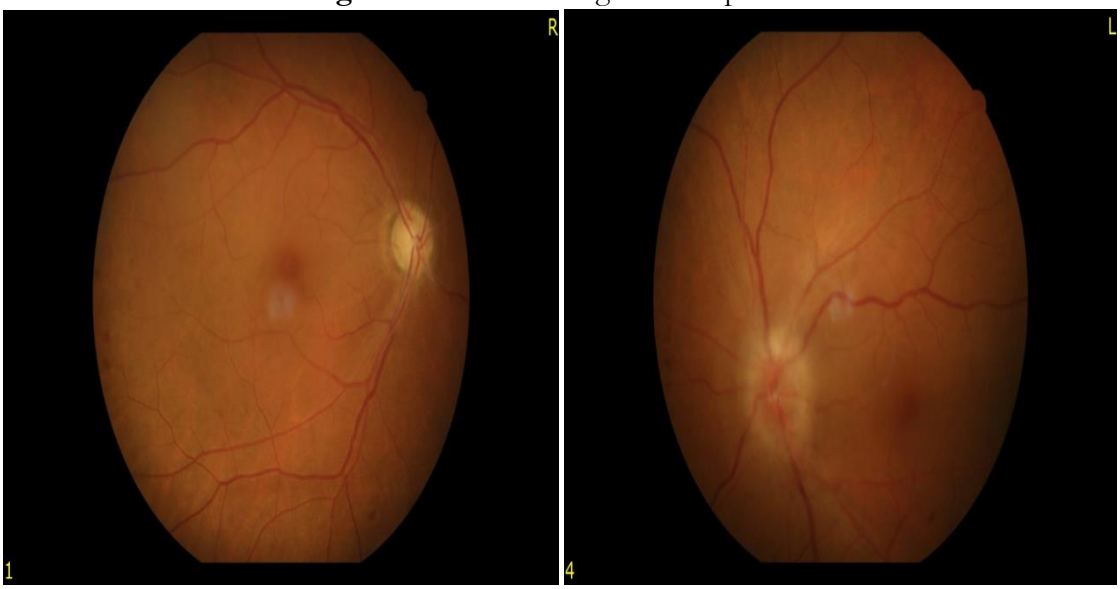

Figure 1b. FFA images of the patient

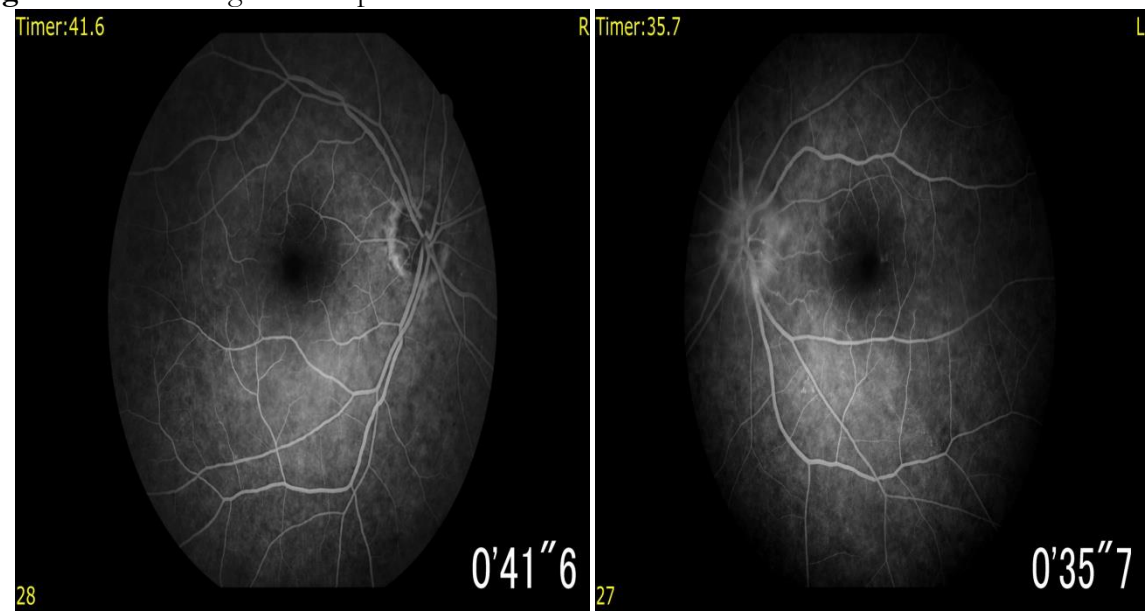

Figure 1c. Optical disc OCT of the patient

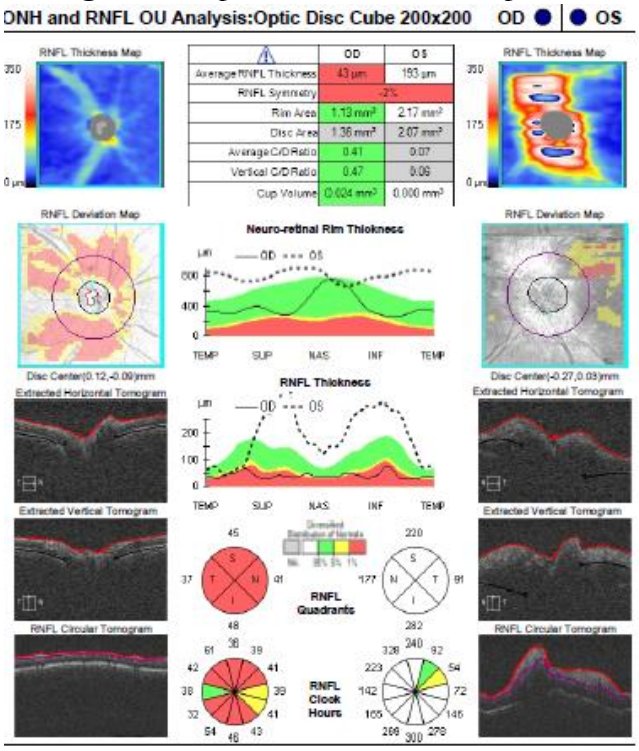

Figure 1d. Visual field of the patient

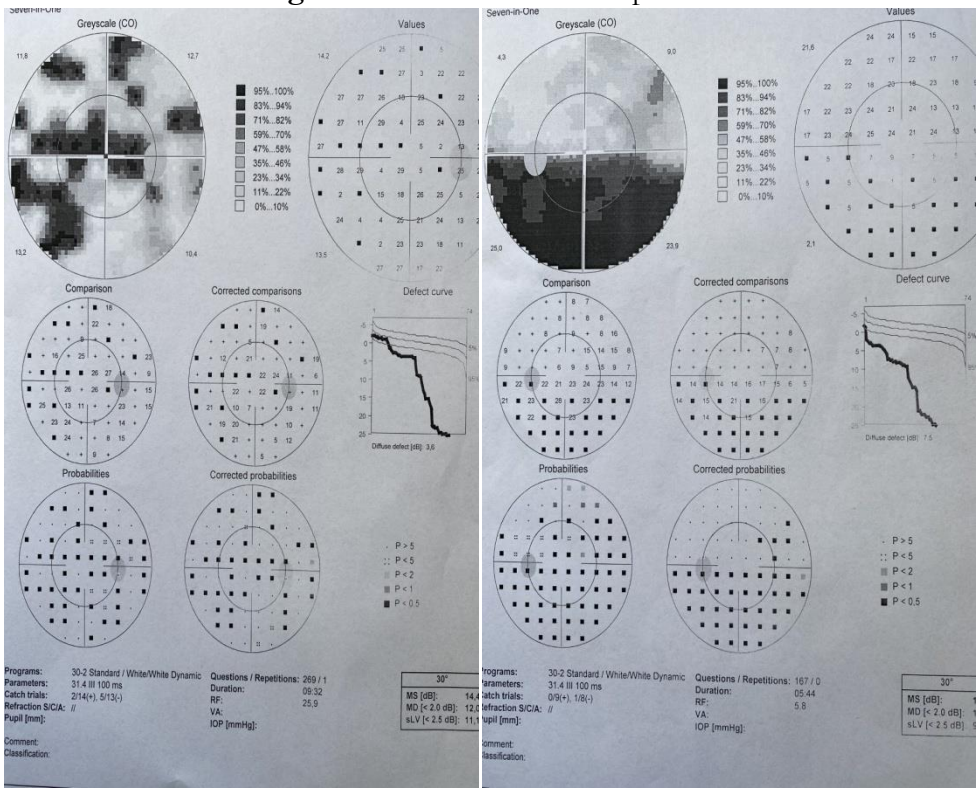

Figure 1e. Orbital MRI of the patient

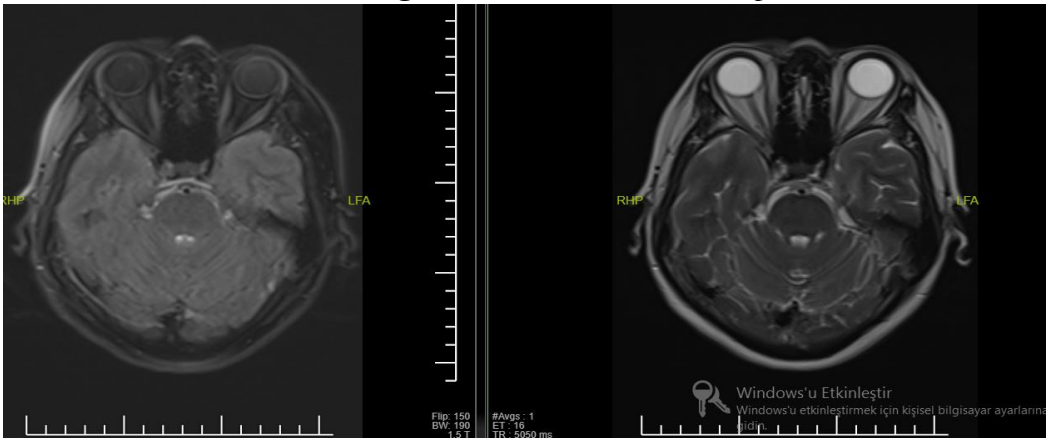

Figure 1f. Blood parameters of the patient

Tetkik Adı	Sonuç	Duru	Birim	Referans Aralığı
WBC	7,79		10^3/µL	4 - 10.5
NEU	4,63		10^3/µL	2 - 7
NEU%	59,4		%	40 - 70
LY%	34,1		%	20 - 50
LYM	2,65		10^3/µL	0.8 - 4
EOS	0,14		10^3/µL	0.02 - 0.5
EO%	1,8		%	0.5 - 5
BASO	0,03		10^3/µL	0 - 1
BASO%	0,3		%	0 - 1
MONO	0,34		10^3/µL	0.12 - 1.2
MON%	4,4		%	3 - 12
RBC	4,72		10^6/µL	3.5 - 5
HGB	12,6		g/dL	12 - 16
HCT	38,0		%	37 - 47
MCV	80,5		fL	80 - 100
MCH	**26,7**	**(D)**	**pg**	**27 - 34**
MCHC	33,2		g/dL	32 - 36
RDW-CV	13,1		%	11 - 16
PLT	156		10^3/uL	150 - 450
MPV	10,9		fL	6.5 - 12
PCT	0,169		%	0.108 - 0.282
PDW	16,5		%	10 - 65
Sedimantasyon	13		mm	0 - 20
GGT	**133**	**(Y)**	**IU/L**	**9 - 36**
Total Protein	71.3		g/L	63 - 86
Albumin	46		g/L	35 - 55
Bilirubin (direkt)	0.25		mg/dL	0 - 0.5
Bilirubin (indirekt)	**0,06**	**(D)**	**mg/dl**	**0.1 - 1**
Bilirubin (total)	0.31		mg/dL	0 - 1
Kalsiyum (Ca)	9.2		mg/dL	8.4 - 10.2
Potasyum	3.8		mmol/L	3.5 - 5.1
Sodyum	146		mmol/L	136 - 146
Klor(Cl)	106		mmol/L	96 - 110
CRP	1.7		mg/L	0.1 - 5
Ürik asit	5.0		mg/dL	2.3 - 8.2
Glukoz (açlık)	**148**	**(Y)**	**mg/dL**	**70 - 105**
BUN	12,0		mg/dl	10 - 20
Kreatinin	0.71		mg/dl	0.6 - 1.1
t GFH (CKD-EPI formülü ile)	>90		-	-
CK	51		U/L	29 - 200
CK-MB	**9.3**	**(D)**	**U/L**	**10 - 25**
AST	**43**	**(Y)**	**U/L**	**5 - 34**
ALT	**56**	**(Y)**	**U/L**	**0 - 55**
ALP	93		U/L	40 - 150
HDL kolesterol	48.8		mg/dL	35 - 60
Trigliserid	153		mg/dL	50 - 200
LDL kolesterol (Hesaplamalı)	112,6		mg/dL	60 - 130
Kolesterol	192		mg/dL	0 - 200

Serbest T3	3.08		ng/L	2.3 - 4.2
Serbest T4	1.31		ng/dL	0.85 - 1.6
TSH	**0.269**	**(D)**	**mIU/L**	**0.55 - 4.78**
Vitamin B12	356		ng/L	214 - 914
Anti HBs	0.37		mIU/mL	0 - 9.9
Anti HCV	0.40		mIU/mL	0 - 0.99
Anti HIV	0.05		mIU/mL	0 - 0.99
Anti CMV IgM	0.14		mIU/mL	0 - 0.99
Anti CMV IgG	**141.6**	**(Y)**	**AU/mL**	**0 - 5.9**
VDRL-RPR	Negatif (-)	-		-
EBV EBNA IgG	15.78		-	-
EBV VCA IgG	41.02		-	-
EBV VCA IgM	0.10		-	-
Anti Rubella IgM	0.27			0 - 1.2
Anti Rubella IgG	**37.4**	**(Y)**	**IU/mL**	**0 - 4.9**
Anti Toxoplasma IgM	0.21			0 - 0.49
Anti Toxoplasma IgG	**6.3**	**(Y)**	**IU/mL**	**0 - 1.6**
HLA B27	NEGATİF	-		-
p-ANCA (antimiyeloperoksidaz) (ELISA)	1.15 NEGATİF		U/mL	-
Adenozin deaminaz aktivitesi (ADA)	16.86		U/L	5.0 - 20.0
c-ANCA (antimiyeloperoksidaz) (ELISA)	0.33 NEGATİF		U/mL	-
Homosistein	9.31		µmol/L	4.65 - 12.22
Anti nükleer antikor (ANA)	NEGATİF		-	NEGATİF -

Discussion

There is no doubt that the vaccine plays one of the biggest roles in the effort to stop the COVID-19 pandemic, which has affected the entire world. Most vaccines are safe, but they usually have a wide spectrum of side effects, and it is equally difficult to prove that the side effects are related to the vaccine.[3] It is reported in the literature that vaccines generally cause side effects through the adjuvants they include, which can happen in two ways. They cause these side effects either by initiating an inflammatory event or by triggering an autoimmune mechanism.[4] We believe that an autoimmune mechanism played a role in this case.

It was reported that central serous chorioretinopathy developed in only one patient as an intraocular complication after receiving the COVID-19 mRNA vaccine (BioNTech, Pfizer).[5] Optic neuropathy development has not been reported in the literature. It was detected that optic neuropathy develops after vaccinations such as influenza, anthrax and rubella. This situation, which may be unilateral as reported in some publications, can sometimes be bilateral. Treatments were generally initiated with pulse steroids and continued with oral treatment for 14 days. Although visual acuity increased in some cases, it generally remained the same or deteriorated.[6,7] We applied the same treatment modality in this case, and the patient's vision remained at the same level and did not improve.

This case is very important in terms of the immediate development after the vaccination. Furthermore, the fact that NAAION is more common in men, and the fact that the age of consent was 60, further distinguishes this case from classical causes of NAAION.[8] DM, which could cause possible NAAION in the patient, was ruled out as a result of the long-term regulation of her blood sugar and internal medicine consultations. Neurological causes were also ruled out as a result of the absence of significant pathologies in cranial and orbital MRIs and neurology consultations. All of the radiological, biochemical and microbiological results indicated that the most likely cause of NAAION was vaccination.

In conclusion, COVID-19 vaccinations, which are administered all over the world, naturally increase the incidence of side effects. This case will contribute to the literature because it is the first case that developed NAAION following the COVID-19 mRNA vaccine (BioNTech, Pfizer).

References

1. Morrow, MJ. Ischemic optic neuropathy. *Continuum.* 2019; *25*(5):1215–1235. doi: https://doi.org/10.1212/CON.0000000000000767
2. Aptel F, Khayi H, Pépin JL, et al. Association of nonarteritic ischemic optic neuropathy with obstructive sleep apnea syndrome: Consequences for obstructive sleep apnea screening and treatment. *JAMA Ophthalmol* 2015; *133*:797–804. https://doi.org/10.1001/jamaophthalmol.2015.0893.
3. Baxter R, Lewis E, Fireman B, et al. Case-centered analysis of optic neuritis after vaccines. *Clin Infect Dis.* 2016; *63*(1):79–81. https://doi.org/10.1093/cid/ciw224
4. Li E, Fisayo A. Bilateral reversible optic neuropathy after influenza vaccination. *J Neuroophthalmol.* 2019; *39*(4):496–497. https://doi.org/10.1097/WNO.0000000000000776.
5. Fowler N, Martinez N, Pallares B, Maldonado R. Acute-onset central serous retinopathy after immunization with COVID-19 mRNA vaccine. *Am J Ophthalmol Case Rep.* 2021; *23*:101136. https://doi.org/10.1016/j.ajoc.2021.101136
6. Huynh W, Cordato D, Kehdi E, et al. Post-vaccination encephalomyelitis: Literature review and illustrative case. *J Clin Neurosci.* 2008; *15*(12):1315–22. https://doi.org/10.1016/j.jocn.2008.05.002
7. Papke D, McNussen P, Rasheed M, et al. A case of unilateral optic neuropathy following influenza vaccination. *Semin Ophthalmol.* 2017; *32*(4):517–523. https://doi.org/10.3109/08820538.2015.1120758
8. Arnold AC. The 14th Hoyt Lecture: Ischemic optic neuropathy: The evolving profile, 1966–2015. *J Neuroophthalmol.* 2016; *36*(2):208–15. https://doi.org/10.1097/WNO.0000000000000395

Current Approach to Molecular Mechanism of Antibiotic Resistance in Enterobacteriaceae

Seyda İĞNAK TARLIĞ

Enterobacteriaceae is a family of gram-negative rods commonly found in nature and intestinal flora of most animals, including humans. Enterobacteriaceae is responsible for clinically significant health-care and community acquired infections in human. β-lactam group drugs are used extensively in the treatment of Enterobacteriaceae-related infections. It is known that the overuse of these antibiotics promotes the problem of antibiotic resistance. Today, bacteria that are members of the Enterobacteriaceae can develop resistance to broad-spectrum beta-lactam antibiotics and carbapenems with inactivating enzymes called beta-lactamases. This does not only cause problems in the treatment of Enterobacteriacea-related infections, but also it increases mortality rates, duration of hospital stay, and health care costs all over the world (Murray, Rosenthal & Pfaller, 2021, De Angelis et al., 2020).

Resistance genes encoded in mobile genetic elements such as transposon, integron, and plasmid cause the spread of antibiotic resistance among different bacterial species. The World Health Organization (WHO) states that multi-drug-resistant Gram-negative bacteria (MDR-GNB), including carbapenem-resistant Enterobacteriaceae (CRE), have priority in new antibiotic research and development. The reason for this high priority is the necessity of infection control measures to reduce the spread of infection in the community and health care services, and the high mortality observed especially in serious infections in immunocompromised individuals (WHO, 2017). Centers for Disease Control and Prevention (CDC) emphasizes that carbapenem-resistant Enterobacteriaceae is a global threat due to the use of toxic and less effective methods in treatment due to their resistance to almost all antibiotics. (CDC, 2019).

Enterobacteriaceae

Members of the Enterobacteriaceae family are gram-negative rods of 0.3-1.0 x 1.0-6.0 μm in size, which do not form spores. Enterobacteriaceae, which have simple nutritional requirements, ferment glucose, reduce nitrate, are catalase positive and oxidase negative. It is commonly found in the intestinal flora of many animals, including humans, and in nature such as soil, water and vegetation, especially in humid environments.

Some Enterobacteriacea members (*Salmonella typhi*, *Shigella dysenteriae*, *Yersinia pestis*) are obligate pathogens and their detection in clinical samples is always associated with infection, while some (*Escherichia coli*, *Klebsiella pneumoniae* etc.) are opportunistic pathogens which cause serious infections, especially in immunocompromised individuals (Murray, Rosenthal & Pfaller, 2021).

Enterobacteriacea may cause community-acquired, hospital-acquired, and health care–associated infections. Enterobacteriaceae strains which are isolated from rectal swab, urine and respiratory tract samples may be associated with colonization rather than infection. The inability to distinguish between colonization and infection easily causes trouble in treating patients, especially in areas where multidrug resistance is common (Paterson & Mathers, 2020).

Mechanisms of Antibiotic Resistance

Antibiotic resistance in microorganisms can be classified into two as natural and acquired resistance.

1) Natural (Intrinsic) Resistance: It depends on the natural characteristics of microorganisms encoded on the chromosome. This type of resistance is independent of the selective pressure of antibiotics and horizontal gene transfer.

Natural resistance can develop with different characteristics encoded on the chromosome such as lack of a target of the drug, not taking the drug into the cell by the bacteria, removal of the drug from the cell by the efflux pump systems, and the presence of enzymes that inactivate the drug. Knowing the natural resistance profiles of microorganisms will prevent unnecessary antibiotic use and thus the development of acquired resistance. For example, *Klebsiella pneumoniae* is naturally resistant to ampicillin. This resistance is thought to be caused by the β-lactamase enzyme, which is a product of the SHV-1 gene encoded on the chromosome. As a result of the intensive use of ampicillin and antibiotics in the same spectrum as ampicillin in hospital settings, *K. pneumoniae* colonization is found in the intestinal flora of the patients. Prolongation of hospital stay and antibiotic use increase the importance of *K. penumonia* in nosocomial infections (Patterson et al., 2000, Paterson & Mathers, 2020, Chaves et al., 2001).

2) Acquired resistance: Acquired resistance can occur as a result of mutation in an existing gene or by the acquisition of new resistance genes through horizontal gene transfer mechanisms such as conjugation, transformation and transduction. Contrary to natural antimicrobial resistance, it first appears in some strains and subpopulations of the bacterial species. The intracellular concentration of the antibiotic can not reach the desired therapeutic concentration due to decreased uptake (down-regulation of porin protein expression, etc.) or increased multi-drug resistance pumps, changes in the target of the drug through mechanisms such as mutation or post translational modification, and enzymatic inactivation of the antibiotic (Paterson & Mathers, 2020, Opal & Pop-Vicas, 2020).

Molecular Mechanisms of Resistance in Enterobacteriaceae

Enzymatic Inhibition of Antimicrobial Activity

Bacteria may gain antimicrobial resistance by breaking down the antibiotic with various enzymes or by impairing their antibacterial activity. There are different types of enzymes with hydrolyzing (β-lactamase), inactivating (aminoglycoside-inactivating enzymes, chloramphenicol acetlytransferase, erythromycin esterase etc.) and modifying (amonoglycoside modifying enzyme) types of activities (Zhu et al., 2022, Opal & Pop-Vicas, 2020).

Enzymatic inactivation of β-lactam antibiotics

Since the discovery of benzylpenicillin in the 1920s, many penicillin derivatives and β-lactam group antibiotics such as cephalosporins, monobactams and carbapenems have been discovered. These bactericidal drugs inhibit bacterial cell wall biosynthesis by covalently binding to transpeptidases (Penicillin Binding Proteins-PBPs) (Bush & Bradford, 2016).

Resistance to β-lactam antibiotics is mainly caused by β-lactamase enzyme that cleaves the amide bond in the β-lactam ring of the antibiotic. This resistance mechanism probably developed by bacteria against natural antimicrobials and has become common due to increased use of antimicrobial drugs in modern medicine.

The genes encoding the β-lactamase enzyme can be encoded on the chromosome or on mobile genetic elements such as plasmid and transposon. It has also been shown that β-lactamase genes can be encoded on the integron. According to the Ambler classification (Ambler, 1980), β-

lactamases are classified based on their amino acid sequence into 4 molecular groups (A, B, C and D). Class B beta-lactamases called metallo-β-lactamases break the amide bond in the beta-lactam ring using zinc $(Zn)^{2+}$, whereas class A, C and D beta-lactamases hydrolyze the beta-lactam ring by using the serine residues in their active sites (De Angelis et al., 2020, Opal & Pop-Vicas, 2020).

Most of the extended-spectrum beta-lactamases are in Ambler group A and are inhibited by clavulonic acid and tazobactam (Zhu et al., 2022). Ampicillin resistance in Gram-negative bacteria increased in 1960s with TEM-1 (Temoneria β-lactamase) beta-lactamase encoded on the plasmid. SHV type β-lactamase associated with TEM-1 in terms of molecular structure has become common especially in *E. coli* and *K. pneumoniae* isolates. TEM-1 is the most common β-lactamase in Gram-negative bacteria, hydrolyzes penicillin and narrow-spectrum cephalosporins in the Enterobacteriacea. The configuration change of a few amino acids in the active site of the TEM-related ESBL enzyme increases the effect of the enzyme against 3rd generation cephalosporins (cefotaxime, cefpodoxime, ceftazidime, ceftriaxone) and monobactam (aztreonam). The first TEM-related ESBL (TEM-3) was reported in 1988. Upto date there are more than 200 TEM-related ESBL resistances identified. These enzymes are mostly observed in members of Enterobacteriaceae such as *E. coli* and *K. pneumoniae*. SHV-1 type β-lactamase is mainly found in *K. penumonia* strains. Point mutations in the active site of SHV-1 β-lactamase, which has 68% similarity to TEM-1 at amino acid level, has been caused the development of SHV type ESBL (Opal & Pop-Vicas, 2020). Some specific OXA β-lactamases (OXA-10, OXA-13, OXA-19) are included in the ESBL group because they hydrolyze extended spectrum cephalosporins (Zhu et al., 2022).

Cefotaxime-M (CTX-M) enzyme has spread rapidly around the world and has become among the most common ESBL in the world. In general, CTX-M hydrolyzes cefotaxime and ceftriaxone better than ceftazidime and is inhibited more by tazobactam than clavulanic acid (Opal & Pop-Vicas, 2020). The ST131 clone carrying the CTX-M-15 gene encoded on the plasmid plays an important role in multidrug resistant *E. coli*-associated infections in Europe and America (Johnson et al., 2010). CTX-M-14 is commonly detected in South East Asia whereas CTX-M-27 in Japan and Europe (Zhu et al., 2022).

AmpC β-lactamases confer resistance to penicillin, narrow-spectrum cephalosporins, oxymino-beta-lactams and cephamycins, but they are not sensitive to beta lactamase inhibitors such as clavulanic acid and tazobactam. While they do not show any effect against cefepime and aztreonam, changes in the active sites with point mutations may increase their effects on cefepime. Although AmpC β-lactamases are listed in the Ambler C group, there are also several members in the A group. They can be encoded on the chromosome or plasmid. Low level AmpC production is observed in *E. coli* strains while there is no AmpC coding gene on the chromosome of *Klebsiella* and *Salmonella* strains. But these bacteria may acquire AmpC genes which are encoded on the plasmids (Opal & Pop-Vicas, 2020, Zhu et al., 2022). Although their production is normally suppressed in Gram-negative rods, inducible Amp-C production may be observed after exposure to β-lactam antibiotics. Amp-C beta lactamase production decreases in a short time after the antibiotic exposure is terminated. However, irreversible overproduction of Amp-C may be seen due to point mutations (Opal & Pop-Vicas, 2020). AmpC enzymes encoded on the plasmid are named according to the drug that provide resistance (CMY, FOX, MOX, LAT), enzyme type (AAC, ACT) or the region they were discovered (MIR-1 or DHA) (Zhu et al., 2022).

Enzymatic inactivation of carbapenem antibiotics

Carbapenemases are β-lactamase enzymes that hydrolyze not only carbapenems but also broad-spectrum penicillins, oxymino-cephalosporins, and cephamycins. Enterobacteriaceae develop resistance by five types of carbapenemases such as Klebsiella pneumoniae carbapenemase (KPC), New-Delhi metallo-beta-lactamase (NDM), imipenem's metallo-beta-lactamase (IMP),

Verona integron encoded metallo-β-lactamase (VIM) and oxacillin carbpenemase (OXA) that are encoded on mobile genetic elements (Opal & Pop-Vicas, 2020).

Although KPC, an Ambler class A serine carbapenemase, was first identified in *K. pneumoniae* strains, it is now commonly detected in *E. coli*, *Citrobacter*, *Enterobacter*, *Salmonella* and *Serratia* strains worldwide (Logan & Weinstein, 2017).

Ambler Class B metallo-β-lactamases (MBLs), which are encoded on the chromosome and break the beta-lactam ring using Zn^{2+}, were mostly detected in environmental bacteria with low pathogenic potential. Most clinically important transmissible carbapenamases are in NDM, IMP and VIM. NDM-1 was first detected in *K. penumoniae* strains in India in 2008, it is now widely observed in many countries because of rapid gene transfer between species (Opal & Pop-Vicas, 2020, Logan & Weinstein, 2017).

OXA-48-like carbapenemases belong to Ambler Class D. Among these variants OXA-48, which is increasingly identified in Enterobacteriaceae, provides weak but significant cabapenemase activity (Poirel, Potron & Nordman, 2012).

Enzymatic inactivation of aminoglycoside antibiotics

Resistance to aminoglycoside antibiotics usually develop through aminoglycoside-modifying enzymes encoded on chromosomes or mobile genetic elements. These enzymes modify aminoglycoside antibiotics by phosphorylation of the OH group, adenylation of the OH group (addition of adenosine monophosphate-AMP), or acetylation of the NH2 group (Clark et al., 2019). According to their modifying properties and activities, they are divided into three groups: acetyltransferases (AAC), nucleotidyl transferases (ANT), and phosphotransferases (APH) (Ramirez & Tolmasky, 2010). There are many enzymes that target specific amino and hydroxyl groups for a activity. Of these, aminoglycoside acetyltransferase (AAC) acting in the 3' region is named AAC(3'), while there is more than one enzyme catalyzing the same reaction, it is named with Roman numerals (For example, AAC(3')-IV). The distribution of aminoglycoside resistance genes in the world is related to the selective pressure of the commonly used antibiotic. APH(3'), which causes decreased susceptibility to kanamycin and streptomycin, spreads in Gram-positive and negative bacteria worldwide.

Aminoglycoside nucleotidyltransferase (ANT)(2") has been associated with multiple nosocomial outbreaks in the United States in 1990. It was determined that *K. pneumonia* strains carrying ANT(2') gene encoded on plasmid are responsible for a significant portion of the outbreaks associated with aminoglycoside resistant Enterobacteriaceae. During epidemics, the plasmid ANT(2') gene was observed to be spread in other strains of the species and Enterobacteriaceae member bacteria

AAC(6') is common in enteric bacteria in East Asia. AAC(3') group enzymes were seen in many epidemics in South America, Western Europe and the United States. (Opal & Pop-Vicas, 2020).

The first bifunctional enzyme (AAC(6')-Ib-cr) that inactivates aminoglycosides and fluoroquinolone (ciprofloxacin) was discovered in 2006 (Robicsek et al., 2006).

Enzymatic inactivation of tetracycline antibiotics

Although tetracycline resistance occurs through mechanisms such as changes in the ribosomal target or efflux pump systems, it is expected that the tetracycline-degrading enzymes called TetX which are seen in environmental bacteria may also be transmissible to strains with clinical importance (Forsberg, 2015). In 2013, the plasmid-encoded tetX gene was shown in Enterobacteriaceae strain, which was the causative agent of urinary tract infection for the first time (Leski & et al., 2013).

Enzymatic inactivation of Macrolide-Lincosamide-Streptogramin antibiotics

Resistance to erythromycin and other antibiotics develops as a change in the target of the drug, removal of the drug from the cell by efflux pump systems, and inactivation of the drug by enzymes. Erythromycin esterase, one of the inactivating enzymes, hydrolyzes the lactone ring of erythromycin. Erythromycin esterase encoded on the plasmid causes high levels of erythromycin resistance. (Ounishi & Courvalin, 1985, Arthur, Autissier & Courvalin, 1986, Opal & Pop-Vicas, 2020). In addition, erythromycin esterase gene that causes high-level macrolide resistance and NDM-1 type carbapenemase were detected in a same *K. pneumoniae* strain (Yong et al., 2009).

Decreased permeability of bacterial membrane

Diffusion channels called porins in the cell membrane are used for hydrophilic antibiotic molecules to pass through the outer cell membrane. Numerous porins (10^5 porin molecules per one *E. coli*) are found on bacterial membranes. Bacteria change the porin number according to the osmolarity of the medium. In the hyperosmolar environment, *E. coli* decreases the expression of larger porins (OmpF) while maintaining the production of small porins (OmpC). The passage of antibiotics across the cell membrane is not only related to the number and structure of porin proteins, but also to the physiochemical structure of the antibiotic. It is difficult for negatively charged, hydrophobic and large antibiotic molecules to pass through porins. While the cell membrane is permeable to small, hydrophilic and zwiitterionic charged antibiotic molecules such as imipenem, it is not permeable to large and higly charged molecules such as carbenicillin (Opal & Pop-Vicas, 2020). The number of OmpF porin proteins is decreased in *E. coli* strains via chromosomal Mar mutation. It is known that decreased OmpF porin expression, as in the marA mutation, causes resistance to beta-lactams, tetracycline, chloramphenicol and hydrophilic fluoroquinolones (Cohen, McMurry & Levy, 1988). Mutations in genes encoding porin protein are known to cause increased resistance to β-lactam antibiotics. Loss of porin proteins (OmpK35, OmpK36) in *K. penumoniae* strains has been shown to increase carbapenem resistance in strains carrying ESBLs (CTX-M or SHV) or carrying AmpC (ACT-1, CMY-2, CMY-4 or DHA-1) enzymes which are encoded on plasmids (Crowley, Benedı´, & Dome´ nech-Sa´nchez, 2002, Lee et al. 2007).

In addition, it was determined that resistance developed against aminoglycoside and carbapenem group antibiotics due to the decrease in outer membrane proteins during treatment. Plasmid-encoded chloramphenicol resistance has been demonstrated in *E. coli* strains due to decreased membrane permeability (Gaffney, Cundliffe & Foster, 1981).

Aminoglycoside antibiotics are taken into the cell by anionic transporters. Positively charged antibiotics can pass through the cytoplasmic membrane due to negatively charged intracellular environment. Before this transition is achieved, a threshold value of negative charge must be present in the cell. In some cases where the proton motive force or energy production is mutated, resistance to aminoglycosides may develop. Aminoglycoside resistant mutants due to electron transport system defects have been observed in *E. coli*, *Salmonella spp.* (Opal & Pop-Vicas, 2020).

Antibiotic efflux pumps

The extrusion of the toxic material such as antibiotics out of the cell by the energy dependent pump systems causes antibiotic resistance by reducing the intracellular concentration of the drug. There are five major subfamilies of efflux pumps; 1) Resistance Nodulation Division (RND) family, 2) Major Facilitator Superfamily (MFS), 3) ATP binding cassette (ABC) superfamily, 4) Small Multidrug Resistance (SMR) family, 5) Multidrug and Toxic Compound Extrusion (MATE) (Thakur V, Uniyal A & Tiwari V, 2021).

The extrusion of the antibiotics out of the cell by the energy dependent pump systems causes resistance by reducing the intracellular concentration of the drug. It is known that AcrAB-TolC

and OqxAB-TolC efflux pumps, which are members of the RND family, cause resistance to fluoroquinolones, β-lactams, novobiocin, tetracycline, erythromycin, linezolid, quinolone, chloramphenicol, nitrofurantoin in members of Enterobactericeae (Thakur V, Uniyal A & Tiwari V, 2021).

It is known that EmrAB-TolC, a member of the MFS type efflux family, provides resistance to novobiocin and fluoroquinolones, while MacAB-TolC, a member of the ABC efflux family, causes macrolide resistance in Enterobacteriaceae (Nishino, Latifi & Groisman, 2006, Kobayashi, Nishino & Yamaguchi, 2001).

While QepA causes low-level fluoroquinolone resistance, it is thought that it may be responsible for the development of high resistance when combined with resistance mechanisms such as DNA gyrase mutations. (Yamane et al., 2007).

There are over 40 identified tetracycline resistance determinants that have been associated with drug efflux. Tet(A) and Tet(B) are the common tetracycline efflux pumps in Gram negative bacteria (Chopra & Roberts, 2001, Grossman, 2016).

Changes in antibiotic targets

The effect of the drug can be prevented by changes made in the target site to which the antibiotic binds. In Enterobacteriacea, resistance to aminoglycoside antibiotics can develop by modifying the spesific sites in 16S rRNA by methyl-transferases. It is known that some genes (armA, rmtA, rmtB, rmtC, rmtD, rmtE and npmA) encoding these methylase enzymes are carried on the plasmids. (Wachino & Arakawa, 2012).

Chloramphenicol inhibits the peptidyl transferase by binding to 23S rRNA which is in the large subunit of the ribosome. Resistance to chloramphenicol in Gram-negative bacteria is generally provided by the enzyme chloramphenicol acetyltransferases (CAT). This enzyme is encoded on the plasmid or chromosome and inactivates the antibiotic by O-acetylation of the 3-hydroxyl group (Clark, Pazdernik & McGehee, 2019).

DNA gyrase (Topoisomerase II) is involved in the super-folding process of chromosomal DNA during cell division. Another enzyme, topoisomerase IV, is involved in the segregation of the bacterial genome in two new cells after cell division. These enzymes consist of 2 subunits, A and B. The A unit is encoded by the gryA gene, while the B region is encoded by gyrB. In enteric bacteria, the gyrA mutation is the most common cause of fluoroquinolone resistance. Plasmid-encoded quinolone resistance has been demonstrated in various members of Enterobacteriacea. This type of resistance develops by the binding of qnr gene (QnrA, QnrB, QnrS) products to DNA gyrase. Plasmid-encoded Qnr genes associated with fluoroquinolone resistance low level fluoroquinolone resistance. However, because the same plasmids may encode the different genes required for the development of resistance to other antibiotics, the problem of multi-drug resistance arises in the clinic (Opal & Pop-Vicas, 2020, Nordman & Poirel, 2005).

Polymyxins, which are cationic peptide antibiotics, change the outer membrane permeability electrostatically by binding to the anionic lipopolysaccharide in the outer membrane in Gram-negative bacteria. Polymyxin B (colistin) is one of the limited number of alternatives in the treatment of multidrug resistant bacteria. Membrane modification by adding cationic groups (4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (pEtN) to the cell membrane reduces the affinity of the drug to its target. Many operons and gene regions responsible for cationic modification of the cell membrane are identified. The most important ones are the dual PhoPQ and PmrAB system. Chromosomal eptA or mcr genes are carried on plasmids cause polymyxin resistance by adding pEtN to lipid A with phosphoethonalamine transferase enzyme. It is known that mcr1-3 genes carried in plasmid spread rapidly in enteric bacteria (Opal & Pop-Vicas, 2020, Hugry et al., 2020).

REFERENCES

Ambler, R.P. (1980). The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*, 289(1036):321-331.

Arthur, M., Autissier, D. & Courvalin, P. (1986). Analysis of the nucleotide sequence of the ereB gene encoding the erythromycin esterase type II. *Nucleic Acids Res*, 14(12), 4987-4999.

Bush, K. & Bradford, P.A. (2016). β-Lactams and β-Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med*, 6(8):a025247.

Centres for Disease Control and Prevention (2019). Antibiotic Resistance Threats in the United States. Accessed (03/11/2022) from https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf.

Chaves, J., Ladona, M.G., Segura, C., Coira, A., Reig, R. & Ampurdanés C. (2001). SHV-1 beta-lactamase is mainly a chromosomally encoded species-specific enzyme in Klebsiella pneumoniae. *Antimicrob Agents Chemother*, 45(10), 2856-2861.

Chopra, I., Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev*, 65(2), 232-260

Clark, D. P., Pazdernik, N. J., & McGehee, M. R. (2019). Plasmids. In *Molecular Biology* (3th edition). (s. 712–748). Academic Press: Elsevier.

Cohen, S.P., McMurry, L.M. & Levy, S.B. (1988). marA locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of Escherichia coli. *J Bacteriol*, 170, 5416-5422.

Crowley,, B., Benedı´, V. J. & Dome´ nech-Sa´nchez, A. (2002).Expression of SHV-2 b-lactamase and of reduced amounts of OmpK36 porin in Klebsiella pneumoniae results in increased resistance to cephalosporins and carbapenems. *Antimicrob Agents Chemother*, 46, 3679–3682.

De Angelis, G., Del Giacomo, P., Posteraro, B., Sanguinetti, M. & Tumbarello, M. (2020). Molecular Mechanisms, Epidemiology, and Clinical Importance of β-Lactam Resistance in Enterobacteriaceae. *Int J Mol Sci*, 21(14):5090. doi: 10.3390/ijms21145090.

Forsberg, K.J., Patel, S., Wencewicz, T.A. & Dantas, G. (2015). The Tetracycline Destructases: A Novel Family of Tetracycline-Inactivating Enzymes. *Chem Biol*, 22(7), 888-97.

Gaffney, D.F., Cundliffe, E. & Foster, T.J. (1981). Chloramphenicol resistance that does not involve chloramphenicol acetyltransferase encoded by plasmids from gram-negative bacteria. *J Gen Microbiol*, 125(1), 113-121.

Grossman, T. H. (2016). Tetracycline Antibiotics and Resistance. *Cold Spring Harb Perspect Med*, 6(4), a025387.

Huang, J., Li, C., Song, J., Velkov, T., Wang, L., Zhu, Y. & Li, J. (2020). Regulating polymyxin resistance in Gram-negative bacteria: roles of two-component systems PhoPQ and PmrAB. *Future Microbiol*, 15(6), 445-459.

Johnson, J.R., Johnston, B., Clabots, C., Kuskowski, M.A. & Castanheira, M. (2010) Escherichia coli sequence type ST131 as the major cause of serious multidrug-resistant E. coli infections in the United States. *Clin Infect Dis*, 51(3):286-294.

Kobayashi, N., Nishino, K., Yamaguchi, A. (2001). Novel macrolide-specific ABC-type efflux transporter in Escherichia coli. *J Bacteriol*, 183(19), 5639-5644.

Lee, K., Yong, D., Choi, Y. S., Yum, J. H., Kim, J. M., Woodford, N., Livermore, D. M. & Chong, Y. (2007). Reduced imipenem susceptibility in Klebsiella pneumoniae clinical isolates with

plasmidmediated CMY-2 and DHA-1 b-lactamases co-mediated by porin loss. *Int J Antimicrob Agents*, 29, 201–206.

Leski, T. A, Bangura, U. B., Jimmy, D. H, Ansumana, R., Lizewski, S.E., Stenger, D. A., Taitt, C. R., Vora, G. J. (2013). Multidrug-resistant tet(X)-containing hospital isolates in Sierra Leone. *Int J Antimicrob Agents*, 42(1), 83-86.

Logan, L.K, Weinstein R.A. (2017) The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. *J Infect Dis*, 215(suppl_1), 28-S36.

Murray, P.R, Rosenthal, K.S, Pfaller, M.A. (2021). Enterobacteriaceae. In *Medical Microbiology*. (Ninth edition). (s.257-270). USA: Elsevier.

Nishino, K., Latifi, T, Groisman,E.A. (2006). Virulence and drug resistance roles of multidrug efflux systems of Salmonella enterica serovar Typhimurium. *Mol Microbiol*, 59, 126-41.

Nordmann, P. & Poirel, L. (2005). Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother*, 56, 463-469.

Opal, SM. & Pop-Vicas, A. (2020). Molecular Mechanisms of Antibiotic Resistance in Bacteria. Bennett, J.E, Dolin, R. & Blaser, M.J. (Eds) In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Blaser. (Ninth ed.) Philadelphia: Elsevier.

Ounissi, H. & Courvalin, P. (1985). Nucleotide sequence of the gene ereA encoding the erythromycin esterase in Escherichia coli. *Gene*, 35(3), 271-278.

Paterson, D.L. & Mathers, A.J. (2020). Infections Due to Other Members of the Enterobacteriaceae, Including Management of Multidrug-Resistant Strains. Goldman, L. & Schafer, A.I. (Eds.) In *Goldman-Cecil Medicine* (26th edition) (s1927-1930). USA: Elsevier.

Patterson, J.E., Hardin, T.C., Kelly, C.A., Garcia, R.C. & Jorgensen, J.H. (2000). Association of antibiotic utilization measures and control of multiple-drug resistance in Klebsiella pneumoniae. *Infect Control Hosp Epidemiol*, 21(7), 455-458.

Paulsen, I.T., Brown, M.H. & Skurray, R.A. (1996). Proton-dependent multidrug efflux systems. *Microbiol Rev*, 60, 575-608.

Poirel, L., Potron, A. Nordman, P. (2012). OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother*, 67(7), 1597-1606.

Ramirez, M. S. & Tolmasky, M. E. (2010). Aminoglycoside modifying enzymes. *Drug Resist Update*, 13, 151–171.

Robicsek, A., Strahilevitz, J., Jacoby, G.A., Macielag, M., Abbanat, D., Park, C.H., Bush, K. & Hooper, D.C. (2006). Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nature Medicine*,12, 83–88.

Thakur, V., Uniyal, A., Tiwari, V. (2021). A comprehensive review on pharmacology of efflux pumps and their inhibitors in antibiotic resistance. *Eur J Pharmacol*, 903, 174151.

Wachino, J. & Arakawa, Y. (2012). Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: An update. *Drug Resist Update*, 15, 133–148.

World Health Organization (2017). Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities. Accessed (21/09/2022) from https://www.who.int/publications/i/item/9789241550178

Yamane, K., Wachino, J., Suzuki, S., Kimura, K., Shibata, N., Kato, H., Shibayama, K., Konda, T, & Arakawa, Y. (2007). New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an Escherichia coli clinical isolate. *Antimicrob Agents Chemother*, 51, 3354-3360.

Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K. & Walsh, T.R. (2009) Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother*, 53(12), 5046-5054

Zhu, Y., Huang, W.E. & Yang Q. (2022). Clinical Perspective of Antimicrobial Resistance in Bacteria. *Infect Drug Resist*, 5:735-746.

New Treatments For Primary Teeth With Excessive Substance Loss

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Introduction:

In poor nations, dental caries is a health issue that affects 60–90% of those with low income levels (Petersen, P. E., & Lennon, M. A. 2004). The breakdown of the enamel-dentin tissues of teeth by the acids created by the fermentation of the carbohydrates ingested by bacteria is known as tooth decay (Margolis and Moreno 1994). Because the structure of the enamel in deciduous teeth differs from that of permanent teeth, caries in deciduous teeth advances more quickly than in permanent teeth (Goldberg, M 2017).

Treatment of caries provides very important benefits such as cleaning the decayed tissues on the tooth surfaces, eliminating the areas where new caries may occur, stopping demineralization, restoring the tooth structures in terms of function, stopping the spread of infection, and preventing tooth loss (Downer et al. 1999; Lenters, van Amerongen, and Mandari 2006). In the article published by Hickel et al. in 2006, it was stated that the probability of failure of restorative treatments in primary teeth is higher than that in permanent teeth. In addition, the falling times of the teeth for which restorations will be made should be considered. In the guide published by the American Academy of Pediatric Dentistry (AAPD) in 2019, two general recommendations were made regarding when dental caries should be restored.

First, the risk of progression and prognosis of the caries should be evaluated specifically for that patient, and the preventive treatments should be supported by restorative treatments. The second recommendation is that the caries lesions should be restored in cases where the enamel cavitation is clinically detected, the caries image reflects from under the enamel, and caries are diagnosed in the radiographic examination ("AAPD | Pediatric Restorative Dentistry" n.d. 2022).

Primary tooth decay that is left untreated spreads quickly and results in material loss. Complex procedures can be used to treat teeth that have caries on many surfaces. Primary teeth may have more failures in caries treatment than permanent teeth. These issues can be attributed to a variety of factors, including improper material use, difficulties with dental hygiene in youngsters, tooth morphological variations, and variations in the chemical composition of teeth.

There are studies and guides showing that covering the entire deciduous molar is more successful in teeth with excessive material loss, such as those with multiple caries, pulp amputation, or root canal treatment ("AAPD | Pediatric Restorative Dentistry," n.d., 2022; Sonmez and Duruturk 2010) .In order for the entire tooth to be covered with prefabricated crowns to give a successful result, it must have a good edge fit and be done in an appropriate way that will not allow plaque accumulation (Sharaf and Farsi 2004) . It was reported in a study conducted by Belduz Kara and YIlmaz in 2014 that SCC types that can be made can cause gingivitis, even though they provide suitable conditions for gingival health (" Beldüz Kara, N., & Yilmaz, Y. 2014).

The American Academy of Pediatric Dentists (AAPD) suggests stainless steel crowns (PSCC) for the repair of teeth with numerous surface cavities .("AAPD | Pediatric Restorative Dentistry," n.d.2022) Additionally, in teeth with significant substance loss, glass ionomer cements (CIS), amalgam, compomer, strip crowns, polycarbonate crowns, and zirconium

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advantages and weaknesses. The material a dentist chooses should be unique to each instance and should take into account cost, durability, conservatism, allergic reactions, and aesthetics.Currently, teeth with appealing appearances and socio-economic.It is regarded as a sign of development (Srinath 2017).

Until permanent teeth erupt, milk teeth perform the functions of phonation, aesthetics, and space holding.Therefore, it is crucial that the deciduous tooth remain in the mouth until it is time to fall out in order for the jaw and permanent dentition to develop properly .(Pinkham JR et al. 2005) Although the same methods are used to restore primary teeth as permanent teeth, morphological and histological distinctions can occasionally lead to divergent preferences.The need for applied aesthetic restorations may vary depending on the tooth's structural qualities (such as its pulp, enamel, and size), the child's cognitive and emotional state, and the material's cost (Srinath 2017; Pinkham JR et al. 2005). Crown restorations were first introduced in 1947 by the Rocky Mountain Company. In 1950, Engel introduced stainless steel crowns (SSC), but it was mostly through William Humphrey.

In 1971, a prominent opinion was ostensibly taken regarding the uses of stainless steel coin bail areas for deep bruises. If the vehicle of the 1970s is correct, it was put forward in the appearance of the ground protection of the SCCs. Webber introduced strip crowns in 1979. In 1983, Hartman developed the polished stainless steel crown for aesthetic anterior crowns. Buy Cheng crowns and Kinder crowns at the correct prices of the 1980s ("Available from: URL:Http://Www.Kinderkrowns.com/about-Us/History/ (Erişilme Tarihi: 30 Mart 2020)," n.d.). In the early 1990s, Randy replaced the band loop on the stainless steel crown by adding loops to the stainless steel crown. Again, the 1990s progressed. Norna Salon was made using a technical technique of dental restoration with a stainless steel crown without caries removal. Today, finding better systems and materials has brought zirconium to the forefront (Fellagh HF. 2016). Because of the high failure rate in restorations of teeth with excessive material loss, crown restoration is more preferred.

We can list the indications for crown restoration as follows;

- In teeth that have undergone endodontic treatment,
- In the treatment of advanced interface caries,
- In the treatment of broken teeth,
- In the presence of multifaceted caries,
- In children treated under general anesthesia,
- In patients with bruxism,
- In the treatment of defects in the cervical regions,
- In children with high caries risk and a lack of cooperation against rotary instruments,
- It is used in teeth undergoing pulpotomy-pulpectomy treatment. Desired properties in ideal primary tooth crown restorations;
- It must be aesthetic,
- chewing function should be preserved,
- The service life should be close to the life of the milk tooth,
- Protected teeth against breakage,
- The antagonist should not cause any wear on the tooth,
- It should be easy to apply,

- It should restore the lost function (Veerakumar R, Pavithra J, and Sekar K, 2017; Fellagh HF, 2016).

Today, the most commonly used crown restorations in the treatment of primary teeth are;

- Stainless steel crowns
- Polycarbonate crowns
- Strip crowns
- Pediatric jaket crowns
- Crowns created with the CAD/CAM system
- Zirconium crowns

Stainless steel crowns:

Restorative treatment may not always give the right response in primary teeth that have been severely damaged by caries. For this reason, in the early 1950s, Dr. Humphrey started to use stainless steel crowns for the first time as placeholders and crown restorations (Humphrey WP 1950). Thus, the frequency of indications for tooth extraction in children decreased, and the teeth began to stay in the mouth for a longer time. The stainless steel crown (SCC) contains 12–30% chromium, 8% nickel, and 1–20% carbon. It is reported that the lifespan of SCCs is longer than amalgams (Einwag and Dünninger 1996). Prefabricated SCCs have been used in the treatment of primary molars since 1950 (Engel R. J. 1950).

Over time, it has become more suitable for the anatomy of primary teeth and has been brought closer to the material structure that will facilitate applications (Ash, M. 1993). SCCs with traditional or veneered aesthetic surfaces are produced in many different sizes, can be corrected, stretched, and shaped with a bur, so they adapt well to crown margins, adhere well to tooth structures, and can be easily adapted to undercut areas. Due to these features, it stands out as a successful restoration type (Full, Walker, and Pinkham 1974; Duggal MS, Curzon MEJ, Fayle SA, Polard MA, and Robertson AJ 1995). It has been stated that SCCs are also produced in sizes suitable for first permanent molars so that they can be applied to permanent teeth. It is possible to shape and adapt these crowns to the teeth, and this is necessary for the success of the treatment. Restorations made with SCC have been considered a good alternative to traditional restorative treatments for many years, considering their durability and prognosis (N. P. T. Innes, Ricketts, and Evans 2007). It was stated by the British Association of Pediatric Dentistry in 2008 that restorations with SCC could be preferred (Kindelan et al. 2008). In a systematic analysis published by Innes et al. in 2015, evidence was revealed that SCCK is the most appropriate restoration method, the effect of which is compared with traditional methods (Nicola P. T. Innes et al. 2015).

The indications for the stainless steel crown are as follows;

- Teeth with a small amount of enamel where adhesive bonding is not possible,
- Caries formation is seen on more than one surface of the tooth,
- Patients with poor oral hygiene,
- Root canal treatment and amputation were applied to milk teeth,
- In the presence of dental anomalies,
- In fractures with excessive substance loss that can be seen in primary and permanent teeth,
- They can be applied in subgingival caries (Einwag and Dünninger 1996; Seale 2002; Tayfun Alaçam, Uzel İ, and Alaçam A 2000).

In a study, Atieh compared the clinical success and retention time of PSC applied to primary molars after pulp treatment and the modified open sandwich technique using resin modified glass ionomer cement and composite resin. In the study, which included 87 children aged between 4 and 7 years, 6, 12, 18, and 24 month controls were performed on 160 restorations. After two years of follow-up, the restoration rates were 95% for PSC and 92.5% for modified open sandwich restoration.

There was no significant difference between the two restorations in terms of marginal fit, proximal contact, occlusion, and recurrent caries criteria. When examined in terms of gingival health, better results were obtained in restorations made with the modified open sandwich technique compared to SCC (Atieh 2008). Webber observed some gingival enlargement in the follow-up of stainless steel crown restorations in a group of patients aged 8–12 years. However, it has been reported that gingival differences may be physiological in the mixed dentition and that the duration of use of the crown does not have a significant effect on gingival tissues (Webber 1974). Sharaf and Farsi, on the other hand, stated that the lack of interface contact by the SCC has no effect on gingival health. They reported that the most obvious factor for gingival health around stainless steel crowns is the level of oral hygiene (Sharaf and Farsi 2004).

Papathanasiou et al. evaluated the clinical success of different restorative materials. In this study, in which 604 restorations were examined in total, it was stated that the residence times of the applied restorations were SCC, amalgam, composite resin, and glass ionomer cement, respectively, from high to low. It has been reported that the mean residence time of SCC and amalgam restorations is 5 years. In this period, the percentage of PSCs remaining in the mouth is 68%, and amalgam restoration is 60%. After four years, the success rate of composite resin restorations is 40% and the average residence time in the mouth is 32 months; it has been reported that the success rate of glass ionomer cement restorations is 5% and the average residence time in the mouth is 12 months (Papathanasiou, Curzon, and Fairpo 1994).

There are studies that indicate that stainless steel crowns (which will be erased as their disadvantages) have a gingival compliance problem or (by looking at their condition inside the mouth) may cause periodontal problems. Tooth loss can also be seen in cases where the physician's clinical experience is insufficient or excessive cuts are made in the tooth. The aesthetic feature of stainless steel crowns is an important disadvantage not only for the anterior deciduous teeth, but also for the posterior deciduous teeth. In order to eliminate this negative feature, open-faced stainless steel crowns and veneered stainless steel crowns have been developed (Kindelan et al. 2008).

Although its durability, long life, and prevention of caries reoccurrence are the biggest advantages of SCC, studies have shown that the aesthetic expectations of patients and their parents cannot be met (Diana Ram, Fuks, and Eidelman, 2003; Townsend et al., 2014; Pani et al., 2016). Since these restorations did not meet the expectations in terms of aesthetics, various materials such as resin composite were tried to cover the buccal and occlusal surfaces, but these studies were not successful in clinical terms (Diana Ram, Fuks, and Eidelman 2003). In a study, it was stated that the aesthetics of dental treatments applied are one of the most important factors for parents (Zimmerman et al. 2009). For this purpose, single-faced crowns, strip crowns, and SCC veneer crowns have been developed (William F. Waggoner, 2002; 2015). Although these new materials contributed aesthetically, they caused some gingival problems (MacLean et al. 2007).

Open-face stainless steel crowns:

Although the aesthetic properties are insufficient and not accepted by parents and children, SCCs have continued to be used to protect the existing teeth in the mouth. With the advancement of technology, the aesthetics of stainless steel crowns have been improved. Open-faced stainless steel crowns are obtained by removing the front face of the stainless steel crowns we apply in the

clinic and placing resin on the removed part. It was first used in the early 1980s (Wiedenfeld, Draughn, and Welford, 1994). In the survey study conducted by Nagarathna et al., 70% of the participating physicians stated that they could make an open-faced stainless steel crown for the anterior region when faced with polyhedral caries. In addition, physicians stated that the ease of application is an advantage, and the child-parents are satisfied with this situation. 81.8% of the children showed a positive interaction during the treatment (Thimmegowda 2018). Disadvantages of open-faced stainless steel crowns; It is stated that the treatment period is long, fractures appear due to occlusal forces, and they are not aesthetically sufficient (Wiedenfeld, K. R., Draughn, R. A., & Goltra, S. E. 1995).

Veneered stainless steel crowns:

Due to the problems encountered with open-faced stainless steel crowns, veneered stainless steel crowns have been developed as an alternative. Veneered stainless steel crowns are a type of crown with an aesthetic appearance that can be cemented in the patient's mouth without any preparation. Initially, it was started to be used in the anterior region of deciduous teeth, and later it was applied to the posterior region of deciduous teeth as well. These crowns are applied in a single appointment and are reported to be less affected by the salivary environment. Since the aesthetic sections are made by the manufacturer, the time spent by the physician on the patient has also decreased (W. F. Waggoner and Cohen 1995).

Disadvantages of veneered stainless steel crowns;

- Contouring is performed only on the palatal or lingual surface, as there may be fractures on the facial surfaces during contouring,

- Color options are limited, so it is difficult to capture natural images,

- It is difficult to apply to children with limited space due to the volume's width.

- More teeth are cut due to passive seating in the tooth structure (W. F. Waggoner and Cohen, 1995; Roberts, Lee, and Wright, 2001) .

Fuks et al. examined the clinical success of veneered stainless steel crowns versus conventional stainless steel crowns over a 6-month period. At the end of the study, it was reported that the crown edge was compatible, the crowns were in the proper position/occlusion, proximal contact was provided, no bone resorption was observed, and no breakage or deformation was observed in the aesthetic part of the veneered stainless steel crowns. They stated that there was a significantly different gingival incompatibility in veneered stainless steel crowns compared to stainless steel crowns (Fuks, Ram, and Eidelman 1999).

Shah et al. examined 46 veneered SCCs they applied in the anterior region of 12 pediatric patients. They stated that the average duration of the crowned teeth in the mouth is 17.5 months (between 5 and 38 months). They reported that 24% of the crowns did not have resin fractures, and 61% of them did not have any fractures. When the gingiva of the crowned teeth were examined, they reported that 61% of the teeth had good gingival health, 24% had redness in the gingival area, and 15% had spontaneous bleeding (Shah, P. V., Lee, J. Y., & Wright, J. T. 2004).

Polycarbonate crowns:

Polycarbonate crowns, which have been widely used since the 1970s, are produced prefabricated as a result of the acrylic resin hardening with heat. As a result of the fabrication process, it is produced thinner than self-curing acrylic resin. Although aesthetically better than stainless steel crowns, they have lost popularity due to their fragility and ease of wear (Stewart, Luke, and Pike 1974). While applying the polycarbonate crown, a prefabricated polycarbonate crown suitable for the prepared tooth is selected and adhered to the tooth with acrylic resin after alignment. Restoration is completed by correcting the margins and polishing. It should be noted

that polycarbonate crowns may break and completely separate from the tooth. Due to this insufficiency, their use is very low (Stewart, Luke, and Pike 1974) . Although the indications for polycarbonate crowns are similar to those for stainless steel crowns, there are situations where they are contraindicated.

We can list them as follows:

- In cases with a deep bite,
- In the presence of progressive resorption in the root,
- In cases of excessive tooth wear or bruxism,
- In teeth with excessive substance loss,
- In cases of periodontal disease,
- In cases where a cross bite is seen,
- It is contraindicated in cases of deep bite (Venkataraghavan, Chan, and Karthik 2014; Nitkin, Rosenberg, and Yaari 1977).

Strip crowns :

Strip crowns have been frequently used in the restoration of primary teeth since the late 1970s. With the development of bonding agents and the increase in the bonding capacity of composites to enamel-dentin, strip crowns have been developed for the treatment of deciduous teeth in the anterior-posterior region in order to achieve tooth form, function, and aesthetics (William F. Waggoner 2002). Especially in anterior group milk teeth with excessive substance loss, strip crowns, which are much better aesthetically and have a wide usage area, are preferred instead of the stainless steel crowns that have been used for years. As an alternative to stainless steel crowns, strip crowns have been produced for posterior teeth, but they have not been used as widely as stainless steel crowns. Strip crowns are based on the selection of the appropriate mold in the form of plastic teeth for the tooth to be applied, the adaptation of the crown after the caries is removed, and the restoration of the entire tooth with a tooth-colored restoration material. After proper shaping, the resin material is cured with a light device. After that, the plastic tooth form is removed, and the remaining part of the tooth is corrected and polished (Citron 1995). The advantages of a strip crown are that it has good aesthetic properties, a shiny and smooth surface can be obtained, it is economical, a result that is very similar to the natural image can be obtained, and it can be applied easily and quickly (Kupietzky 2002). Tate et al., in a retrospective study investigating the restoration success of primary teeth treated with general anesthesia, examined the records of 504 patients and found PSCs to be significantly more successful than restorative methods such as amalgam, composite, and strip crowns. While the failure seen in SCCs was 8%, it was reported as 51% in strip crowns (Tate et al. 2002).

The main disadvantages are that the application technique is sensitive, blood and saliva contamination reduces the success of the restoration, sufficient dental tissue is needed for proper adhesion, and there are difficulties in its application because it requires cooperation in young children (Diana Ram, Fuks, and Eidelman 2003).

A sensitive technique is required during the application of strip crowns. In order to give the material a good shape and a suitable color, it is important to prevent its contamination with blood and saliva. In order for the bonding material and the composite to bond properly, the tooth tissue remaining after the preparation must be in sufficient quantity. At the same time, it can be difficult to apply strip crowns to very young children due to cooperation problems (Kupietzky 2002; D. Ram and Peretz 2000).

Pediatric jaket crowns:

It is an alternative type of crown used after severe caries or trauma. They are copolyester-containing, flexible, and naturally tooth-colored crowns. They can be shaped by cutting with scissors. Thanks to the fact that some of the preparations can be made outside the mouth, the reduction of intraoral working time provides a significant advantage during its use, especially in children with a lack of cooperation. They are placed on the prepared tooth by applying acid and a bonding agent. If isolation can be achieved, either with composite resin cement or, if not, with resin modified glass ionomer cement, the jaket crown is adapted to the tooth and cementation is performed. The disadvantages of the pediatric jaket crown include wear by occlusal forces and discoloration over time. The cause of failure is usually the separation of the crown from the cement. When the copolyester crown is separated, the composite or resin-modified glass ionomer cement applied as an adhesive remains on the tooth and appears as a crown without the need for restoration (Daniels, Sim, and Simon 1966).

Crowns created with CAD/CAM:

In the last 25 years, CAD-CAM has become increasingly popular in dentistry. This technology enables the production of restorations with sufficient durability and a natural appearance with an application process that is easier, faster, and reduces the risk of human error (Zaruba and Mehl 2017).

Many restorative materials have been used to occlude primary teeth with excessive material losses, but a material that meets all expectations in terms of both durability and aesthetics has not been found. Due to the disadvantages of existing crowns, the need to produce crowns that can be prepared and applied more practically in parallel with technological developments and that have a much better aesthetic appearance has emerged. Today, the use of crowns prepared based on computer-aided design and production has come to the fore. The basis of this system; collection of data in a computer environment, the creation of a three-dimensional model, the realization of designs in the light of these data, and production using the realized designs. Computer-assisted shaping/computer-assisted restorations are called Computer-Assisted Design and Computer-Assisted Manufacture (CAD/CAM). CAD/CAM technologies were introduced to the world of dentistry in the 1980s. Since then, these technologies have continued to progress. Today, CAD/CAM systems cover indication areas such as inlays, onlays, laminate veneers, partial crowns, full crowns, and bridge systems. Although CAD/CAM restorations have begun to be used in pediatric dentistry clinical practice, their use in the restoration of primary teeth is not common, except for research (Ayşe METE 2014).

Inlay, onlay, laminate veneer crowns, bridges, skeletal structures of removable partial dentures, abutment in implant-supported prostheses, and infrastructure design and production in crown-bridge and hybrid prostheses constitute the indication areas of CAD-CAM systems. Materials currently used in CAD-CAM systems; ceramic (alumina, zirconia, and porcelain based) blocks, metal, and composite resin. Titanium and noble metals are used as restoration infrastructure (Willer, Rossbach, and Weber 1998; Piwowarczyk et al. 2005). Advantages of CAD-CAM systems; shortening the treatment time by reducing the number of sessions, eliminating the need for temporary crown preparation, reducing the rate of error, allowing the use of high quality materials, editing different parameters (such as restoration thickness, cement spacing) by the user, and archiving patient and restoration data (Miyazaki et al., 2009; Christensen, 2001). The disadvantages of CAD-CAM systems are the high production costs, the need for equipment and experienced personnel, the sometimes poor aesthetics of monochromatic blocks, and the difficulty of transferring to the computer environment in teeth with deep subgingival margin extension (Christensen 2001; Duret and Preston 1991) . The fact that intraoral scanners in CAD-CAM systems have increasingly smaller designs allows their use in pediatric patients (Zaruba and Mehl

2017). CAD/CAM restorations are known to have become a common treatment for permanent teeth in children, but there are limited case reports of their use in primary teeth (Stines 2008).

CAD/CAM technology, restoration, and protective tooth preparation concepts should be considered together. Thus, unnecessary removal of the hard tissue of the tooth is avoided, and restorations that are much more compatible and better processed can be obtained in a single session and are specific to the tooth to be restored. (Tsitrou and van Noort 2008) In a study in which the crown prepared by the CAD/CAM method in primary molars was compared in vitro, if there is extensive coronal destruction in primary molars, full coronal restorations prepared with the Chairside Economical Restorations of Esthetic Ceramic 3 (CEREC) system using polymeric CAD/CAM resin blocks are preferred because they are both aesthetically pleasing, have low preparation costs, and can be prepared at the bedside and in a single session. It has been reported that it can be done (Ayşe METE 2014).

Pediatric Zirconium Crowns

Zirconium, symbol Zr and atomic weight 40, is a chemical element first discovered in Sri Lanka in the 18th century. The minerals of zirconium are zirconium silicate and zirconium oxide. Zirconium silicate is also called zircon, and zirconium oxide is also called zirconia. It has been reported that zirconium is not seen in nature in its pure form, but can be found in many different compounds. Zirconium is a highly resistant metal against temperature, wear, and corrosion. The purity of zirconium metal increases its mechanical properties. Since zirconium is a reactive metal, an oxide layer forms on its surface when it comes into contact with air or a solution. The oxide layer formed provides the zirconium with resistance to corrosion. It has been observed that the physical properties of zirconium are much superior to those of other ceramics. It is known that zirconium started to be used as a biomaterial in 1969 with the construction of hip prostheses by Hellmer and Driskell (Piconi and Maccauro 1999). Today, it is reported that stainless steel crowns are the most durable restorations that can be used in the treatment of deciduous molars and can protect the tooth structure the most. Despite the many positive features of SCC, researchers continue to develop new materials due to its poor aesthetics. Recently, zirconium crowns have been produced in order to eliminate the deficiencies in the restoration of milk teeth and to meet the aesthetic needs of children and parents. These materials can be used in the treatment of both milk incisors and milk molars. Prefabricated zirconium crowns were first introduced in 2008 under the brand name EZ-Pedo™ (Loomis, California, USA). The metal reflection from the gingiva, color mismatch, the presence of an artificial appearance in the stainless steel crown, and the absence of a personalized crown that can meet the aesthetic expectations of adults have led to the desire to produce a new material. With a patented system called Zir-Lock retention, grooves that provide mechanical retention were opened in the crown were opened and they aimed to increase clinical success. They produced narrowed crowns for first and second primary molars whose mesio-distal size was reduced due to caries. Later, pediatric zirconium crowns with various features were produced by companies such as BruxZir (Glidewell), Lava™ Premium (3M ESPE; Katana ML; Kuraray), and NuSmile (Huston, TX, USA) (Prashant Babaji 2015). Tetragonal zirconium oxide polycrystals (yttria-stabilized tetragonal zirconia polycrystal: Y-TZP), stabilized by yttrium oxide, pass from one crystal phase to another, forming a resistant structure that stops crack formation and prevents crack propagation. Due to the high fracture resistance of the zirconium material, zirconium crowns are quite strong (Denry and Holloway 2010). The ready-made form of zirconium crowns released for children has been developed to be much stronger and more durable than the enamel structure. The transparency of zirconium crowns, thanks to their "translucent" feature, allows for the solution of the color problem that appears in the teeth after very good aesthetics and pulpal treatment (Tote et al. 2015). Holsinger et al., in a study in which they examined the clinical success and parental satisfaction of pediatric zirconium crowns used in anterior teeth, evaluated them according to their retention, gingival effect, color, contour, edge harmony, and abrasion on the opposite tooth. In the controls made as a result of an average of 21 months of follow-up, it

was observed that no loss of retention was observed in any of the crowns, and 96% of them were present in the mouth. It was stated that no inflammation was observed in the gingival index (96%), no marginal alignment problem was observed in 86%, the color difference with the adjacent tooth (64%), was unnoticeable, secondary caries did not occur in the tooth with the zirconium crown, and the opposing tooth They reported no wear and tear. Parents also reported that the tooth with pediatric zirconium crowns had a natural tooth appearance (89%); it has been determined that children and parents are visually satisfied (Holsinger et al. 2016). In their case report, Cazaux et al. applied EZ-Pedo™/EZCrown™ pediatric zirconium crowns to the first primary molar after pulpotomy and followed up until the tooth exfoliated (29 months). They reported that 3 days after cementation, the gingival tissues regained their former health and appearance, there was no wear on the opposing tooth during the follow-up, and the tooth with a pediatric zirconium crown exfoliated at the same time (2 weeks apart) as the symmetrical tooth.

However, the researchers emphasized that it is often not easy to apply new materials in clinical practice, the application time of pediatric zirconium crowns is longer than that of stainless steel crowns in the first use, and the procedure becomes much faster and easier as clinical practice is gained over time. They reported that the application of the pediatric zirconium crown to the lower primary molars is easier than to the anterior teeth or upper primary molars. It is also emphasized that patient cooperation should be appropriate and that sedation may help crown application in uncooperative patients (Lopez Cazaux et al. 2017).

CONCLUSION:

Various treatment methods have been developed in cases of excessive substance loss in the teeth of pediatric patients. Factors such as the age of the child, aesthetic and functional needs, cost, ease of application, duration of treatment, and the number of teeth that require restoration constitute important criteria in determining the treatment methods to be applied. While aesthetics is in the foreground of a treatment method to be applied in the anterior region, function comes to the fore in the posterior region. There are not many randomized clinical studies on pediatric crowns.

According to the results of the existing studies, when stainless steel crowns and zirconium crowns are compared, it is known that patient satisfaction is higher with zirconium crowns, but due to the fact that the cost comes to the fore, stainless steel crowns and their more aesthetic modifications continue to be used today.

References :

"AAPD | Pediatric Restorative Dentistry." n.d. Accessed November 1, 2022. https://www.aapd.org/research/oral-health-policies--recommendations/pediatric-restorative-dentistry/.

Ash M. 1993. *Wheeler's Dental Anatomy, Physiology and Occlusion*. 7th ed. Philadelphia, Pa., U.S.A.: W.B. Saunders Co;

"Assessment of Oral Hygiene and Periodontal Health around Posterior Primary Molars after Their Restoration with Various Crown Types - PubMed." n.d. Accessed November 1, 2022. https://pubmed.ncbi.nlm.nih.gov/24164167/.

Atieh, Momen. 2008. "Stainless Steel Crown versus Modified Open-Sandwich Restorations for Primary Molars: A 2-Year Randomized Clinical Trial." *International Journal of Paediatric Dentistry* 18 (5): 325–32. https://doi.org/10.1111/j.1365-263X.2007.00900.x.

"Available from: URL:Http://Www.Kinderkrowns. Com/about-Us/History/ (Erişilme Tarihi: 30 Mart 2020)." n.d.

Ayşe METE. 2014. "Süt Azı Dişlerinde CAD/CAM Yöntemiyle Hazırlanan Kuronların in Vitro Şartlarda Karşılaştırılması." TEZ, Erzurum: Atatürk Üniversitesi.

"Chairside Veneering of Composite Resin to Anterior Stainless Steel Crowns: Another Look - PubMed." n.d. Accessed November 2, 2022. https://pubmed.ncbi.nlm.nih.gov/7593886/.

Citron, C. I. 1995. "Esthetics in Pediatric Dentistry." *The New York State Dental Journal* 61 (2): 30–33.

Daniels, L. M., J. M. Sim, and J. F. Simon. 1966. "Plastics in Pedodontics." *Dental Clinics of North America*, July, 365–75.

Denry, Isabelle, and Julie A. Holloway. 2010. "Ceramics for Dental Applications: A Review." *Materials* 3 (1): 351–68.

Downer, M. C., N. A. Azli, R. Bedi, D. R. Moles, and D. J. Setchell. 1999. "How Long Do Routine Dental Restorations Last? A Systematic Review." *British Dental Journal* 187 (8): 432–39. https://doi.org/10.1038/sj.bdj.4800298a1.

Duggal MS, Curzon MEJ, Fayle SA, Polard MA, Robertson AJ. 1995. "Restorative Techniques in Paediatric Dentistry." *Restorative Techniques in Paediatric Dentistry. London, U.K.: Martin Dunitz;* 1: 79–83.

Duret, F., and J. D. Preston. 1991. "CAD/CAM Imaging in Dentistry." *Current Opinion in Dentistry* 1 (2): 150–54.

Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. n.d. "Effective Use of Fluorides for the Prevention of Dental Caries in the 21st Century: The WHO Approach - PubMed." Accessed November 1, 2022. https://pubmed.ncbi.nlm.nih.gov/15341615/.

Einwag, J., and P. Dünninger. 1996. "Stainless Steel Crown versus Multisurface Amalgam Restorations: An 8-Year Longitudinal Clinical Study." *Quintessence International (Berlin, Germany: 1985)* 27 (5): 321–23.

Engel RJ. 1950. "Chrome Steel as Used in Children's Dentistry." *Chron Omaha Dist Dent Soc*, no. 13: 255–58.

Fellagh HF. 2016. "Evaluation of Full Coronal Esthetic Restorations in Primary Incisors: Clinical Success, Parental Satisfaction, In Vitro Fracture Resistance and Bacterial Adhesion." *Yeditepe University, Institute of Health Sciences, Department of Pediatric Dentistry. Istanbul,* 1–224.

Fuks, A. B., D. Ram, and E. Eidelman. 1999. "Clinical Performance of Esthetic Posterior Crowns in Primary Molars: A Pilot Study." *Pediatric Dentistry* 21 (7): 445–48.

Full, C. A., J. D. Walker, and J. R. Pinkham. 1974. "Stainless Steel Crowns for Deciduous Molars." *Journal of the American Dental Association (1939)* 89 (2): 360–64. https://doi.org/10.14219/jada.archive.1974.0409.

Goldberg M. 2017. "Deciduous Tooth and Dental Caries. Annals Pediatr Child Health" 5 (1): 1120–25.

Holsinger, Daniel M., Martha H. Wells, Mark Scarbecz, and Martin Donaldson. 2016. "Clinical Evaluation and Parental Satisfaction with Pediatric Zirconia Anterior Crowns." *Pediatric Dentistry* 38 (3): 192–97.

Humphrey WP. 1950. "Use of Chrome Steel in Children's Dentistry." *Dental Survey*, no. 26: 145–49.

Innes, N. P. T., D. N. J. Ricketts, and D. J. P. Evans. 2007. "Preformed Metal Crowns for Decayed Primary Molar Teeth." *The Cochrane Database of Systematic Reviews*, no. 1 (January): CD005512. https://doi.org/10.1002/14651858.CD005512.pub2.

Innes, Nicola P. T., David Ricketts, Lee Yee Chong, Alexander J. Keightley, Thomas Lamont, and Ruth M. Santamaria. 2015. "Preformed Crowns for Decayed Primary Molar Teeth." *The Cochrane Database of Systematic Reviews*, no. 12 (December): CD005512. https://doi.org/10.1002/14651858.CD005512.pub3.

Kindelan, S. A., P. Day, R. Nichol, N. Willmott, S. A. Fayle, and British Society of Paediatric Dentistry. 2008. "UK National Clinical Guidelines in Paediatric Dentistry: Stainless Steel Preformed Crowns for Primary Molars." *International Journal of Paediatric Dentistry* 18 Suppl 1 (November): 20–28. https://doi.org/10.1111/j.1365-263X.2008.00935.x.

Kupietzky, Ari. 2002. "Bonded Resin Composite Strip Crowns for Primary Incisors: Clinical Tips for a Successful Outcome." *Pediatric Dentistry* 24 (2): 145–48.

Lenters, M., W. E. van Amerongen, and G. J. Mandari. 2006. "Iatrogenic Damage to the Adjacent Surfaces of Primary Molars, in Three Different Ways of Cavity Preparation." *European Archives of Paediatric Dentistry: Official Journal of the European Academy of Paediatric Dentistry* 7 (1): 6–10. https://doi.org/10.1007/BF03320808.

Lopez Cazaux, Serena, Isabelle Hyon, Tony Prud'homme, and Sylvie Dajean Trutaud. 2017. "Twenty-Nine-Month Follow-up of a Paediatric Zirconia Dental Crown." *BMJ Case Reports* 2017 (June): bcr-2017-219891. https://doi.org/10.1136/bcr-2017-219891.

MacLean, Jeanette K., Cariann E. Champagne, William F. Waggoner, Marcia M. Ditmyer, and Paul Casamassimo. 2007. "Clinical Outcomes for Primary Anterior Teeth Treated with Preveneered Stainless Steel Crowns." *Pediatric Dentistry* 29 (5): 377–81.

Margolis, H. C., and E. C. Moreno. 1994. "Composition and Cariogenic Potential of Dental Plaque Fluid." *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists* 5 (1): 1–25. https://doi.org/10.1177/10454411940050010101.

Miyazaki, Takashi, Yasuhiro Hotta, Jun Kunii, Soichi Kuriyama, and Yukimichi Tamaki. 2009. "A Review of Dental CAD/CAM: Current Status and Future Perspectives from 20 Years of Experience." *Dental Materials Journal* 28 (1): 44–56. https://doi.org/10.4012/dmj.28.44.

Nitkin, D. A., H. M. Rosenberg, and A. M. Yaari. 1977. "An Improved Technique for the Retention of Polycarbonate Crowns." *ASDC Journal of Dentistry for Children* 44 (2): 108–10.

Pani, Sharat Chandra, Abdulrahman Al Saffan, Sultan AlHobail, Fares Bin Salem, AlBara AlFuraih, and Mohammad AlTamimi. 2016. "Esthetic Concerns and Acceptability of Treatment Modalities in Primary Teeth: A Comparison between Children and Their Parents." *International Journal of Dentistry* 2016: 3163904. https://doi.org/10.1155/2016/3163904.

Papathanasiou, A. G., M. E. Curzon, and C. G. Fairpo. 1994. "The Influence of Restorative Material on the Survival Rate of Restorations in Primary Molars." *Pediatric Dentistry* 16 (4): 282–88.

Piconi, C., and G. Maccauro. 1999. "Zirconia as a Ceramic Biomaterial." *Biomaterials* 20 (1): 1–25. https://doi.org/10.1016/s0142-9612(98)00010-6.

Pinkham JR, Casamassimo PS, Nowak AJ. St., Fields Jr HW, McTigue, Louis: Saunders., and Waggoner WF. 2005. *Restorative Dentistry for the Primary Dentition. In: Pediatric Dentistry: Infancy Through Adolescence*. 4th Edition.

Piwowarczyk, Andree, Peter Ottl, Hans-Christoph Lauer, and Timo Kuretzky. 2005. "A Clinical Report and Overview of Scientific Studies and Clinical Procedures Conducted on the 3M ESPE Lava All-Ceramic System." *Journal of Prosthodontics: Official Journal of the American College of Prosthodontists* 14 (1): 39–45. https://doi.org/10.1111/j.1532-849X.2005.00003.x.

Prashant Babaji. 2015. *Different Crowns Used in Pediatric Dentistry. In: Babaji P. Crowns in Pediatric Dentistry*. Vol. 1. New Delhi, India: Jaypee Brothers Medical Publishers;

Ram, D., and B. Peretz. 2000. "Composite Crown-Form Crowns for Severely Decayed Primary Molars: A Technique for Restoring Function and Esthetics." *The Journal of Clinical Pediatric Dentistry* 24 (4): 257–60. https://doi.org/10.17796/jcpd.24.4.u1784716nx571p82.

Ram, Diana, Anna B. Fuks, and Eliecer Eidelman. 2003. "Long-Term Clinical Performance of Esthetic Primary Molar Crowns." *Pediatric Dentistry* 25 (6): 582–84.

Roberts, C., J. Y. Lee, and J. T. Wright. 2001. "Clinical Evaluation of and Parental Satisfaction with Resin-Faced Stainless Steel Crowns." *Pediatric Dentistry* 23 (1): 28–31.

Seale, N. Sue. 2002. "The Use of Stainless Steel Crowns." *Pediatric Dentistry* 24 (5): 501–5.

Shah, P. V., Lee, J. Y., & Wright, J. T. (2004). Clinical success and parental satisfaction with anterior preveneered primary stainless steel crowns. Pediatric dentistry, 26(5), 391–395.

Sharaf, Aly A., and Najat M. Farsi. 2004. "A Clinical and Radiographic Evaluation of Stainless Steel Crowns for Primary Molars." *Journal of Dentistry* 32 (1): 27–33. https://doi.org/10.1016/s0300-5712(03)00136-2.

Sonmez, D., and L. Duruturk. 2010. "Success Rate of Calcium Hydroxide Pulpotomy in Primary Molars Restored with Amalgam and Stainless Steel Crowns." *British Dental Journal* 208 (9): E18; discussion 408-409. https://doi.org/10.1038/sj.bdj.2010.446.

Srinath, Srithi. 2017. "Different Crown Used For Restoring Anterior Primary Teeth: A Review." *J. Pharm. Sci.* 9: 4.

Stewart, R. E., L. S. Luke, and A. R. Pike. 1974. "Preformed Polycarbonate Crowns for the Restoration of Anterior Teeth." *Journal of the American Dental Association (1939)* 88 (1): 103–7. https://doi.org/10.14219/jada.archive.1974.0018.

Stines, Suzette Marie. 2008. "Pediatric CAD/CAM Applications for the General Practitioner. Part 1." *Dentistry Today* 27 (9): 130, 132–33.

Tate, Anupama Rao, Man Wai Ng, Howard L. Needleman, and George Acs. 2002. "Failure Rates of Restorative Procedures Following Dental Rehabilitation under General Anesthesia." *Pediatric Dentistry* 24 (1): 69–71.

TAYFUN ALAÇAM, Uzel İ, and Alaçam A. 2000. *Fazla Madde Kayıplı Süt Dişlerinde Tedavi Seçenekleri. In: Endodonti*.

Thimmegowda, Umapathy. 2018. "THE UTILITY OF OPEN-FACED ANTERIOR STAINLESS STEEL CROWN RESTORATION AMONG PEDIATRIC DENTISTS AS A LUCRATIVE ESTHETIC OPTION IN PRIMARY INCISORS," March.

Tote, J., A. Gadhane, Gautam Das, Smriti Soni, Kritika Jaiswal, and Gaurav Vidhale. 2015. "Posterior Esthetic Crowns in Peadiatric Dentistry." *Int J Dent Med Res* 1 (6): 197–201.

Townsend, Janice A., Patrick Knoell, Qingzhao Yu, Jian-Feng Zhang, Yapin Wang, Han Zhu, Sean Beattie, and Xiaoming Xu. 2014. "In Vitro Fracture Resistance of Three Commercially Available Zirconia Crowns for Primary Molars." *Pediatric Dentistry* 36 (5): 125–29.

Tsitrou, E. A., and R. van Noort. 2008. "Minimal Preparation Designs for Single Posterior Indirect Prostheses with the Use of the Cerec System." *International Journal of Computerized Dentistry* 11 (3–4): 227–40.

Veerakumar R, Pavithra J, and Sekar K. 2017. "Esthetic Crown in Paediatric Dentistry: A Review. IJIDS" 2 (2): 44–62.

Venkataraghavan, Karthik, John Chan, and Sandhya Karthik. 2014. "Polycarbonate Crowns for Primary Teeth Revisited: Restorative Options, Technique and Case Reports." *Journal of the Indian Society of Pedodontics and Preventive Dentistry* 32 (2): 156–59. https://doi.org/10.4103/0970-4388.130981.

Waggoner, W. F., and H. Cohen. 1995. "Failure Strength of Four Veneered Primary Stainless Steel Crowns." *Pediatric Dentistry* 17 (1): 36–40.

Waggoner, William F. 2002. "Restoring Primary Anterior Teeth." *Pediatric Dentistry* 24 (5): 511–16.

. 2015. "Restoring Primary Anterior Teeth: Updated for 2014." *Pediatric Dentistry* 37 (2): 163–70.

Webber, D. L. 1974. "Gingival Health Following Placement of Stainless Steel Crowns." *ASDC Journal of Dentistry for Children* 41 (3): 186–89.

Wiedenfeld, K. R., R. A. Draughn, and J. B. Welford. 1994. "An Esthetic Technique for Veneering Anterior Stainless Steel Crowns with Composite Resin." *ASDC Journal of Dentistry for Children* 61 (5–6): 321–26.

Wiedenfeld, K. R., Draughn, R. A., & Goltra, S. E. (1995). Chairside veneering of composite resin to anterior stainless steel crowns: another look. ASDC journal of dentistry for children, 62(4), 270–273.

Willer, J., A. Rossbach, and H. P. Weber. 1998. "Computer-Assisted Milling of Dental Restorations Using a New CAD/CAM Data Acquisition System." *The Journal of Prosthetic Dentistry* 80 (3): 346–53. https://doi.org/10.1016/s0022-3913(98)70136-2.

Zaruba, Markus, and Albert Mehl. 2017. "Chairside Systems: A Current Review." *International Journal of Computerized Dentistry* 20 (2): 123–49.

Zimmerman, J. A., R. J. Feigal, M. J. Till, and J. S. Hodges. 2009. "Parental Attitudes on Restorative Materials as Factors Influencing Current Use in Pediatric Dentistry." *Pediatric Dentistry* 31 (1): 63–70.

A stomachache experience

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1-Introduction

Mesenteric ischemia is a condition that causes necrosis in the intestines due to insufficient oxygenation of the small and large intestines as a result of deterioration in mesenteric vascular blood flow. The most common condition of cronic mesenteric ischemia (CMI) is atherosclerosis in the splannic arteries, especially in advanced age (Jaster, 2016). In addition to atherosclerosis, vasculitides, fibromuscular dysplasia, radiation, aneurysms, arteriovenous fistulas, aortic dissection or coarctation, and congenital anomalies of the splannic vessels may also cause CMI. In diagnosis, doppler ultrasonography is being used with increasing frequency in the curvature of ripples in the deep and superior mesenteric artery (SMA). Angiography still continues to be the most sensitive method (Mastoraki, 2016). Although severe stenosis or complete obstruction of at least two of the three main splanic arteries is usually present due to the development of collateral segments, it may not have arisen in the classroom. Acute mesenteric vein thrombosis and consequently bowel infarction is one of the most feared cardiac controls. This kind of ability may not be easy to develop. Because, like many patients, there may be limitations due to common collaterals.Surgical revascularization and percutaneous transluminal angioplasty can be performed in two of the 3 splanchnic vessels in the mesenteric angiography in a patient who does not have any other gastrointestinal system disease and usually has symptoms of abdominal angina(Foley,2016; Barret ,2015). A stent can be inserted during angioplasty. Bypass graft application to patients with S controls CMI is expressed as a safe treatment option(Flis,2016). The average early operative mortality rate in elective revascularization is around 5% (0-12%). 80% of patients survive at 3-year follow-up. In the 3 years after revascularization, 15% of patients have s-graft features. Endo vessels have shelters in preferred places (Moghadamyeghaneh,2015).

2-Case Report

1.st application

51 year old male patient; presents to the emergency department with the complaint of abdominal pain. Abdominal examination of the patient was unremarkable, mild leukocytosis (wbc: 12300) in the complete blood count and hematuria in the complete urinalysis, the urinary system was evaluated as normal in the abdominal usg. In the right lower quadrant of the abdomen, at the level of the terminal ileum and cecum, increased thickness of the intestinal wall, increased peristalsis, and fluid distension were observed, and abdominal CT is performed upon the recommendation of the radiologist. In the General Surgery consultation, it was learned that the

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abdominal pain and severity of the patient that started after the meal increased. The patient did not have a cardiac disease, has a one pack/day smoking history and a history of substance abuse. Due to widespread tenderness and rebound in the abdomen, the patient was admitted to the general surgery clinic with the diagnosis of acute abdomen, and preoperative preparations and resuscitation were started. Oral intake of the patient, whose ASA 2 anesthesia risk was determined, was stopped and fluid resuscitation, nasogastric decompression and antibiotherapy was started. The patient was discharged voluntarily without waiting for the results of the examinations. In the patient's entire abdominal contrast-enhanced CT examination; Fluid distention, dilatation and air fluid levels were observed in the ileal loops and were evaluated as compatible with ileus, while the increase in fringing-like density in the mesentery fat areas was recommended to be evaluated primarily in terms of inflammatory ileitis and terminal ileitis.

2.st application

Four days later, she came to the emergency room with the complaint of abdominal pain again. In general surgery consultation; There was pain and rebound in all quadrants of the abdomen. Contrast-enhanced tomography of the entire abdomen was requested, considering mesenteric vascular disease. Here, an exophytic localized irregular noncalcified plaque that causes more than 80% stenosis in the lumen of the superior mesenteric artery (approximately 2 cm segment at the exit of the SMA) was observed (Picture 1: stenosis in SMA). Thromboembolism causing total occlusion of the end branches of the SMA was observed in the pelvic region. All this Considering the imaging results and the patient's history, he was referred to another center for the treatment of stenosis in the SMA with interventional radiological methods for the treatment of abdominal pain due to intestinal hypoperfusion due to microemboli and narrowing of the SMA.

3.st application

A stent was placed in the SMA by endovascular method in an external center.

4.st application

The patient, who has applied to our clinic with the complaint of abdominal pain again 2 months later, states that this abdominal pain, which increases with meals, prevented him from eating anymore , he was getting weaker and he cannot stand these pains. Imaging examinations of the patient, who was hospitalized because of diffuse tenderness in the abdomen, showed a stent in the SMA and left common iliac artery (Picture 2: CT view of the stents). In the left lower quadrant, thickening of the intestinal wall, distension and edema, and minimal free fluid between the intestinal loops were observed. Oral intake of the patient was stopped, the patient is relieved by the continuation of anticoagulant treatment, fluid resuscitation and the use of analgesics.

5.st application

One month after discharge, the patient, who was admitted to another center with complaints of abdominal pain and inability to pass gas and stool, has followed up with the diagnosis of ileus. The abdomen distended and tenderness in the left lower quadrant and a palpable mass in the rectum were felt. Laboratory examinations were normal, imaging examination (whole abdomen with contrast CT) has cholelitiasis, cortical cyst in bilateral kidneys, collection area associated with the anterior wall of the middle part of the rectum, a stent was present in the proximal 4 cm segment of the SMA and its lumen was open, the lumen of the stent in the left common iliac artery. Occluded throughout, extending between the small intestines in the retrovesical space in the pelvis, continuing towards the pelvic wall on the left, the large 50*30 cm dimensions, which cannot be clearly distinguished from the neighboring intestines, were observed to be related to each other. The patient, who was followed up with antibiotherapy with the diagnosis of pararectal abscess, was discharged with anticoagulant treatment after his general condition improved and the size of the abscess decreased in the control.

6.st application

The diagnosis of acute abdomen was operated in an external center and small bowel perforation has been observed. Small bowel resection, ileostomy and mucous fistula have been performed.

7.st application

The patient was a cachectic appearance, insufficient oral intake, and fluid and electrolyte imbalance. He stated that he has lost 30 kg of weight in the last 6 months. Hyponatremia was treated.

3. Discussion

There are 3 arteries in the mesenteric system. The celiac artery is the superior mesenteric artery (SMA) and the inferior mesenteric artery (IMA). Celiac artery; proximal of stomach and duodenum, SMA; distal duodenum, jejunum, ileum, up to 2/3 of transverse colon, IMA; Starting from the 1/3 distal transverse colon, it supplies the rectum and proximal anal canal. There is rich collateral circulation between the branches of the mesenteric vessels. Therefore, clinical findings occur when 2 or 3 main arteries are occluded or have critical stenosis. Most commonly, the obstruction of the SMA, which is described as acute mesenteric ischemia, is encountered as a result of embolism or thrombosis developing on the basis of chronic atherosclerosis. In addition, when bowel ischemia is encountered without an occlusive lesion, non occlusive mesenteric ischemia is defined. Although it is thought that almost all ischemia originates from the arterial system, intestinal ischemia of venous origin can be seen. Acute mesenteric ischemia accounts for 1 out of 1000 hospitalized patients. It is a fatal disease if left untreated. Although early intervention provides a complete recovery, it is a difficult disease to diagnose (Tilsed,2016). It is a disease that should be kept in mind when the patient has recurrent episodes of abdominal pain that starts after meals and lasts for 1-3 hours, as in our case example of CMI, which has been admitted to the hospital 7 times and was followed up every time. Although the pain may be mild at first, it can become more severe in weeks and months. Postprandial vasodilation reduces peripheral resistance, but flow cannot increase in the presence of proximal fixed occlusive lesions, resulting in transient ischemic pain. This pain has been described as bowel angina. Weight loss may occur because patients are hesitant to eat. Nonspecific findings such as hypoalbuminemia, hypoproteinemia, and hypocholesterolemia caused by malnutrition may be encountered. Our case was cachectic at the 4th admission and did not want to take oral. In our patient, a stent was inserted during angioplasty with an endovascular treatment approach. There are publications stating that surgical or endovascular interventions have similar success rates (4, 7), but in some publications, bypass grafting is indicated as a safe treatment option in patients with symptomatic CMI(6).

4. Conclusion

Chronic mesenteric ischemia, which we do not encounter very often in our general surgery practice; Due to the difficulty of treatment, the patient progresses to death with intestinal ischemia at the end of the treatments and follow-ups for abdominal pain attacks and weight loss symptoms.

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Conflicts of interest

The authors declare that there are no potential conflicts of interest relevant to this article

Refarences

Flis V, Mrdža B, Štirn B, Milotič F, Kobilica N, Bergauer A. Revascularization of the superior mesenteric artery alone for treatment of chronic mesenteric ischemia. Wien Klin Wochenschr. 2016 ;128(3-4):109-13.

Moghadamyeghaneh Z, Carmichael JC, Mills SD,et al. Early Outcome of Treatment of Chronic Mesenteric Ischemia. Am Surg. 2015 ;81(11):1149-56.

Barret M, Martineau C, Rahmi G,et al. Chronic Mesenteric Ischemia: A Rare Cause of Chronic Abdominal Pain. Am J Med. 2015;128(12):1363-8

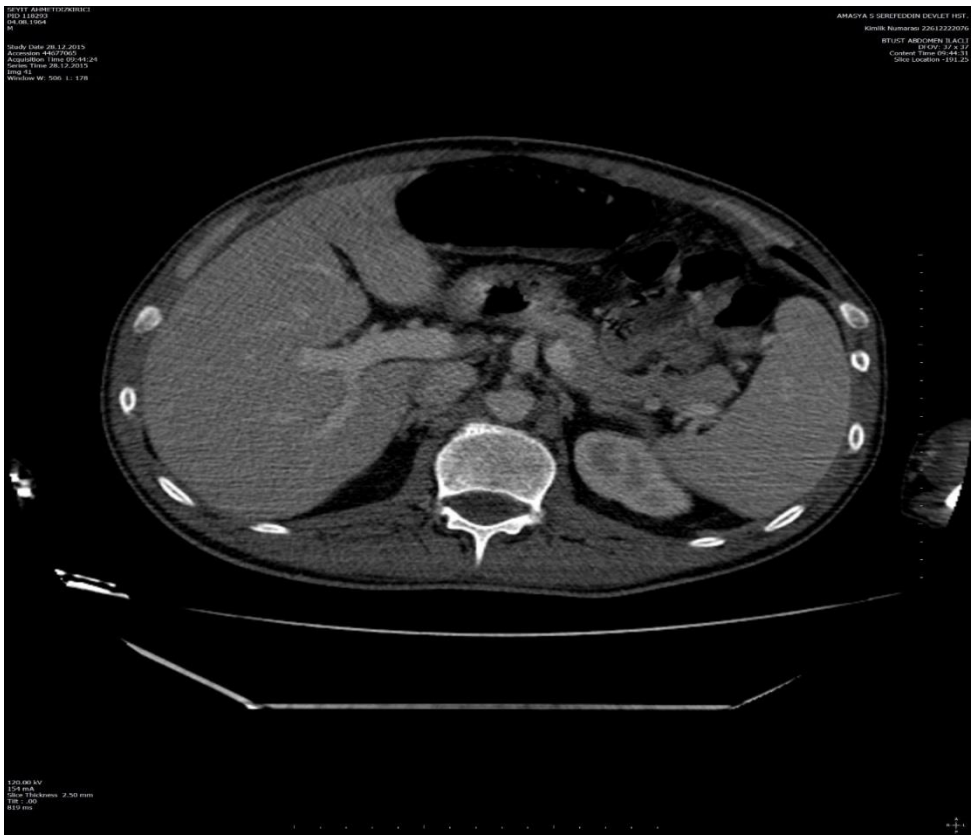
J. V. T. Tilsed · A. Casamassima · H. Kurihara. ESTES guidelines: acute mesenteric ischaemia. Eur J Trauma Emerg Surg (2016) 42: 253–270

Jaster A, Choudhery S, Ahn R, Sutphin P. et al. Anatomic and radiologic review of chronic mesenteric ischemia and its treatment. Clin Imaging. 2016 16;40(5):961-969.

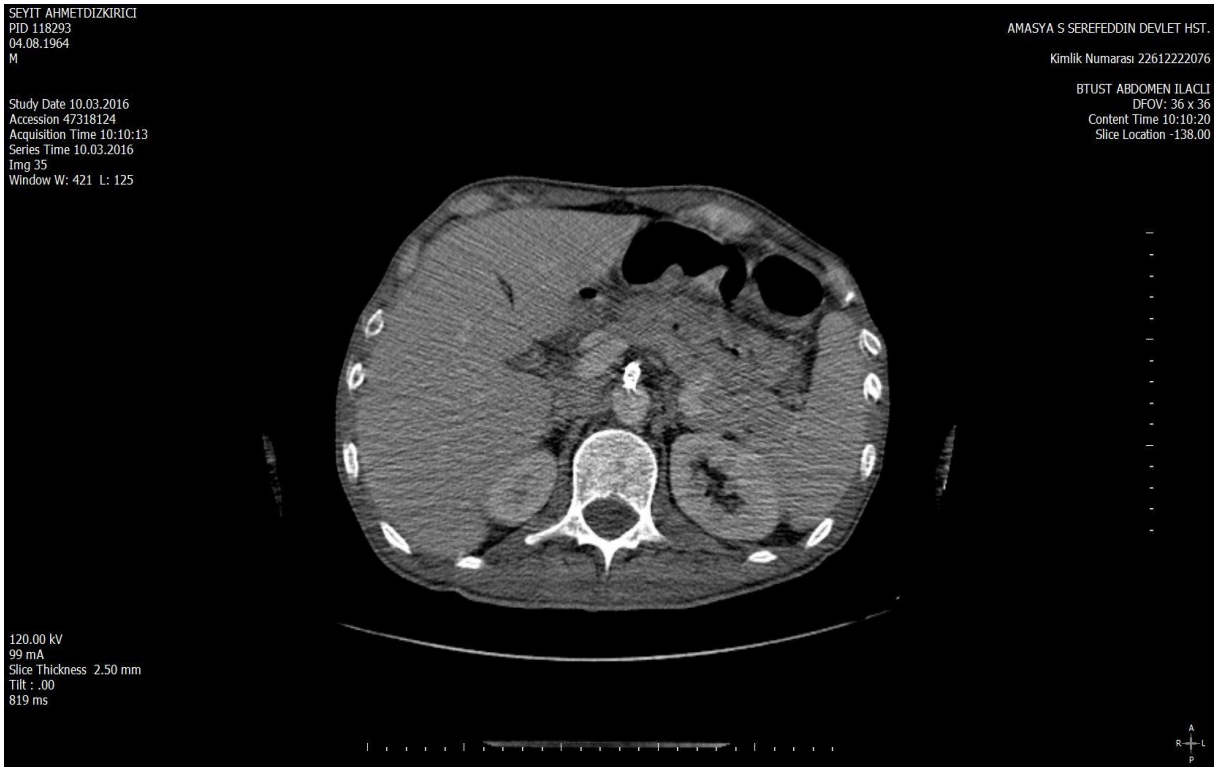
Mastoraki A, Mastoraki S, Tziava E, et al. Mesenteric ischemia: Pathogenesis and challenging diagnostic and therapeutic modalities. World J Gastrointest Pathophysiol. 2016 15;7(1):125-30.

Foley TR, Rogers RKEndovascular Therapy for Chronic Mesenteric Ischemia. Curr Treat Options Cardiovasc Med. 2016 ;18(6):39.

Zacharias N, Eghbalieh SD, Chang BB, et al. Chronic mesenteric ischemia outcome analysis and predictors of endovascular failure. J Vasc Surg. 2016 ;63(6):1582-7.



Picture1: stenosis in SMA



Picture 2: CT view of the stents

State-Trait Anxiety Levels Of Teachers During The Covid-19 Pandemic İn Kirşehir, Turkey

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Introduction

Anxiety is a feeling of unease, which can be experienced by the individual in the face of events that are assumed to be dangerous or threatening without any apparent reason. Anxiety is an unpredictable, unpleasant, and overwhelming feeling, which is associated with sad feelings about the future or anticipation of something bad about to happen. It is a feeling of nervousness and stress because of generalized thoughts around bad things that could happen with no identifiable cause (Şahin, 2019). Anxiety disorders are described in DSM-V and categorized as different types such as generalized anxiety, panic, and social anxiety disorder, agoraphobia, specific phobia, and separation anxiety disorder (DSM-V, 2013). Everybody can feel anxious time to time. Anxiety is examined in two categories, which are the "state anxiety" and the "trait anxiety". Trait anxiety is a generalized and persistent state of anxiety that is not tied to a specific event or situation. In contrast, state anxiety occurs before or during the so-called jeopardous situations. This is the anxiety experienced by almost every individual, which others can understand, and which can be attributed to logical reasons (Yıldız, 2010).

Infectious disease outbreaks induce anxiety and worry, causing individuals to feel ashamed, unsuccessful, and weak (Sim, 2010). Furthermore, psychological reactions occurring during outbreaks vary in a spectrum extending from extreme forms of anxiety and fear to feelings of indifference. The long-term psychological effects of outbreaks include the weakening of social and interpersonal ties. The psychological consequences of such periods are not always short-lived. Experiences in such periods can cause severe and persistent mental disorders as well (Huang & et al., 2020, Zhu & et al., 2020). Anxiety, mood and post-traumatic stress disorders are the most common mental distresses in epidemics (Tükel, 2020). Indeed, health anxiety experienced during outbreaks is an anticipated situation. People may develop a state of mental exhaustion due to uncontrollable health anxiety, which consequently can induce a vicious cycle. Another form of health anxiety during outbreaks is the anxiety of transmitting the disease to someone (Pierce, 2020). COVID-19 outbreak is the most recent one that emerged in China in 2019 and then spread around the world turning into a pandemic. When pneumonia cases of unknown origin increased in Wuhan (China), China reported this situation to the World Health Organization (WHO) on 31 December 2019. Chinese scientists named this coronavirus "2019-nCoV" on January 7, 2020. Spread around the world in a short time, the coronavirus started to have been identified in many people in Turkey as of March 10, 2020. From January 3, 2020 to January 10, 2022, there were 9,977,561 confirmed cases of COVID-19 in Turkey, with 83,702 deaths reported to WHO (WHO, COVID-19 Dashboard, 2022). The COVID-19 pandemic caught people unprepared and defenseless, affecting them globally and reaching alarming levels all over the world. The lives of many people were lost or disabilities occurred, leading to the development of psychological problems (Pektekin, 2020).

In the study of Nelson et al. (2020) in March 2020, it was revealed that 67.3% of 9009 participants were very much or extremely worried about COVID-19 (Nelson & et al., 2020). In the study of Lai et al. (2020) in China, 44.6% of healthcare professionals were found to experience anxiety (Lai & et al., 2020). Anxiety also arises from loneliness and limited social contacts during quarantine and isolation periods of outbreaks. People start experiencing anxiety because they cannot run their business and fail to meet the needs of their dependents (Brooks & et al., 2020, Huremović, 2019). Emotional responses to COVID-19 are experienced by all working individuals from all social levels, primarily healthcare professionals and people that are directly affected by the untoward consequences of the pandemic (Sim, 2010, Huang & et al., 2020, Zhu & et al., 2020, Stuijfzand & et al., 2020, Pappas & et al., 2009)

During the COVID-19 pandemic, schools have spent their best efforts to carry out educational activities online. All educational activities were interrupted, affecting students, teachers, and parents remarkably. Literature review revealed that many studies reported that the COVID-19 pandemic induced many feelings affecting the mental status of students unfavorably, such as learning and exam anxiety, worry, fear, extreme levels of stress, irritability, aggression, insomnia (Kara, 2020, Sintema, 2020, Cao & et al., 2020, Daniel, 2020). However, the number of studies investigating the problems experienced by teachers during the COVID-19 pandemic is not adequate despite their key roles in education. Studies available in the literature are mostly about the views of teachers on education (Ozamiz-Etxebarria & et al., 2021, Wakui & et al., 2021, Wong & et al., 2021, Çakın & Akyavuz, 2020, Çiçek, Tanhan & Tanrıverdi, 2020, Li, 2020, Stachteas & Stachteas, 2020). It has been determined that more international studies are needed to determine the effect of the epidemic on teachers' mental states (Ozamiz-Etxebarria & et al., 2021). The teacher with a high level of anxiety cannot fully devote herself to her task. Excessive anxiety causes the individual to become incompetent and result in disagreeable and irritable behavior in the workplace. On the contrary, lack of anxiety causes the individual to behave inconsiderately. Therefore, the anxiety level of the teacher in the workplace should not be at extremes (Başar, 1999).

Cooperation across professional associations, including those of psychiatrists, nurses, psychologists, and social workers, plays a crucial role in the resolution of psychosocial problems occurring during the pandemic. In this context, the Online Mental Health Program for Coronavirus Infection (KORDEP) was implemented to provide psychosocial support to people affected by the COVID-19 pandemic in Turkey. This program is considered an important step in coping with difficulties occurring while we live the pandemic (Pektekin, 2020). With awareness about the anxiety levels of teachers, such psychological support programs may provide more effective guidance.

Based on these considerations, we designed this study to examine the anxiety levels of teachers during the COVID-19 pandemic.

Patients and Methods

The research was conducted on 1704 volunteering teachers working in primary, secondary, and high schools, and in institutions providing pre-school education in Kırşehir (located in central Anatolia) province in the 2020-2021 academic year. Teachers were reached by the snowball sampling method. With this method, teachers participating in the research were asked to distribute data collection tools to their colleagues.

Research data were collected using an online demographics form, the State Anxiety Inventory (SAI) (STAI FORM TX-I), and the Trait Anxiety Inventory (TAI) (STAI FORM TX-II). The design and adaptation of the inventories into Turkish was performed by Öner and Le Compte (Öner & Compte, 1983). Both inventories comprise 20 items to be scored using a 4-point Likert

scale. Each item can be rated from 1 to 4. Response options in SAI were as follows: (1) not at all, (2) somewhat, (3) moderately so, (4) very much so. Response options in TAI were as follows: (1) almost never, (2) sometimes, (3) often, (4) almost always. The inventories included direct and reversed items or items phrased in the semantically opposite direction. The reversed ones were the items 1, 2, 5, 8, 10, 11, 15, 16, 19, and 20 in SAI. In TAI, the reversed ones were the items 21, 26, 27, 30, 33, 36, and 39. The minimum and maximum scores on both inventories could be 20 and 80, respectively. A high score indicates high anxiety, a small score indicates low anxiety. In this study, Cronbach's alpha values of the inventories were calculated as 0.951 for SAI and 0.891 for TAI. It has been reported that a scale with an alpha coefficient of 0.80-1.0 is highly reliable (Alpar, 2011). According to the calculated alpha coefficients for this study, our results are reliable.

Data collection forms were delivered to teachers online. Before teachers started filling out the online data collection forms, the aim of the research was explained to them in accordance with the Declaration of Helsinki and consent was obtained from participants. It took about 10-15 minutes for teachers to fill out the forms.

The data of the study were evaluated in computer environment. Kolmogorov-Smirnov, Shapiro-Wilk, Levene test, percentage, mean, standard deviation, independent t test, ANOVA, Duncan multiple comparison test and Cronbach's alpha coefficient were used in the analyzes.

Results

Of the teachers, 47.1% were women and 52.9% were men. Descriptive statistics of demographics and comparative answers to inventory items are presented in Table 1. As seen in Table 1, the effects of the gender variable on both SAI and TAI scores were statistically significant ($p<.001$). Women's state and trait anxiety scores were higher than those of men. The number of children of a participant affected the state anxiety level ($p<.05$). The state anxiety levels of those with only one child were higher than those of the rest of the participants. The effect of the number of children had a statistically insignificant effect on the trait anxiety level ($p>.05$). The participants' marital status, education level, length of service, and the type and location of the school of employment did not affect their state and trait anxiety levels ($p>.05$). The effect of job tasks performed by the participants at the time of the study on the state and trait anxiety levels was statistically significant ($p<.05$ and $p<.001$, respectively). The state and trait anxiety levels of participants working as administrators were lower compared to those performing other jobs.

Table 1. Descriptive characteristics and comparison of scale scores (N=1704)

Demographics	characteristics	n (%)	Mean±SD	p
Gender				
State Anxiety	Men	802 (47.1)	39.17±11.46	0.000
	Women	902 (52.9)	41.93±11.04	
Trait Anxiety	Men	802 (47.1)	40.28±7.70	0.000
	Women	902 (52.9)	43.38±7.71	
Marital Status				
State Anxiety	Single	163 (9.6)	41.49±11.60	0.307
	Married	1541 (90.4)	40.54±11.29	
Trait Anxiety	Single	163 (9.6)	43.01±8.95	0.063
	Married	1541 (90.4)	41.80±7.72	
Number of children				
State Anxiety	0	179 (10.53)	40.71±10.42*a	0.017
	1	377 (22.18)	41.31±11.52*a	
	2	898 (52.85)	40.94±11.58*a	
	3	191 (11.24)	38.67±10.48*ab	
	4+	54 (3.17)	37.44±10.81*b	
Trait Anxiety	0	179 (10.53)	42.24±8.68	0.185
	1	377 (22.18)	42.39±8.08	

	2	898 (52.85)	41.94±7.64	
	3	191 (11.24)	40.94±7.32	
	4+	54 (3.17)	40.57±8.45	
Educational status				
State Anxiety	Bachelor	1532 (89.9)	40.54±11.37	0.347
	Postgraduate	168 (9.9)	41.41±10.86	
Trait Anxiety	Bachelor	1532 (89.9)	41.92±11.37	0.936
	Postgraduate	168 (9.9)	41.97±7.13	
Length of service				
State Anxiety	1-5 years	114 (6.7)	39.15±11.71	0.151
	6-15 years	711 (41.7)	41.17±10.71	
	16-25 years	614 (36.0)	40.68±11.56	
	26+ years	265 (15.6)	39.71±12.10	
Trait Anxiety	1-5	114 (6.7)	42.44±8.87	0.054
	6-15	711 (41.7)	42.50±7.74	
	16-25	614 (36.0)	41.44±7.75	
	26+	265 (15.6)	41.23±7.86	
Type of school of employment				
State Anxiety	Pre-school	89 (5.2)	41.32±11.17	0.722
	Primary school	453 (26.6)	40.92±11.29	
	Secondary school	576 (33.8)	40.10±11.12	
	High school	509 (29.9)	40.79±11.60	
	Other	77 (4.5)	41.0±11.41	
Trait Anxiety	Pre-school	89 (5.2)	42.13±8.16	0.796
	Primary school	453 (26.6)	42.07±7.74	
	Secondary school	576 (33.8)	41.86±7.92	
	High school	509 (29.9)	41.69±7.78	
	Other	77 (4.5)	42.80±8.24	
Location of school	Village	85 (5.0)	41.02±11.21	
	Town	46 (2.7)	43.19±10.68	
State Anxiety	District	440 (25.8)	40.80±11.17	0.406
	City center	1133 (66.5)	40.43±11.41	
	Village	85 (5.0)	43.05±8.69	
Trait Anxiety	Town	46 (2.7)	42.95±8.28	0.428
	District	440 (25.8)	41.84±8.16	
	City center	1133 (66.5)	41.83±7.65	
Job title	Teacher	1486 (87.2)	40.86±11.31	
State Anxiety	Administrator teacher	169 (9.9)	38.28±10.82	0.015
	Other	49 (2.9)	41.73±12.31	
	Teacher	1486 (87.2)	42.17±7.75	
Trait Anxiety	Administrator teacher	169 (9.9)	39.57±8.18	0.000
	Other	49 (2.9)	42.38±8.58	

*Groups with significant differences in between, as identified by Duncan's multiple comparisons, are marked with different letters in the same column.

Descriptive statistics of 40 items in both inventories used in the study are presented in Table 2. The mean scores of participants on SAI and TAI were 40.6(11.3) and 41.9(7.8), respectively. The state-trait anxiety levels of teachers were moderate.

Table 2. Mean scores from the State-Trait Anxiety Inventory

Scale	Mean
State Anxiety Inventory	40.63±11.32 (min= 20, max= 80)
Trait Anxiety Inventory	41.92±7.85 (min=20, max=71)

Discussion

In this study, teachers' anxiety levels were found to be of moderate severity. Studies in the literature support this finding. According to the results of a study conducted during the SARS epidemic in Hong Kong, people were found to experience moderate levels of anxiety (Leung & et al., 2003). It was previously reported that teachers' anxiety about the COVID-19 pandemic was of moderate severity (Çiçek & et al., 2020). In the study performed by Wong et al. (2021), it was found that teachers experienced depression and stress, and their anxiety was mostly about themselves and their family members. In that study, it was found out that teachers also had anxiety about distance education (Wong & et al., 2021).

According to Fuller (1969), teachers' feelings of anxiety occur at certain time points normally (Fuller, 1969). Teachers experience anxiety when faced with certain concerns such as using time effectively in the classroom, completing activities effectively, and maintaining discipline in the classroom (Gangal, 2020). Teachers may also develop anxiety in response to some situations, such as incompetency in the field of the job task, lack of knowledge and experience, or the inability to use relevant knowledge and equipment (Uçak & Bindak, 2017). Nevertheless, teachers need to control the level of anxiety they experience in the workplace. It is a favorable finding that teachers' anxiety levels did not increase during the pandemic and remained at moderate levels. Teachers, who perform education and training activities online and remotely during the pandemic, may experience some difficulties in communication. The methods used by teachers for student motivation regarding education and the maintenance of good health are very important for the assessment and improvement of educational activities. It has been observed that teachers have readily adapted themselves to the use of distance education during the pandemic. The fact they could manage the process well might help prevent them from developing increased anxiety levels.

In this study, we found that the marital status, education levels, and lengths of service of teachers did not affect their state and trait anxiety levels. These findings are consistent with those reported in the literatüre (Çiçek & et al., 2020, Sakaoğlu & et al., 2020, Çifçi & Demir, 2020, Bulut & Duman, 2021). In a study performed by Sakaoğlu et al. (2020), the examination of anxiety levels by education status showed that there were no differences between the state and trait anxiety scores. Similar to the findings of the present study, it was also reported in the literature that the level of anxiety increases as the education level of teachers increases (Sakaoğlu & et al., 2020). In the study performed by Bulut and Duman (2021), no relationships were found between the length of service and the level of anxiety of participants (Bulut & Duman, 2021). Some studies reported higher anxiety levels in female, young, and single teachers compared to their male, older, and married counterparts during the COVID-19 pandemic (Çiçek & et al., 2020, Çifçi & Demir, 2020). The present study found that women's anxiety levels were higher than that of men. In studies conducted on different sample groups in the literature, it was found out that women were more worried than men during the COVID-19 pandemic (Bulut & Duman, 2021, Çölgeçen & Çölgeçen,2020, Kayaoğlu & et al., 2021).

In the present study, the state anxiety levels of teachers with only one child are higher than those of their counterparts. Being a child without siblings in a family means receiving all focused attention and care. The low anxiety levels of parents with no children or more than one child suggest that attention directed to the only child in the family can be potentially excessive. Furthermore, such increased anxiety levels may result from the fear of the likelihood of leaving the only child all alone in case of conditions that could put the parents in peril.

Among the participants, the state and trait anxiety levels of teachers in administrative positions were lower compared to the rest. This may be due to the better command of the pandemic-related

subjects by the administrators, who acquire information through frequent meetings and by closely following up the state and government policies about the pandemic.

Following the start of the COVID-19 pandemic in the world and our country, telepsychiatry applications allowing telephone calls or online video calls were readily implemented, providing access to all healthcare professionals and anyone affected by the pandemic. This has led to the development of a mental support mechanism for individuals. Solidarity and charity work in society are critical in alleviating the pandemic-related problems(Tükel, 2020).

Conclusion

It is pleasing that teachers have not developed severe levels of anxiety and worry during the pandemic. This has helped prevent the serious interruptions in the education system. During periods of outbreaks, people should be in social, psychological and, when necessary, material solidarity. In such a process, people should do their parts.

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REFERENCES

Alpar R. Multivariate Statistical Methods. Detail Publishing, 3rd ed. Ankara: 2011; pp 853.

American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental Disorders. 5th ed. (DSM 5). Washington: 2013.

Başar H. Classroom management. M.E.B. Publications, İstanbul: 1999.

Brooks SM, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet 2020; 395: 912-20.

Bulut Ç, Duman S. Investigation of job motivation and anxiety levels of youth and sports provincial directors' employees during the Covid-19 pandemia process. Gaziantep University Journal of Sport Science 2021; 6: 178-95.

Çakın M, Akyavuz EK. The Covid-19 process and its reflection on education: An analysis on teachers' opinions. Int J Soc Sci Educ Res 2020; 6: 165-86.

Cao W, Fang Z, Hou G, Han M, Xu X, Dong J, et al. The psychological impact of the COVID-19 epidemic on college students in China. Psychiatry Res 2020; 287: 1-5.

Çiçek İ, Tanhan A, Tanrıverdi S. COVID-19 and education. The Journal of National Education 2020; 49: 1091-104.

Çifçi F, Demir A. The effect of home-based exercise on anxiety and mental well-being levels of teachers and pre-service teachers in COVID-19 pandemic. African Educ Res J 2020; 8: 20-8.

Çölgeçen Y, Çölgeçen H. Evaluation of anxiety levels arising from Covid-19 Pandemic: The case of Turkey. Turkish Studies 2020; 15: 261-75.

Daniel SJ. Education and the COVID-19 pandemic. Prospects 2020; 49: 91-6.

Fuller FF. Concerns of teachers: A developmental conceptualization. Am Educ Res J 1969; 6: 207-26.

Gangal M. The effects of practice-based classroom management training and pedagogical documentation implementation on preschool teachers' classroom management skills. Hacettepe University Primary Education Department Preschool Education Program Ph.D. Thesis, 2020, Ankara.

Huang JZ, Han MF, Luo TD, Ren AK, Zhou XP. Mental health survey of medical staff in a tertiary infectious disease hospital for COVID-19. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2020; 38: 192-5.

Huremović D. Mental health of quarantine and isolation. In: Huremović D, ed. Psychiatry of pandemics, a mental health response to infection outbreak. Switzerland: Springer: 2019.

Kara Y. Students' experiences in the pandemic duration: The case of Bakırköy district. Eurasian Journal of Researches in Social and Economics 2020; 7: 165-76.

Kayaoğlu K, Polat H, Karakaş SA, Altun ÖŞ. Levels of anxiety and hopelessness in nurses due to COVID-19 infection. Türkiye Klinikleri Journal of Nursing Sciences 2021; 13: 958-70.

Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, et al. Factors associated with mental health outcomes among health care workers exposed to Coronavirus disease 2019. JAMA Netw Open 2020; 3: 1-12.

Leung GM, Lam TH, Ho LM, Ho SY, Chan BHY, Wong IOL, et al. The impact of community psychological responses on outbreak control for severe acute respiratory syndrome in Hong Kong. J Epidemiol Community Health 2003; 57: 857-63.

Li Q, Miao Y, Zeng X, Tarimo CS, Wu C, Wu J. Prevalence and factors for anxiety during the coronavirus disease 2019 (COVID-19) epidemic among the teachers in China. J Affec Disord 2020; 277: 153-8.

Nelson LM, Simard JF, Oluyomi A, Nava V, Rosas LG, Bondy M, et al. US public concerns about the COVID-19 pandemic from results of a survey given via social media. JAMA Intern Med 2020; 180: 1020-2.

Öner N, Le Compte A. Handbook of State and Trait Anxiety Inventory. Boğaziçi University Publications, İstanbul, 1983.

Ozamiz-Etxebarria N, Mondragon NI, Bueno-Notivol J, Moreno MP, Santabárbara J. Prevalence of anxiety, depression, and stress among teachers during the COVID-19 pandemic: A rapid systematic review with meta-analysis. Brain Sci 2021; 11: 1172-86.

Pappas G, Kiriaze IJ, Giannakis P, Falagas ME. Psychosocial consequences of infectious diseases. Clin Microbiol Infect 2009; 15: 743-7.

Pektekin Ç. The importance of psychiatric nursing in the Covid 19 pandemic process. Journal of Psychiatric Nursing 2020; 11: 163-4.

Pierce M, Hope H, Ford T, Hatch S, Hotopf M, John A, et al. Mental health before and during the COVID-19 pandemic: A longitudinal probability sample survey of the UK population. Lancet Psychiatry 2020; 7: 883-92.

Şahin M. Fear, anxiety and anxiety disorders. Eurasian Journal of Researches in Social and Economics 2019; 6: 117-35.

Sakaoğlu HH, Orbatu D, Emiroğlu M, Çakır Ö. Spielberger State and Trait Anxiety level in healthcare professionals during the Covid-19 outbreak: A case of Tepecik Hospital. Journal of Tepecik Education and Research Hospital 2020; 30: 1-9.

Sim K, Chan YH, Chong PN, Chua HC, Soon SW. Psychosocial and coping responses within the community health care setting towards a national outbreak of an infections disease. J Psychosom Research 2010; 68: 195-202.

Sintema EJ. Effect of COVID-19 on the performance of grade 12 students: Implicationsfor STEM education. Eurasia J Math, Sci Tech Educ 2020; 16: 1-6.

Stachteas P, Stachteas C. The psychological impact of the COVID-19 pandemic on secondary school teachers. Psychiatriki 2020; 31: 293-301.

Stuijfzand S, Deforges C, Sandoz V, Sajin CT, Jaques C, Elmers J, et al. Psychological impact of an epidemic/pandemic on the mental health of healthcare professionals: a rapid review. BMC Public Health 2020; 20: 1230-48.

Tükel R. Mental health during the COVID-19 pandemic Turkish Medical Association Sixth Month Evaluation Report; 2020 [Online] 2020 [Cited 2022 Nov 23]. Available from: URL: https://www.ttb.org.tr/kutuphane/covid19-rapor_6.pdf.

Uçak K, Bindak R. Professional anxiety levels of physical education and sport teacher candidates (Ondokuz Mayıs University Sample). Journal of National Sport Sciences 2017; 1: 44-54.

Wakui N, Abe S, Shirozu S, Yamamoto Y, Yamamura M, Abe Y, et al. Causes of anxiety among teachers giving face-to-face lessons after the reopening of schools during the COVID-19 pandemic: A cross-sectional study. BMC Public Health 2021; 21: 1-10.

WHO. WHO Coronavirus Disease (COVID-19) Dashboard. [Online] 2022 [Cited 2022 Nov 23]. Available from: URL: https://covid19.who.int/.

Wong KY, Sulaiman T, Ibrahim A, Mohd AGK, Hussin OH, Jaafar WMW. Secondary school teachers' psychological status and competencies in e-teaching during Covid-19. Heliyon 2021; 7: 1-8.

Yıldız E, Yeniçeri EN, Öngel K. Application of State-Trait Anxiety Scale (STAI-TX) in randomly selected individuals and results. Smryna Medical Journal 2019; 1: 19-24.

Zhu Z, Liu Q, Jiang X, Manandhar U, Luo Z, Zheng Xu, et al. The psychological status of people affected by the COVID-19 outbreak in China. J Psychiatr Research 2020; 129: 1-7.

Intracalcanaeal Lipoma

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Introduction

The lower extremity is one of the most common anatomical sites for bone and soft tissue tumors and tumor-like lesions. Primary bone tumors are rare, and most are benign lesions. Of these, osteochondroma, enchondroma, osteoid osteoma, chondroblastoma, giant cell tumor, chondromyxoid fibroma, liposclerosing myxofibroid tumor, and intraosseous lipoma are the most common lesions.

Intraosseous lipoma, among the primary benign bone tumors, is less commonly diagnosed than other primary bone tumors and may occur incidentally in radiological examinations performed for another reason if they are asymptomatic. Therefore, the true incidence cannot be determined clearly.

The primary and only reason for patients presenting with intracalcaneal lipoma to apply to the clinic is chronic heel pain that persists despite analgesic therapy and worsens with walking. In this benign bone tumor where spontaneous regression is possible, the most critical reason that leads the orthopedist to surgery is the patient's complaint of pain and, more importantly, the radiological resemblance of the lesion to other bone tumors. In particular, intracalcaneal lipomas can be radiologically mistaken for aneurysmal bone cysts, giant cell tumors, unicameral bone cysts, pseudocysts, and bone infarcts (Arkun, 2016; Gero & Kahn, 1988). In intraosseous lipomas, for which curettage and grafting are sufficient, no recurrence is observed after surgery (Levin et al., 1996).

Bone-related lipomas are localized in intraosseous, cortical, or parosteal zones and originate from lipocytes. The most common one is intraosseous localization. This rare lesion accounts for 0.1% of tumoral lesions of the bone (Hassani et al., 2014; Unni & Inwards, 2010). The sex predilection in intraosseous or intramedullary lipoma is controversial (Goto et al., 2002). Intraosseous lipoma was defined in three stages by Milgram et al. (Milgram, 1988a) according to the pathological changes in the internal structure of the lesion (Table 1). An intraosseous lipoma detected in Stage 3 can be mistaken for a bone infarction.

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Table 1. Milgram Staging

Stage 1	There are sharply demarcated lipocytes of mature homogeneous adipose tissue replacing trabecular bone. A radiolucent lesion with fine marginal sclerosis and slightly expanded well-defined margins can be observed on plain radiographs.
Stage 2	Foci of necrosis and calcified areas may be determined in the lesion center of mature adipose tissue cells. At this stage, the cystic area accompanies the lesion.
Stage 3	There is increased fat necrosis, coarse calcifications, myxomatous degeneration of fat cells, and larger cystic areas. There is an increased density of calcification in an expanded, well-defined, radiolucent lesion on direct radiography. The sclerotic zone around the lesion is thicker.

Case Presentation

A 52-year-old male patient, who was admitted to the outpatient clinic with right plantar heel pain, described the symptom as right heel pain that increased significantly after getting up in the morning and walking a few steps and got worse after a long-standing activity or lifting a heavy load. Although he could not remember when this pain started, it was recorded that the patient's complaints had increased in the last year, and there was no previous trauma to his right heel in the anamnesis.

On physical examination, there was pain on palpation, especially on the plantar face. No tenderness was detected on the medial and lateral face of the heel. Ankle and subtalar joint ranges of motion were complete both actively and passively. A preoperative visual pain scale was performed on the patient. The patient rated his pain with a score of 7 to 8 out of 10. Two-way radiography of the ankle was requested from the patient. On the radiograph, it was seen that there was a 25x30 mm unicameral expansile lesion in the calcaneal medulla, hypodense compared to the intratrabecular bone density of the calcaneus, and hyperdense ossification foci in the center of the lesion (Figure 1). Contrast-enhanced ankle magnetic resonance imaging (MRI) was performed for further examination. MRI images reported that a lesion compatible with lipoma was observed in the medulla, which was 36x20 mm in size in the calcaneus, isointense in well-defined T1W, suppressed in fat-suppressed sequences (Figure 2a,2b). After intravenous contrast agent injection, two cystic foci, the largest of which was 9x5 mm, were noted in the center of this lesion. Both MRI and plain X-ray findings favored Milgram grade-3 intraosseous lipoma. (Figure 2c,2d,2e,2f)

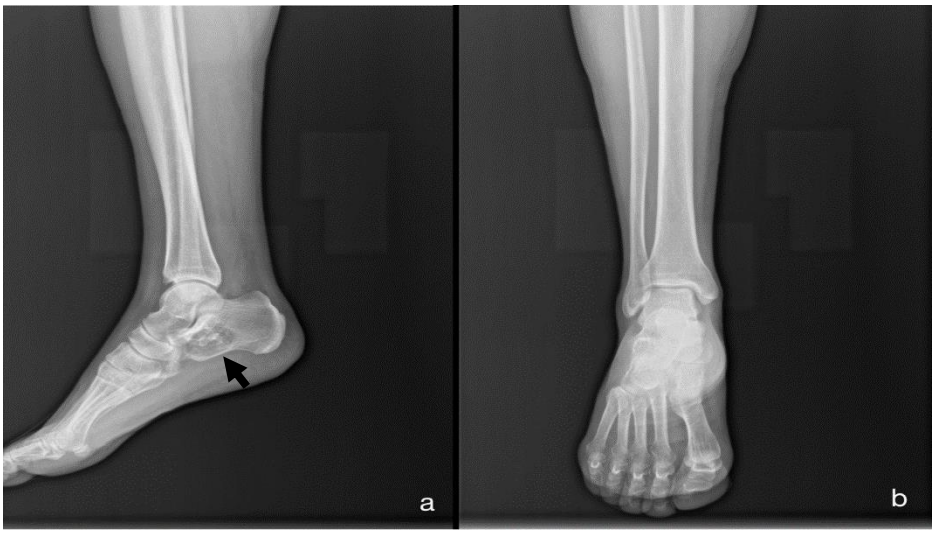

Figure 1 a: Cystic lesion is seen where the arrow points in lateral ankle X-Ray view, b: Anteroposterior (AP) ankle X-Ray view

As a result of the physical examination and tests, no infective or degenerative foot and ankle pathologies were considered in the patient, and the diagnosis of intracalcaneal lipoma was made. The patient was explained that the pain in this disease could be corrected surgically. The complications that may occur were explained to the patient by giving preoperative counseling services. Surgical preparation of the patient, whose informed consent was obtained, was planned.

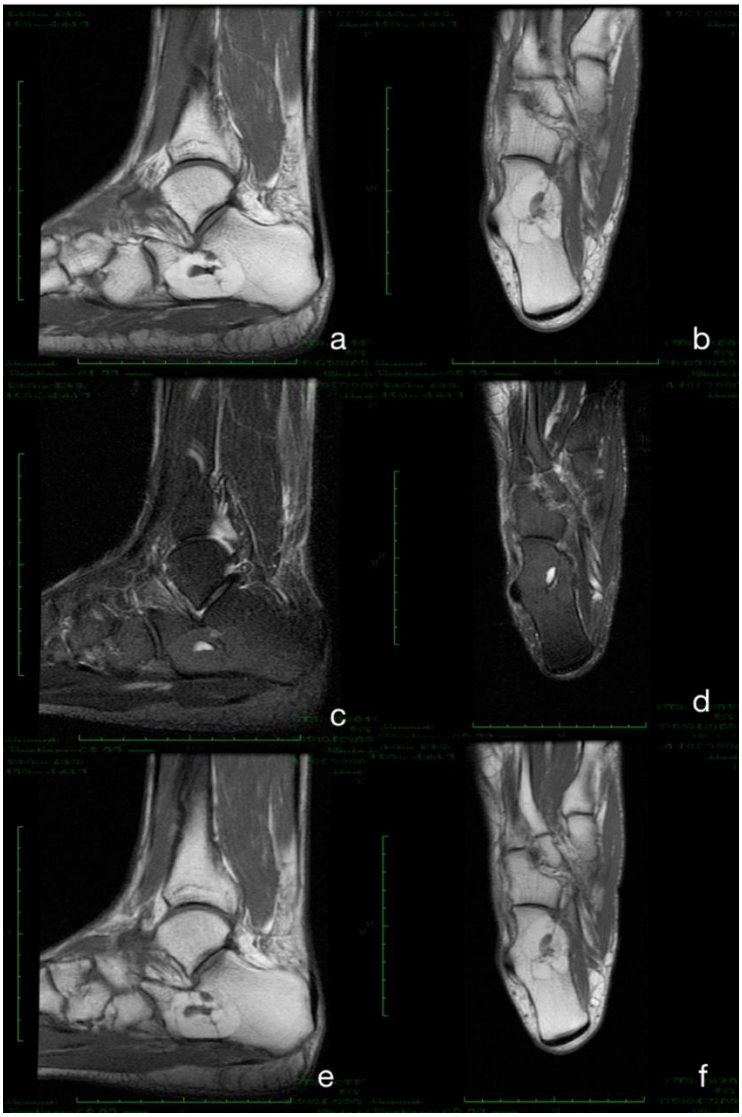

Figure 2 Magnetic resonance (MR) imaging a: Sagittal T1 FSE Non- contrast (NC) MR view, b: Axial T1 FSE NC MR view, c: Sagittal T2 FSAT MR contrast view, d: Axial T2 FSAT MR contrast view, e: Sagittal T1 FSE MR contrast view, f: Axial T1 FSE MR contrast view

It was decided whether the patient would be treated with curettage grafting alone or plating in addition to this treatment based on Mirels' prophylactic detection criteria (Mirels, 1989). A locked calcaneal plate was applied as prophylactic fixation material to the patient who scored 9 according to this criterion. The patient was placed in the lateral decubitus position. The mass in the calcaneus was reached with an extended lateral approach. After the calcaneal cortex was removed, the fluid-filled yellow cystic structure was aspirated, and an excisional biopsy was performed to send the mass like fat globulins in the cyst to pathology. It was observed that the area around the evacuation area was sclerotic. The surrounding of this sclerotic wall was decorticated with the help of a drill, and the connection of this evacuated area with the bone medulla was ensured. A bone graft was then placed in this area. To increase the efficiency of the graft, tricalcium phosphate, a synthetic ceramic graft with osteoconductive properties, and an autogenous bone marrow graft with osteoinductive properties

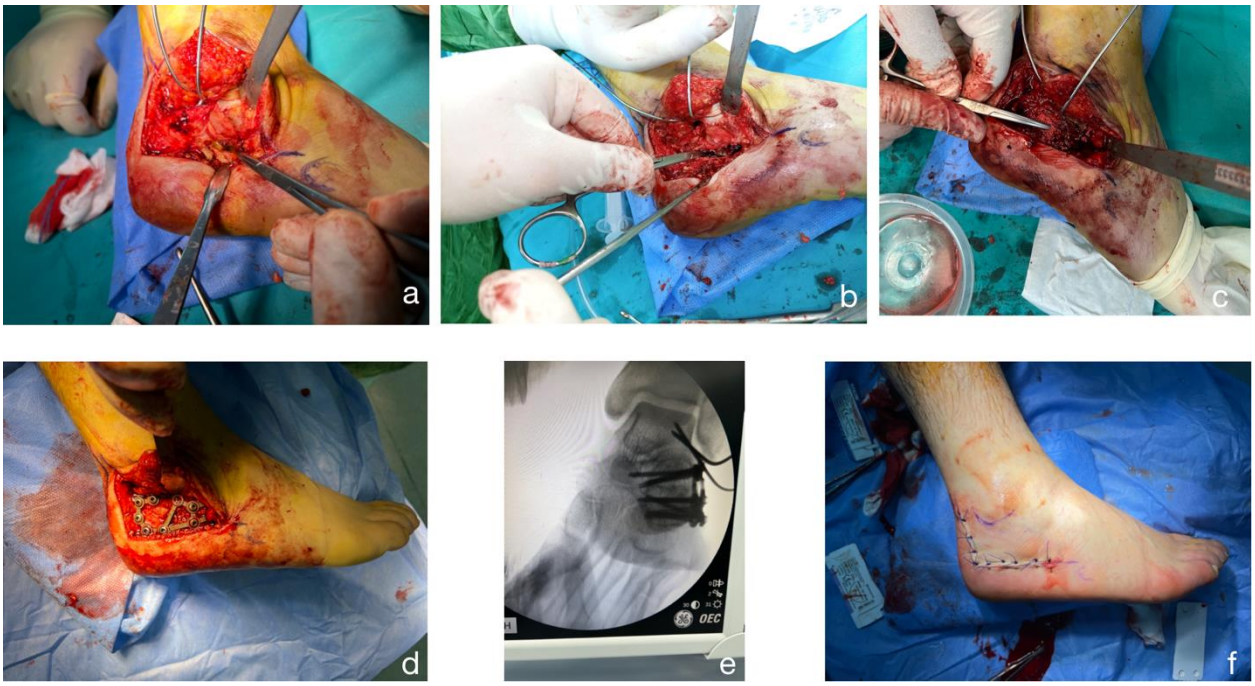

Figure 3 a: Intraoperative view of the cystic structure before opening in the calcaneus' cortex, b: Intraoperative view of the cystic structure after opening in the calcaneus' cortex, c: Greafted after curretage placement of the cystic structure in the calcaneus, d: Applied view of locked calcaneal anatomical plate, e: Intraoperative fluoroscopic AP view after plate use f: Wound closure of extended lateral approach view

were combined. Then, a locked calcaneal anatomical plate was applied, and the operation was completed without complications. (Figure 3)The patient was followed up with a splint for three weeks after the operation, and weight bearing was started approximately five weeks after the operation. Complete healing was determined radiologically seven weeks after the operation. (Figure 4) Clinically, the patient was followed up for 20 weeks after the operation. The patient's visual pain scale was measured. The patient's pain was recorded by giving a value between 2 and 3 points out of 10. As a result of the postoperative controls, it was decided that the bone graft area was healed, both by showing that bone remodalization was achieved on the radiograph and by evaluating the patient's clinic.

Discussion

As reported in the literature, intraosseous lipomas develop in less than 0.1% of all bone tumors and have an incidence between 0.1% and 2.5% among primary bone tumors (Hassani et al., 2014; Unni & Inwards, 2010). The first case was reported in the femoral shaft by Brault in 1868 (Milgram, 1988b). The second case was published in the femur diaphysis in 1901, and the third was published in the fibula in 1910 (Unni & Dahlin, 1996). Intraosseous lipomas are most commonly located in the subtrochanteric area of the proximal femur, second most frequently in the calcaneus (Hong et al., 1999).

Intraosseous lipomas are asymptomatic and are often detected incidentally. As in our case, 70% of the cases in symptomatic patients present with pain, and it is thought that this pain is caused by micro-fractures in the trabecular network of the calcaneus due to axial loading (Goto et al., 2002).

When the literature is reviewed, neoplastic and non-neoplastic pathologies such as simple bone cysts, aneurysmal bone cysts, osteoblastoma, enchondroma, chondromyxoid fibroma, non-ossifying fibroma, giant cell tumor, chondroblastoma, fibrous dysplasia, and chondrosarcoma are considered in the differential diagnosis (Futani et al., 2007). Pain is the primary surgical indication in these masses, and among the surgical options, curettage of the cystic area followed by grafting or cementing, if necessary, is preferred to fill the defect area. After filling the defect area, it was decided to use fixation

material according to Mirels' prophylactic fixation criterion (Azarsina et al., 2019) in some studies against the risk of fracture of this bone area.

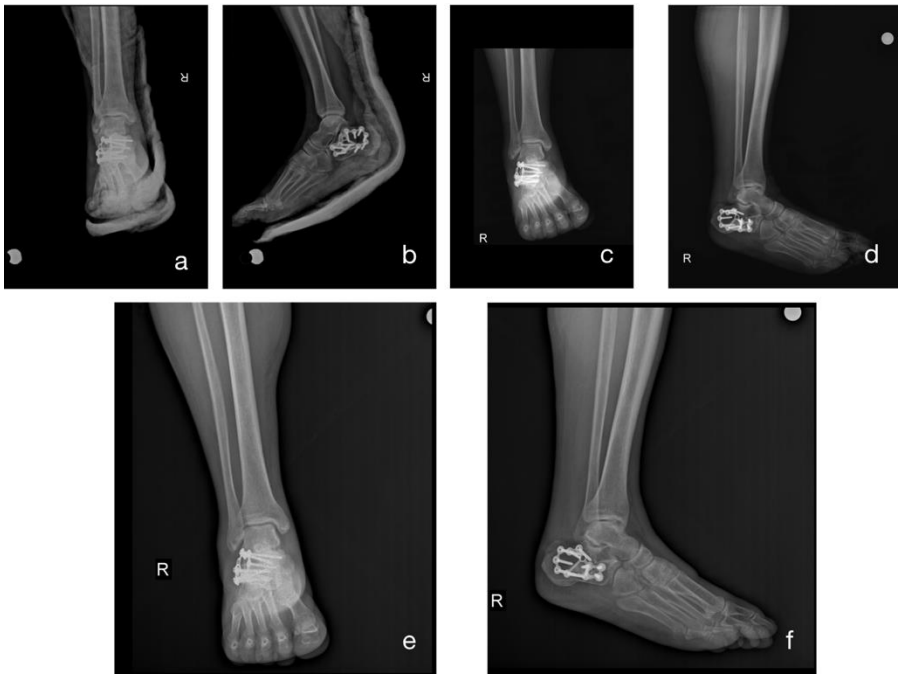

Figure 4 a: Early postoperative (EP) AP ankle X-Ray view, b: EP lateral ankle X-Ray view, c: 5 week after operation AP ankle X-Ray view, d: 5 week after operation oblique ankle X-Ray view, e: 7 week after operation AP ankle X-Ray view, f: 7 week after operation oblique ankle X-Ray view

In contrast, in some studies, it was decided to detect the mass after the curettage of the mass with the concept of a critically sized cyst (Pogoda et al., 2004).

In this case, the patient was evaluated as Milgram stage 3. The use of fixation material was evaluated according to Mirels criteria. According to this criterion, getting 9 points, grafting after curettage, and locking calcaneal plate placement as fixation material were planned.

Whether surgical treatment is required in intracalcaneal lipoma is still a controversial issue. However, these patients go for surgical treatment because they are radiologically like some other bone tumors and because of pain complaints. In surgery, the choice of grafting or cementing after curettage should be made in line with the surgeon's experience. The use of implants after grafting or cementation should be decided by evaluating both Mirels criteria and the criteria in the concept of critical size cyst.

REFERENCE

Arkun, R. (2016). Alt Ekstremite Tümörleri. *Trd Sem*, *4*(3), 516-539. https://doi.org/10.5152/trs.2017.461

Azarsina, S., Biglari, F., Hassanmirzaei, B., Ebrahimpour, A., & Hakakzadeh, A. (2019). Intraosseous Lipoma of Calcaneus, Rare Cause of Chronic Calcaneal Pain: A Case Report. *Arch Bone Jt Surg*, *7*(5), 469-473.

Futani, H., Fukunaga, S., Nishio, S., Yagi, M., & Yoshiya, S. (2007). Successful treatment of bilateral calcaneal intraosseous lipomas using endoscopically assisted tumor resection. *Anticancer Res*, *27*(6c), 4311-4314.

Gero, M. J., & Kahn, L. B. (1988). Case report 498: Intraosseous lipoma of the distal end of the fibula with focal infarction. *Skeletal Radiology*, *17*(6), 443-446. http://inis.iaea.org/search/search.aspx?orig_q=RN:20015906

Goto, T., Kojima, T., Iijima, T., Yokokura, S., Motoi, T., Kawano, H., Yamamoto, A., & Matsuda, K. (2002). Intraosseous lipoma: a clinical study of 12 patients. *J Orthop Sci*, *7*(2), 274-280. https://doi.org/10.1007/s007760200046

Hassani, M., Gharehdaghi, M., Khooei, A. R., Ghodsi, E., & Nazarzadeh, H. (2014). Bilateral intraosseous tumor of the calcaneus with imaging-pathologic discordance a case report and literatures review. *Arch Bone Jt Surg*, *2*(3), 238-242.

Hong, J., Andersson, J., Ekdahl, K. N., Elgue, G., Axén, N., Larsson, R., & Nilsson, B. (1999). Titanium is a highly thrombogenic biomaterial: possible implications for osteogenesis. *Thromb Haemost*, *82*(1), 58-64.

Levin, M. F., Vellet, A. D., Munk, P. L., & McLean, C. A. (1996). Intraosseous lipoma of the distal femur: MRI appearance. *Skeletal Radiol*, *25*(1), 82-84. https://doi.org/10.1007/s002560050039

Milgram, J. W. (1988a). Intraosseous lipomas: radiologic and pathologic manifestations. *Radiology*, *167*(1), 155-160. https://doi.org/10.1148/radiology.167.1.3347718

Milgram, J. W. (1988b). Intraosseous lipomas. A clinicopathologic study of 66 cases. *Clin Orthop Relat Res*(231), 277-302.

Mirels, H. (1989). Metastatic disease in long bones. A proposed scoring system for diagnosing impending pathologic fractures. *Clin Orthop Relat Res*(249), 256-264.

Pogoda, P., Priemel, M., Linhart, W., Stork, A., Adam, G., Windolf, J., Rueger, J. M., & Amling, M. (2004). Clinical relevance of calcaneal bone cysts: a study of 50 cysts in 47 patients. *Clin Orthop Relat Res*(424), 202-210. https://doi.org/10.1097/01.blo.0000128297.66784.12

Unni, K. K., & Dahlin, D. C. (1996). *Dahlin's bone tumors: general aspects and data on 11,087 cases*. Lippincott Williams & Wilkins.

Unni, K. K., & Inwards, C. Y. (2010). *Dahlin's bone tumors: general aspects and data on 10,165 cases*. Lippincott Williams & Wilkins.

Comparison of the Relationship of Health Expenditures and Educational Women Labor in the Eu and Turkey

Belma UZUN[1]

Introduction

Health expenditures have two fundamental importances in terms of public health. The first of these is to provide the best health service with the least cost and the second is to use all public resources for public health expenditures. For this reason, health expenditures are a common and important issue not only in public administration and health sciences, but also in economics and other sciences (Akar, 2014; Baltagi and Moscone, 2010; Hansen and King, 1996). In addition, when private health expenditures are evaluated together, the issue of the rights of individuals and the duties of the state comes to the fore (Bedir, 2016; Tıraşoğlu and Yıldırım, 2012; Barros, 1998).

When the concepts of family, health and expenditure are considered together, it is necessary to mention women and women's employment. The woman, who is the most important support in the family, keeps the home together and shows higher sensitivity about health than the father. For this reason, although the issue of women's employment and health expenditures is constantly questioned in the literature, there has not been enough work in this area. Existing studies are mostly descriptive and inferential studies, and there is a need for important and comprehensive quantitative studies in this field (Zafar, 2016; Wallerstein, 2001; Pyke, 1997; Jacobs, 1996).

Another important issue of health expenditures is the development and economic levels of countries. In this regard, the differences in healthcare services, especially between countries with different development and economic levels, have been a subject that has been constantly questioned from past to present. While these differences in health expenditures and the provision of health services affect social issues, especially welfare level and migration between countries, marketing of health services and ethical issues come to the fore (Akar, 2014; Baltagi and Moscone, 2010; Hansen and King, 1996).

Although the impact of women on health expenditure is significant, there have not been enough studies that address the country's level of development, women's education and employment, and private and public health expenditures. Therefore, in this section, it is aimed to examine and compare the relationship between the employment of women with qualified education and health expenditures by examining the data of the last two decades for Turkey and the EU.

Methods

Data sets from the World Bank country reports prepared for Turkey and the EU were used in the research. The dependent variable of the study was the employment rates of women with advanced education, the independent variables were private and public health expenditures, and the mediator variable was the region. The World Bank codes and research abbreviations of the data sets used in the research are as follows:

DPHE: Domestic private health expenditure (% of current health expenditure)

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DGHE: Domestic general government health expenditure per capita, PPP (current international $)

FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

Mean and standard deviation values were used to define the research data, z-scores and Mann Whitney U test were used for difference analysis. The distribution of highly educated women's employment and public and private health expenditures in the EU and Turkey over time were analyzed by parity analysis. Spearman's rho correlation analysis was performed in relational screening analysis. All analyzes were performed in SPSS 25.0 for windows program with 95% confidence interval and 0.05 significance level.

Results

DPHE, DGHE and FLFAE parameter values of the World Bank data according to years for the EU was given in the Table 1.

Table 1. DPHE, DGHE and FLFAE parameter values of the World Bank data according to years for the EU

Year	DPHE	DGHE	FLFAE
2000	25,23483	1351,965	79,1385
2001	25,02405	1449,235	78,72267
2002	25,06142	1552,601	79,01636
2003	25,28506	1619,654	79,29355
2004	25,80745	1683,923	79,38567
2005	25,64737	1769,808	79,05619
2006	25,98621	1902,315	77,28668
2007	26,12054	2001,471	77,2156
2008	26,08649	2164,82	77,35802
2009	25,68963	2296,991	77,24013
2010	25,7159	2358,137	76,94199
2011	26,12269	2432,181	76,93885
2012	26,45646	2468,166	77,03842
2013	26,19247	2579,157	76,84752
2014	26,10058	2652,882	76,55166
2015	25,88034	2725,272	76,5149
2016	25,25888	2914,406	76,53435
2017	25,20599	3055,053	76,33586
2018	25,03294	3260,8	76,25819
2019	25,08523	3414,582	76,32368
2020			75,19678
2021			75,9898

DPHE: Domestic private health expenditure (% of current health expenditure); DGHE: Domestic general government health expenditure per capita, PPP (current international $); FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

The World Bank data includes data for DPHE and DGHE until 2019, whereas FLFA data is given for 2021. DPHE, private health expenditure is given as percentage of total health expenditure, whereas DGHE, government health expenditure is given as current USD. FLFAE was also given as percentage.

DPHE, DGHE and FLFAE parameter values of the World Bank data according to years for Turkey was given in the Table 2.

Table 2. DPHE, DGHE and FLFAE parameter values of the World Bank data according to years for Turkey

Year	DPHE	DGHE	FLFAE
2000	38,32404	273,2744	
2001	32,74863	305,187	
2002	30,02564	333,0195	
2003	28,79287	345,981	
2004	28,29536	386,2162	70,28
2005	32,25196	397,9779	69,09
2006	31,1485	486,4609	67,03
2007	31,21626	539,2389	67,8
2008	27,30696	613,7765	68,61
2009	19,50121	690,3937	69,31
2010	22,0016	687,4658	69,49
2011	20,87164	728,9692	69,57
2012	20,82859	730,9283	69,48
2013	21,65038	768,4596	70,77
2014	22,39487	808,6069	71,14
2015	21,86664	828,4014	71,22
2016	21,55788	891,0722	71,1
2017	22,28973	906,6701	72,34
2018	22,61773	891,7586	71,37
2019	22,08143	924,6903	71,35
2020			65,39
2021	38,32404	273,2744	

DPHE: Domestic private health expenditure (% of current health expenditure); DGHE: Domestic general government health expenditure per capita, PPP (current international $); FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

For Turkey, FLFAE data was given between 2004 to 2020. Since DPHE was given as percentage of total health expenditures, it may be argued that data of the research does not include deflator effect, due to percentage and ratios are used for private health expenditure and advanced educated female population.

DPHE, DGHE and FLFAE levels of the EU and TR with difference analysis results were given in the Table 3.

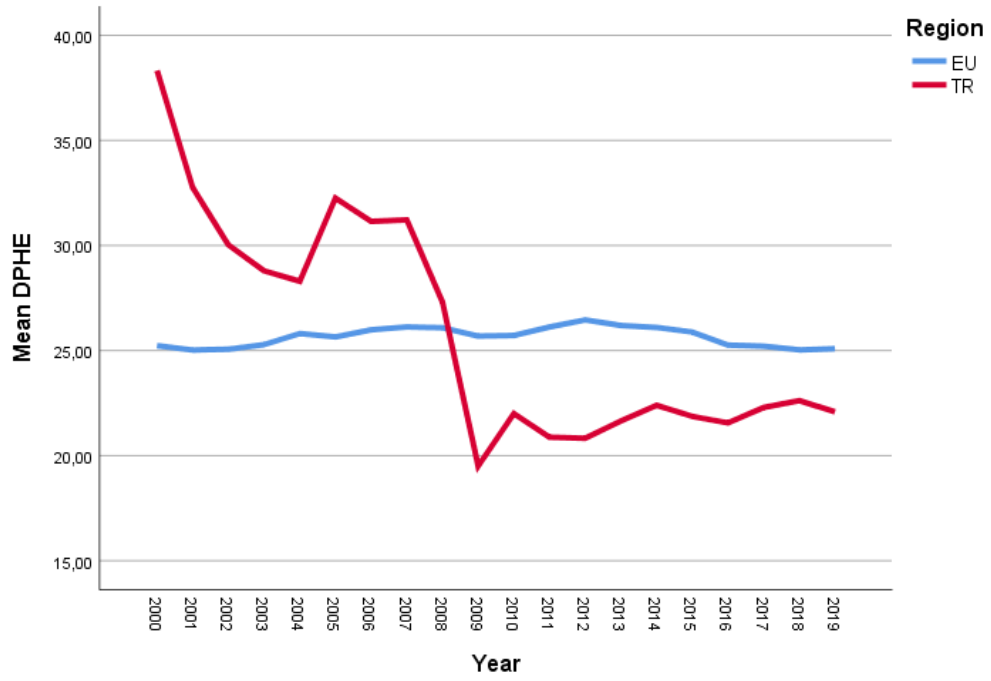
Table 1. DPHE, DGHE and FLFAE levels of the EU and TR with difference analysis results

	EU		TR		Z score	p value
Region	Mean	Std. Deviation	Mean	Std. Deviation		
DPHE	25,65	0,46	25,89	5,34	-0.541	0.602
DGHE	2282,67	611,72	626,93	224,92	-5.410	0.000
FLFAE	77,33	1,22	69,73	1,79	-5.296	0.000

DPHE: Domestic private health expenditure (% of current health expenditure); DGHE: Domestic general government health expenditure per capita, PPP (current international $); FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

Mann Whitney U test results showed that DPHE mean differences were insignificant between the EU and Turkey ($p<0.05$). On the other hand, DGHE and FLFAE levels were significantly lower in Turkey ($p>0.05$).

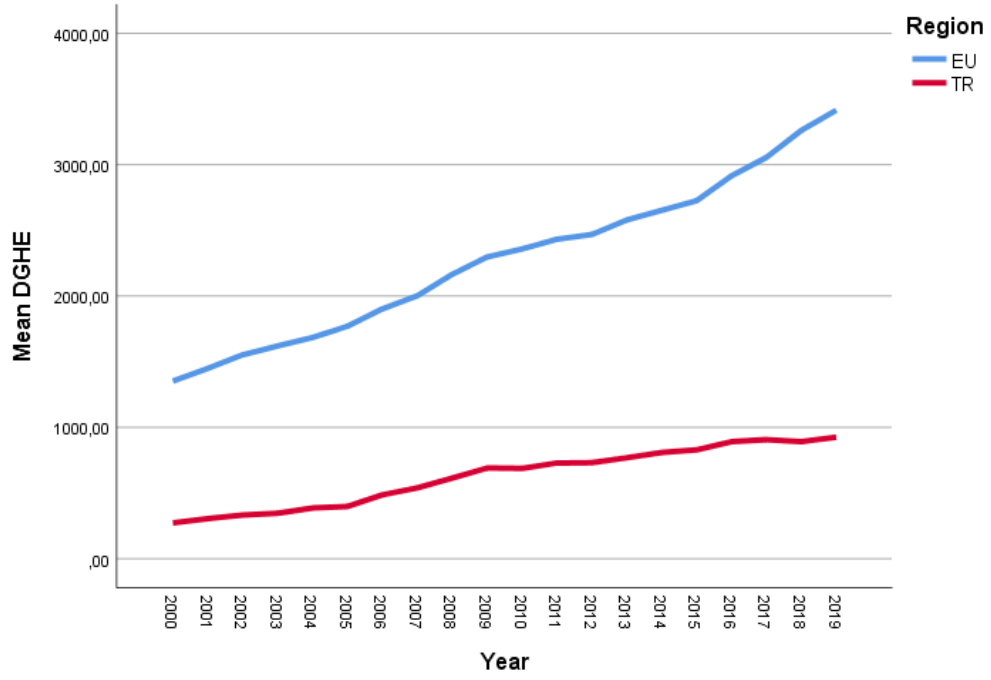
DPHE means and differences between the EU and Turkey according to year were shown in the Scheme 1.



Scheme 1. DPHE means and differences between the EU and Turkey according to year

Scheme 1 shows that private health expenditures in the EU is stable between 2000 and 2020 years, whereas private health expenditures of Turkey has sharply decreased from 2000 to 2009.

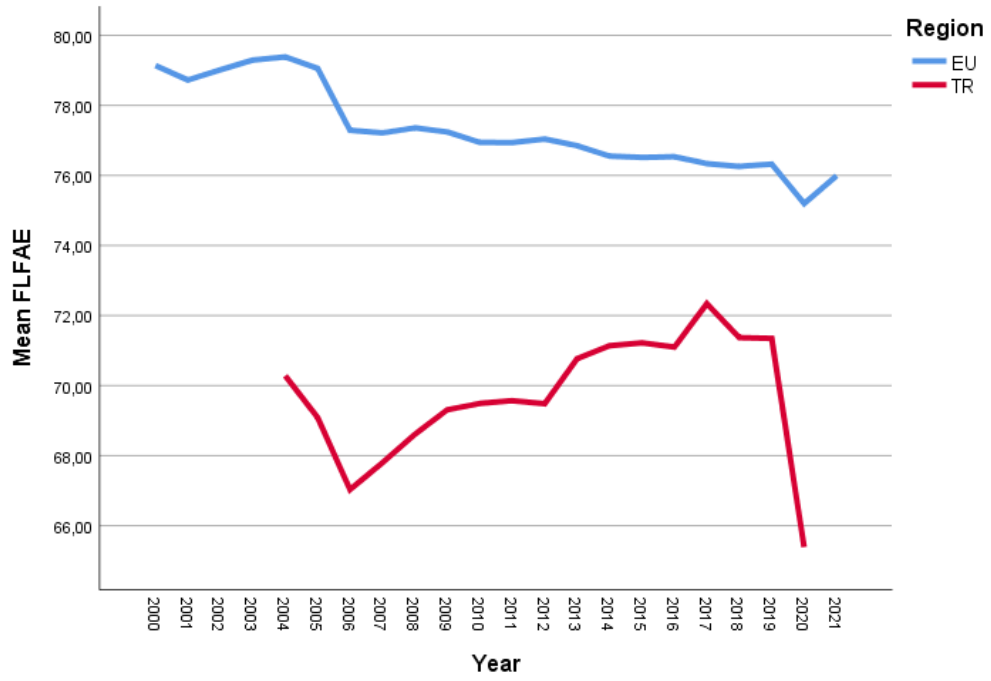
DGHE means and differences between the EU and Turkey according to year were shown in the Scheme 2.



Scheme 2. DGHE means and differences between the EU and Turkey according to year

Both the EU and Turkey have the same linear increasing trend in government health expenditures. Here, the EU has the higher means than Turkey, and its slope is also higher, showing higher increase rates in government health expenditures.

FLFAE means and differences between the EU and Turkey according to year were shown in the Scheme 3.



Scheme 3. FLFAE means and differences between the EU and Turkey according to year

Although advanced educated female rate in Turkey was increasing from 2005 to 2018, there was a sharp decrease in 2018 followed by 2020 decreases. Global crisis and Covid-19 pandemy are two important events of these milestones.

Spearman's rho correlation analysis results for relationship between region and research parameters were shown in the Table 2.

Table 2. Spearman's rho correlation analysis results for relationship between region and research parameters

	Region	
	r	**p**
DPHE	-0.087	0.595
DGHE	-0.866**	0.000
FLFAE	-0.859**	0.000

**p<0.01 DPHE: Domestic private health expenditure (% of current health expenditure); DGHE: Domestic general government health expenditure per capita, PPP (current international $); FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

Spearman's rho correlation analysis results showed that government health expenditures (r=-0.866; p<0.01) and advanced educated female labor rates (r=-0.859; p<0.01) were significantly correlated with the region.

Spearman's rho correlation analysis results for relationship between advanced educated female labor and health expenses were given in the Table 3.

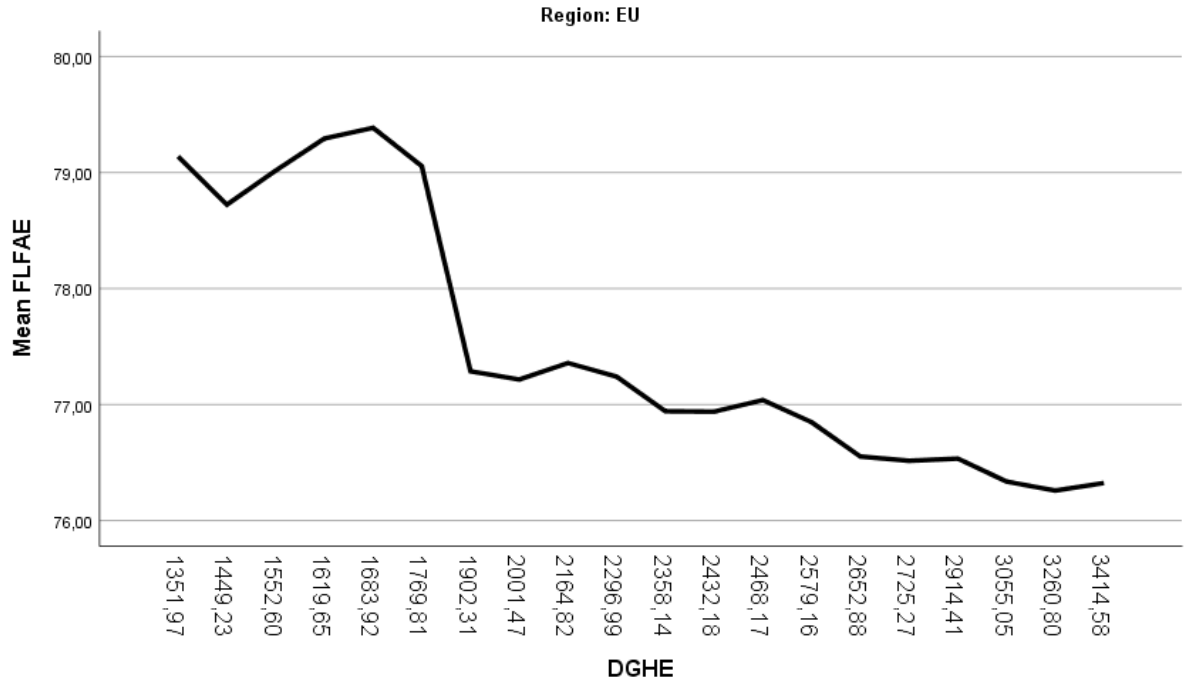
Table 3. Spearman's rho correlation analysis results for relationship between advanced educated female labor and health expenses

	The EU		TR	
FLFAE	**r**	**p**	**r**	**p**
DPHE	-0.009	0.970	-0.271	0.311
DGHE	-0.949**	0.000	0.844**	0.000

**p<0.01 DPHE: Domestic private health expenditure (% of current health expenditure); DGHE: Domestic general government health expenditure per capita, PPP (current international $); FLFAE: Labor force with advanced education, female (% of female working-age population with advanced education)

In the EU, government health expenditure and advanced educated female rate were significantly and negatively correlated (r=-0.949; p<0.01). However, health expenditure and advanced educated female rate were significantly and positively correlated (r=0.844; p<0.01) in Turkey.

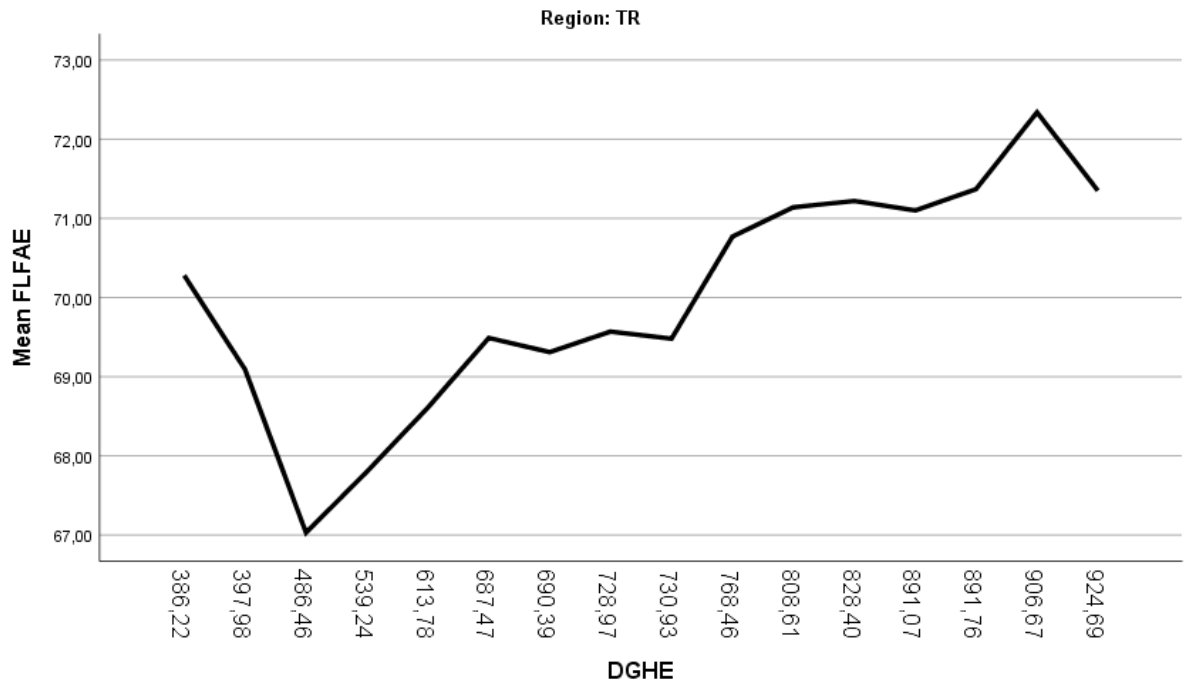
Correlation between FLFAE and DGHE for the EU was shown in the Scheme 4.



Scheme 4. Correlation between FLFAE and DGHE for the EU

According to Scheme 4, it was obvious that there a significant decrease in government health expenditure according to advanced educated female population in the EU. There may be some reasons for this negative correlation, but the result shows that increase in advanced educated female population causes decrease in health expenditures.

Correlation between FLFAE and DGHE for Turkey was shown in the Scheme 5.



Scheme 5. Correlation between FLFAE and DGHE for Turkey

In Turkey, on the other hand, as the highly educated female workforce increases, the state's health expenditures also increase. In general, it is seen that the participation of women with advanced education in employment increases the share of the public in the health expenditures of the country.

Discussion

In this study, it was aimed to examine the effect of women's employment and education level on health expenditures and to compare Turkey and EU countries, in this context, the value of the relationship between the employment of women with qualified education and private and public health expenditures in the last two decades was analyzed.

Women's employment is an issue that has been emphasized all over the world especially in recent years, and it is a subject that is regularly supported by national and international authorities. Women's employment is important not only in terms of economic and development, but also in terms of understanding the place of women in the social structure and fairness in the sharing of public values between men and women. Past experiences have revealed that women's employment and education is not only a social issue, but also important in terms of women's rights (Stromquist, 2015; Suen, 2013; Nikkhah, 2010).

The education level of women in the society also positively affects the level of education in the family and the family education in which individuals receive their first education. Therefore, it can be stated that both the education and employment of women play an important role in social development. On the other hand, while providing this education, it is necessary to accept that women have duties in the family and these duties should also be evaluated. The process of motherhood is an important process for every woman, both emotionally and spiritually, whether she has taken her economic freedom or has a low, middle or high socio-economic level. This process negatively affects both education and employment and career in business life (Bhagavatheeswaran et al, 2016; Sundaram et al, 2014). Even the studies of the World Health Organization that only breast milk is necessary for six months causes women to choose between career and motherhood, or to have difficulties in both.

Health expenditures are also an indicator of the extent to which individuals in a society have access to health services and the quality of the services provided (French, 2012; Di Matteo, 2004). In fact, since health is a global public good, although the relationship between expenditures on health services and quality should not be dependent on economic values, in reality, health services have always been closely related to the economy and countries all over the world have access to health services according to their economic income (Devlin ve Hansen, 2001; Blomqvist and Carter, 1997).

Due to this relationship of health services with the economy, some health expenditures, including private and public health expenditures or basic and private health expenditures, have been collected in a certain group. In fact, although this situation aims to present health expenditures effectively and to use public resources more efficiently, on the contrary, it has caused a serious economic concern in the health sector. There are publications and opinions that contain serious criticisms on this subject.

The first thing that comes to mind about the economic impact of health services on women's employment and education is the thought that women who work and earn their own money will spend more. Indeed, studies have shown that since working women have more economic power, health expenditures, especially cosmetics and beautification products, increase. However, these studies have very limited scope and content and more general studies have not been done. In this regard, in this study, data containing the sample in a wider framework were obtained in the field.

The results obtained in the research reveal that the relationship between the employment of educated and qualified women and public health expenditures between Turkey and the EU is in the opposite direction. In other words, it is seen that the EU and Turkey's employment of qualified women and related health expenditure policies are in the opposite direction of each other.

Conclusion

According to the general evaluation of the results obtained, it is seen that women's education and employment affects the health expenditure on the public. While public health expenditures decrease as women's education and employment increase in EU countries, in Turkey, on the contrary, as women's employment and education increases, the burden of health on the public increases. Therefore, it can be stated that qualified and educated women spend more on health care than women who are not employed and have low education.

The fact that the effect of women's employment and education on private health expenditures is not significant, but has an effect on public health expenditures, shows that health policies as a country are shaped according to the education and employment of women, and that working women cover health expenditures through health expenditures they provide from public resources, not their income.

Many factors can cause this situation. At the beginning of these, although women are enabled to participate in employment and the economic system, they may still use the income they earn economically for family expenses. In addition, there may be faulty or mismanagement of public health expenditure policies. Therefore, it would be beneficial to examine these issues in further research and field studies.

REFERENCES

Akar, S. (2014). Türkiye'de Sağlık Harcamaları, Sağlık Harcamalarının Nisbi Fiyatı ve Ekonomik Büyüme Arasındaki İlişkinin İncelenmesi. *Yönetim ve Ekonomi Dergisi.* 21(1), 311-322.

Baltagi, BH and Moscone, F. (2010). Healthcare expenditure and income in the OECD reconsidered: Evidence from panel data. *Economic Modelling,* 27, 804-811.

Barros, PP. (1998). The black box of healthcare expenditure growth determinants. *Health Economics,* 7, 533-544.

Bedir, S. (2016). Healthcare expenditure and economic growth in developing countries. *Advances in Economics and Business,* 4(2), 76-86.

Bhagavatheeswaran, L., Nair, S., Stone, H., Isac, S., Hiremath, T., Raghavendra, T., ... Watts, C. (2016). The barriers and enablers to education among scheduled caste and scheduled tribe adolescent girls in northern Karnataka, South India: A qualitative study. *International Journal of Educational Development,* 49, 262–270

Blomqvist, A. G., & Carter, R.A.L. (1997). Is healthcare really a luxury?. *Journal of Health Economics,* 16, 207-229.

Devlin, N and Hansen, P. (2001). Healthcare spending and economic output: Granger causality. *Applied Economics Letters,* 8(8), 561-564.

Di Matteo, L. (2004). What drives provincial health expenditure?. *Canadian Tax Journal,* 52(4), 1102-1120.

French, D. (2012). Causation between health and income: A need to panic. *Empirical Economics,* 42, 583-601.

Hansen, P and King, A. (1996). The determinants of healthcare expenditure: A cointegration approach. *Journal of Health Economics,* 15, 127-137.

Jacobs, J. A. (1996). Gender inequality and higher education. *Annual Review of Sociology,* 22(1), 153-186

Nikkhah, H.A., Redzuan, M., & Abusamah, A. (2010). The Effect of Women's SocioDemographic Variables on Their Empowerment, *Journal of American Science,* 6(11), 426-434

Pyke, S. W. (1997). Education and the "woman question." *Canadian Psychology,* 38(3), 154.

Stromquist, N. P. (2015). Women's Empowerment and Education: Linking Knowledge to Transformative Action. European Journal of Education, 50(3), 307–324.

Suen, S. (2013). The education of women as a tool in development: challenging the African maxim. *Hydra,* 1, 60–76.

Sundaram, M. S., Sekar, M. & Subburaj, A. (2014). Women Empowerment: Role of Education. *International Journal in Management & Social Science,* 2(12), 76–85.

Tıraşoğlu, M., Yıldırım, B. (2012).Yapısal Kırılma Durumunda Sağlık Harcamaları ve Ekonomik Büyüme İlişkisi: Türkiye Üzerine Bir Uygulama. *Electronic Journal of Vocational Colleges,*2,111-117.

Wallerstein, N. (2001). Powerlessness, Empowerment and Health: Implications for Health Promotion Programs. *American Journal of Health Promotion,* 6(3), 197-205.

Zafar, Z. (2016). Women Empowerment in South Asia : The Role of Education in Empowering Women in India, *Journal of Indian Studies.* 2(2), 103–115.

Current Approaches in the Treatment of Lumbar Disc Herniation

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Introduction

The spine is a bone structure consisting of cervical, thoracic, lumbar and coccyx, which are connected to each other in a regular way called vertebrae. The robust and flexible structure that connects the vertebrae and serves as an intervertebral cushion is called a disc. Its outer layer is called annulus fibrosus and the inner layer is composed of connective tissue called nucleus pulposus in gel structure. As age progresses, the annulus fibrosus located in the inner part loses its water content and cannot fully fulfill its task. Thus, the annulus fibrosus herniates outward, forming the pathology we call disc herniation. It usually causes complaints of lower back and leg pain.

The most important complaint in lumbar disc herniation is pain, and patients usually complain of a blunt pain in the lower back. The pain is sometimes severe enough to prevent movement and causes paravertebral muscle spasm. This pain is due to the stretching of the posterior annulus fibrosus and the posterior longitudinal ligament, which cover the pain fibers. The pain that occurs in the form of sciatica is the result of the pressure of the disc material on the nerve roots. The pain characteristically increases with sitting, walking around, coughing, sneezing and stretching. The severity of the pain depends on the location, amount and pressure effect of the herniation (Aydogan, 2005).

Low back pain is very widely observed in society. Due to the high incidence of low back pain, it causes loss of labor force and serious economic losses. Intervertebral disc degeneration, which causes lumbar disc herniation (LDH), is the main source of low back pain (Amin, Andrade & Neuman, 2017). Early diagnosis and treatment plan are crucial as the frequency of occurrence of LDH in society is high.

Lumbar disc herniation constitutes one of the important social and economic problems of today, especially due to the fact that it gives symptoms with findings such as low back pain, leg pain, strength defects and hypoesthesia (Guven, Cırak, Isik & Kiymaz, 1999). In addition, it is clear that the cost of health expenditures is very high considering the loss of labor force (Cetinkaya, 2005). Low back pain (LBP) accounts for about 15% of the leave/reports (Ozbayir, 2010).

Examination

The examination begins with an inspection in patients presenting with low back pain. Patients with lumbar disc herniation may have typical postural deformities. The patient takes positions aimed at relaxing the nerve root under pressure (Toplamaoglu, 2005). The pain aggravates by being pressing on spinous protrusions in the lower back, and spasm of the paravertebral muscles is felt by palpation (Zileli & Gulmen, 2002).

Methods used in the neurological examination of a patient with lumbar disc herniation are nerve stretching tests (straight leg lifting, Laseque, counter – Laseque, Bragard, knee extension

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while sitting, Naffziger, Kemp and femoral nerve stretching tests) muscle strength measurement, atrophy measurement, examination of reflexes and sensory examination.

Magnetic resonance imaging (MRI) (Hardy, 1997), computed tomography (CT) (Hardy, 1997), myelography, discography, bone scintigraphy, direct lumbar radiography (Zileli, 2001) and electromyography (EMG) are used in the diagnosis of lumbar disc herniation

Treatment

Despite the different etiology and pathogenesis, the principles of treatment in lumbar pain syndromes are quite similar. These general principles and measures are bed rest, physical agents, massage, traction, manipulation, orthoses, exercise, waist school and sports activities (Kirazli, 2002).

Surgical treatment is recommended for patients who do not respond to medical and conservative treatment, who have a serious deterioration in the quality of life, who have a deficit and severe pain. Most patients with severe clinical symptoms due to lumbar disc herniation show significant improvement within a month. The purpose of surgery is to accelerate the healing process of patients. Surgical treatment of lumbar disc herniation in well-selected patients has high success and low complication rates (Zileli, 2002). The purpose of surgical treatment is mainly to relieve pain. With surgical treatment, it is aimed to improve motor and sensory losses in 10-20% of patients. Low back pain is less affected by surgery, but sciatica goes away well in many patients. Light motor losses do not constitute an indication for surgery in patients other than patients who develop motor defects on the basis of a narrow canal (Zileli, 2002).

1-Precise surgical indications:

- *Cauda equina* syndrome: 0.2-2%
- Severe muscle weakness (low foot): 5-20%
- Progressive motor deficit

2-Relative surgical indications:

- Severe sciatica unresponsive to conservative treatment
- Motor deficit that has settled along with positivity in leg stretching tests
- Disc herniation on the basis of a narrow canal
- Recurrent neurological deficit
- Social indication

Factors that negatively affect surgical success include psychosocial disorders, the goal of making gains from the workplace, female patients and suspected instability of the L4-5 disc hernia (Zileli, 2002).

There is still no consensus on the medical and surgical treatment of intervertebral disc herniation, which is often encountered in the lumbar region. Many different methods have been used in the surgical treatment of lumbar disc herniation, and today different surgical procedures are being applied with new technologies. Also, among these surgical treatments, today the most commonly performed one is discectomy by microsurgical method. With this common surgical method, it is aimed to remove the nerve root compression created by the herniated disc by causing minimal damage to normal tissues (Thongtrangan et al., 2004).

Today, microsurgical discectomy is widely accepted as the gold standard of lumbar disc herniation surgery. However, in parallel with the increasingly widespread use of endoscopic techniques in surgery over the past 30-40 years, especially the studies conducted by surgeons such

as Yeung and Rütten with the devices and techniques they have developed, have ensured that complete endoscopic approaches are a serious alternative to microsurgical operations.

Complete endoscopic lumbar disc surgery can be examined under two main headings: Transforaminal (TF) ve Interlaminar (IL). Although there is no indication difference between microsurgical and endoscopic interventions, the choice of these two endoscopic techniques is based on some criteria. . Although the TF approach seems to be the ideal choice in terms of providing direct access to the target tissue (disk material), in cases where the ruptured fragment extends beyond the lower border of the upper pedicle or forward from the middle of the lower pedicle, and in patients whose foramen is located above the level of the iliac bone on direct X-rays, many endoscopic surgeons, especially Rütten, recommend that the IL approach be preferred. Again, the IL technique stands out as an approach to which surgeons with neurosurgical origin and microsurgical discectomy experience are more accustomed and predisposed to anatomy.

There is usually no need for bed rest after surgery. Patients can be discharged on the same day or the following day.

The complications of endoscopic disc herniation operations are not different from microsurgical discectomies. Motor and sensory neurological problems are reported in less than 3%. A dura injury can be seen, but usually does not require repair. As a result, there is no significant difference in success and complications between complete endoscopic lumbar disc herniation and microsurgical decectomy with an interlaminar approach. However, factors such as increased patient comfort, shorter hospitalization time, excellent anatomical image brought by high technology are increasingly enabling endoscopic surgery to stand out.

With the development of technology, different surgical approaches are coming to the fore in the treatment of lumbar disc herniation. Although microsurgical discectomy is still the gold standard among existing technologies, it is open to different new approaches in lumbar disc herniation treatments with developing technologies.

REFERENCES

Amin RM, Andrade NS, Neuman BJ. (2017). Lumbar disc herniation. *Current reviews in musculoskeletal medicine*., *4*(10), 507-16.

Aydogan N. (2005). *Lomber Disk Hernisi Nedeniyle Cerrahi Girişim Uygulanan Hastaların Taburculuk Aşamasındaki Bilgi Gereksinimleri.* Yüksek Lisans Tezi, Ankara: Hacettepe Universitesi Sağlık Bilimleri Enstitüsü, Cerrahi Hastalıkları Hemşireliği Programı.

Cetinkaya FB. (2005). *Lomber Disk Hernili Hastalarda Egzersiz ve Elektrik Stimülasyonunun Etkinliği.* Uzmanlık Tezi, Istanbul: TC. Sağlık Bakanlığı 70. Yıl Istanbul Fizik Tedavi ve Rehabilitasyon Eğitim ve Araştırma Hastanesi.

Guven MB, Çırak B, Isik HS, Kiymaz N. (1999). Lomber disk hernilerinde retrospektif bir çalışma. *Van Tıp Dergisi*, *1*(6), 20-23.

Hardy Jr. RW. (1997). Lumbar discectomy: surgical tactics and management of complications. In: Frymoyer JW, Ducker TB, Hadler NM, Kostuik JP, Weinstein JN, Whitecloud III TS (Eds.). *The adult spine: principles and practice* vol 2, 2nd ed. Philadelphia: Lippincott-Raven Publishers. p.1947-59.

Kirazlı Y. (2002). Lomber disk hastalığında fizik tedavi ve rehabilitasyon. Zileli M, Özer AF (Editörler). *Omurilik ve omurga cerrahisi* cilt1. 2nci baskı, Izmir:Meta Basım Matbaacılık Hizmetleri; s.661-74.

Ozbayir T. (2010). *Nörolojik travmalar. Dahili ve Cerrahi Hastalıklarda Bakım.* Ed: Karadakovan A, Aslan FE, Adana: Nobel Kitabevi. 1245-1274.

Thongtrangan I, Le H, Park J, Kim DH. (2004). Minimally invasive spinal surgery: a historical perspective. *Neurosurg Focus, 1*(16), 1-10.

Toplamaoglu H. (2005). Spinal dejeneratif hastalıklar. Aksoy K, Palaoğlu S, Pamir N, Tuncer R (Editörler). *Temel Nöroşirürji* cilt II. Ankara: Buluş Tasarım ve Matbaacılık Hizmetleri; s.1056-62.

Zileli M, Gulmen V. (2002). Lomber disk hernisinde yakınma ve bulgular. Zileli M, Özer AF (Editörler). *Omurilik ve omurga cerrahisi* cilt1. 2nci baskı, Izmir:Meta Basım Matbaacılık Hizmetleri;. s.635-46.

Zileli M. (2001). Lomber disk hastalığında klinik, tanıya yaklaşım ve cerrahi endikasyonlar. Özer AF (Editör). *Lomber disk hastalığı.* 2nci baskı, Logos Tıp Yayıncılık; s.116-41.

Zileli M. (2002). Lomber disk hastalığında tedavi endikasyonları ve hasta yönetimi. Zileli M, Özer AF (Editörler). *Omurilik ve omurga cerrahisi* cilt1. 2nci baskı, Izmir:Meta Basım Matbaacılık Hizmetleri; s.647-60.

Occupational Safety and Health Awareness Among Healthcare Professionals[1]

Gülseren GÜNAYDIN[2]
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Introduction

The significance of occupational safety and health in the workplace is growing in the rapidly changing and evolving global environment. Studies on occupational health and safety are crucial in the healthcare profession, as in all working domains. Occupational safety and health refer to protecting employees from all hazardous risks, including keeping them safe and sustaining their health, giving social and psychological support, and employing qualified persons in the right jobs. Establishing a suitable working atmosphere and striving to work within this scope also fall under the occupational health and safety standards (Eroğlu et al., 2022:914; MEB, 2017:3). The primary objectives of occupational safety and health studies are to protect employees from any potential risks originated from the working environment, identify these risks in advance, and take the necessary and precautionary measures. It is also viable to enumerate the aspect of sustaining employees physically and psychologically from work-related accidents and diseases among the objectives of occupational health and safety (Çavuş and Keskin, 2020:629). Therefore, it is essential to raise employee awareness of occupational safety and health, to expand their awareness statuses, to translate this state into a positive and permanent behavior among employees, and to accept this culture throughout the entire organization to restrain the event of accidents and occupational diseases in the workplace and provide a safe workplace atmosphere (Yilmaz, 2019:2).

MATERIAL AND METHOD

The study objective was to assess occupational safety and health awareness among healthcare professionals. The study was carried out between April 5 and September 15, 2015, among the staff members of five public hospitals in the center of Trabzon province. By calculating the study target population as 256 regarding all staff members who worked in these facilities between the study dates, 168 nurses, 62 doctors, and 26 healthcare technicians comprised the total sample size. Participants gave their written consent, allowing the study to be conducted in compliance with ethical standards. The study obtained its study permit from the General Secretariat of the Public Hospitals Association (Trabzon) on May 20, 2015, and received its Ethics Committee approval from the Scientific Research and Publication Ethics Committee of GŞÜ on April 30, 2015. The study employed an occupational safety and health awareness form, whose data were compiled by

[1] This study was reproduced from the master's thesis and presented previously as an oral (summary) presentation at the 'First International 11th Congress of Health and Hospital Administration.'

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Bayılmış in 2013, and a survey form designed by researchers within the parameters of the literature and retaining information about the field studied. The data collecting forms included a section with multiple questions potentially to identify the demographic characteristics of the participants and further comprised other parts retaining information on occupational safety and health awareness. Face-to-face interviews were the primary tools to acquire the research data. The information gathered through the data collecting form was coded, entered into the IBM SPSS Statistics 22 package program, and then analyzed. The data analysis included frequency, percentage, chi-square tests, and arithmetic mean.

RESULTS

This section outlines the study findings. Information related to the participants' demographic features was summarized below: Considering the participants' ages, 24.6% ranged from 18 to 25, whereas 30.1% were between 26-30, 34% were between 31-40, and 11.3% were 41 and over. Of all the participants, 26.2% were male, and 73.8% were female. Regarding their marital statuses, 60.2% of the participants were married, whereas 39.8% were single. Additionally, while 15.2% of the participants were solely high school graduates, 11.7%, 16.8%, 35.5%, and 20.7% of them had a doctoral degree, master's degree, bachelor's degree, and associate's degree, respectively. Furthermore, 61.3% of the participants worked in a mixed manner, whereas 16.4% were on call, 19.5% were during the day shift, and 2.7% were on the night shift. Finally, the work experiences of the participants were as follows; 3.5% were less than a year, 42.2% were between 1-5 years, 21.5% were between 6-10 years, and 32.8% had 11 years or more experience.

Table 1. *OSH awareness levels of the study group*

	Frequency	**Ratio**	**Percentage in Valid Responses**	**Cumulative Percentage**
Very Poor	11	4.3	4.3	4.3
Poor	42	16.4	16.4	20.7
Average	105	41	41	61.7
Good	77	30.1	30.1	91.8
Very Good	21	8.2	8.2	100
Total	256	100	100	

* OSH: Occupational Safety and Health

According to the data in Table 1, the OSH awareness level of 79.3% of participants was at a satisfactory level (average + good + very good).

Table 2. *Overall assessment levels of the study group on workplace OS*

	Frequency	**Ratio**	**Percentage in Valid Responses**	**Cumulative Percentage**
Very poor	19	7.4	7.5	7.5
Poor	52	20.3	20.4	27.8
Average	119	42,2	46.7	74.5
Good	59	19,5	23.1	97.6
Very Good	7	4,3	2.7	100
Total	256	100	100	

*****OS: Occupational safety

While 2,7% of the participants stated that their OS levels in the working environment were very good, 23,1% responded as good, and 46,5% as average.

Table 3. *Awareness levels about the existence of an OHS Board in the workplace*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
No	50	19,5	20	20
Yes	206	80,5	80	100
Total	256	100	100	

Almost 77.7% of the participants were aware of the existence of an OHS board in the workplace.

Table 4. *Satisfaction levels of participants from workplace OHS activities*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
Very poor	27	10.5	10.5	10.5
Poor	60	23.4	23.4	34.0
Average	108	42,2	42.2	76.2
Good	50	19,5	19.5	95.7
Very Good	11	4,3	4.3	100.0
Total	256	100	100	

About 66% of the participants (average + good + very good) responded that they were satisfied; however, 33,9%(poor + very poor) of them stated that they were unsatisfied with the OHS activities in their workplaces.

Table 5. *Participants' occupational risks awareness levels at workplace*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
Very poor	7	2.7	2.7	2.7
Poor	15	5.9	5.9	8.6
Average	66	25.8	25.8	34.4
Good	129	50.4	50.4	84.8
Very Good	39	15.2	15.2	100.0
Total	256	100	100	

Almost 91.4% of the participants (average + good + very good) stated that they were quite aware of the occupational risks, whereas 8.6% (poor + very poor) responded that they had a limited level of risk awareness.

Table 6. *Participants' awareness level about their legal rights in the event of an occupational accident*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
Very poor	23	9.0	9.0	9.0
Poor	41	16.0	16.1	25.1
Average	114	44.5	44.5	69.6
Good	64	25.0	25.0	94.6
Very Good	14	5.4	5.4	100
Total	256	100	100	

Considering the data in Table 6, 74.9% of the participants (average + good + very good) indicated that they were aware of their legal rights, while 25% (poor + very poor) responded as they were unaware.

Table 7. *Distribution of the participant's response to the query that 'my health would be a priority in the face of a hazardous situation'*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
Strongly Disagree	0	0	0	0
Disagree	12	4.7	4.7	4.7
Undecided	29	11.3	11.4	16.1
Agree	137	53.5	53.1	69.3
Strongly Agree	78	30.5	30.7	100.0
Total	256	100	100.0	

About 84% of the participants responded as either 'agree' or 'strongly agree' to the query questioning whether personal health would be a priority in the face of a hazardous situation.

Table 8. *Distribution of the participant's response to the query that 'necessary precautions have been taken in the workplace in the face of a dangerous scenario'*

	Frequency	Ratio	Percentage in Valid Responses	Cumulative Percentage
Strongly Disagree	4	1,6	1.6	1.6
Disagree	48	18,8	18.9	20.5
Undecided	94	36.7	36.2	56.7
Agree	91	35.5	35.8	92.5
Strongly Agree	19	7.4	7.5	100.0
Total	256	100	100.0	

According to data in Table 8, 36.7% of the participants responded as 'undecided' about this query, whereas 18,8% and 4% selected 'disagree' and 'strongly disagree' options, respectively.

DISCUSSION

The primary objective of this study was to examine occupational safety and health awareness among health workers. According to the study findings, the employees' OHS awareness level was highly satisfactory, with 41% of the participants responding as an average, and 38.3% stating as either good or very good options. About 80% of employees became aware of a workplace occupational safety and health committee. Similarly, a majority of the participants (72.3%) stated that there was an occupational security mechanism in their workplace, with 46.5%, 23.1%, and 2.7% reporting to have an average, a good, and very good level, respectively. The Occupational Health and Safety Law (OSHA) No. 6331 explicitly states the requirement of establishing an OSH Board, one of the OSH practices in the workplace (Ozden, 2022: 141). In 2020, Gül, Özalp, and Andsoy reported that the occupational safety awareness of healthcare professionals in the private sector was higher than that of public institutions (Gül, Özalp, and Andsoy, 2020:39). This study, on the other hand, revealed that a sizable portion of the participants posed higher satisfaction with the workplace OSH activities (4.3%, 19.5%, and 42.2% selected very good, good, and average options). Only 23.4% and 10.5% of the participants responded as poor and very poor, respectively, indicating low satisfaction with workplace OSH practices. Correspondingly, a study by Öngel in 2022 reported that employees were satisfied with workplace occupational health and safety measures (Öngel, 2022:1646). Furthermore, Yıldız, 2020 stated that the implementations for occupational safety had a favorable influence on the occupational safety performance of the employees (Yıldız, 2020: 574). The awareness level of the participants about workplace occupational risks was also extremely high, as 15.2%, 50.4%, and 25.8% of the participants responded as very good, good, and average, respectively. Only 8.6% of the participants responded

to this query as poor or very poor options. Similarly, a substantial quantity of participants remarked that they were aware of their legal rights in case of an occupational accident, with responses of 5.1%, 25%, and 44.9% of very good, good, and average options, respectively. Only 25% of the participants answered this query as poorly (16%) and very poorly (9%). As reported, the awareness of occupational health and safety favorably impacts the quality of the task achieved (Kahveci and Gültekin, 2022:105). In response to the query 'my health would be a priority in the face of a hazardous situation,' 84% of the employees responded with either 'agree' or 'strongly agree' options. Regarding the question of 'necessary precautions have been taken in the workplace in the face of a dangerous scenario,' however, 36.7% of the participants chose the 'undecided' option. In their study in 2018, Tan and Çalışkan emphasized that employees who serve as managers in organizations had positive thoughts about occupational safety and health practices (Tan and Çalışkan, 2018:38).

CONCLUSION AND RECOMMENDATIONS

The current study findings proved that the healthcare professionals under consideration posed average and higher awareness of occupational health and safety in their workplace. However, given the risks and hazards associated with the workplace, priority should always be given to sustaining a high degree of occupational health and safety. In this context, it is crucial and advised that all occupational safety and health procedures, including those for managers and all workers, be fully implemented by rules specified in the laws, with a focus on establishing, adopting, sustaining, and examining an occupational safety and health culture in the workplace.

REFERENCES

Eroğlu, G., Şüküroğlu, E. E., Günaydın, M. & Şüküroğlu, S. (2022). İş Güvenliği Kültürünün İş Verimliliği Üzerine Etkisi: Pres Fabrikası Örneği. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi*, 11(3), 913-926. Doi: 10,37989/gumussagbil.1136462.

Gül, A., Özalp, Ş. & Işık Andsoy, İ. (2020). Sağlık Kurumlarında İş Güvenliğinin Değerlendirilmesi. *Zeynep Kamil Tıp Bülteni,* 51(1), 35-39. Doi: 10.16948/zktipb.411568.

Kahveci, S. & Gültekin, Z. (2022). Liman Çalışanlarının İş Sağlığı Ve Güvenliği Farkındalık ve Bilinç Düzeylerinin İş Performansına Etkisi. *Ergonomi,* 5(2), 98-107. Doi: 10.33439/Ergonomi.1127818.

Keskin, R. & Çavuş, Ö. H. (2020). İş Sağlığı ve Güvenliği Eğitimlerinin Sağlık Sektöründe Güvenlik Kültürü Üzerindeki Etkilerinin Analizi. *Yönetim ve Ekonomi Dergisi.* 27(3), 627-644. Doi: 10,18657/yonveek.592878.

Öngel, G. (2022). İnşaat Sektörü Çalışanlarının İş Sağlığı ve Güvenliği Uygulamaları Hakkındaki Görüşleri. *Electronic Journal Of Social Sciences,* 21(84), 1632-1650. Doi: 10.17755/esosder.1094786

Özden, B. (2022). Asıl İşveren Alt İşveren İlişkisinde İş Sağlığı ve Güvenliği Kurullarının Oluşumu ve İşleyişi. *İstanbul Medeniyet Üniversitesi Hukuk Fakültesi Dergisi*, 7 (2022): 139-167.

T.C. Millî Eğitim Bakanlığı. Mesleki Gelişim (2017). *İş Sağlığı ve Güvenliği.* Ankara.

Tan, F. Z. & Çalışkan, S. (2018). Yöneticilerin İş Sağlığı ve Güvenliği Uygulamalarına Yönelik Algılarının Analizi Üzerine Bir Araştırma. *Karaelmas Journal of Occupational Health and Safety*, 2(1), 31-44. Doi:10.33720/kisgd.384136.

Yıldız, A. (2020). Hastane İş Güvenliği Uygulamalarının Sağlık Çalışanlarının İş Güvenliği Performansına Etkisi. *Akademik Araştırmalar ve Çalışmalar Dergisi (Akad),* 12(23), 566-578. Doı: 10.20990/Kilisiibfakademik.709754.

Yılmaz, F. (2019). İş Sağlığı ve Güvenliği Uygulamalarının Çalışma Yaşamına Etkisi. *Ohs Academy,* 2 (1), 1-10. Retrieved From Https://Dergipark.Org.Tr/Tr/Pub/Ohsacademy/İssue/44841/539852.

The Nutrients of Formula Milk in Terms of Meeting the Needs of an Individual

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Introduction

Every baby born at the time of mother's milk has all of the nutritional elements needed for normal development which is enough for the first 6 months and it Is the most suitable natural food for newborns and infants. It can be supplemented with continuous formula and additional foods after 6 months (Köksal & Gökmen, 2000).

The types of formulas in the market, their production processess and marketability have been standardized according to Codex Alimentarius. These products may lead to unhealthy conditions such as an increase of infection risk and a higher rate of obesity when compared to the breast milk. Prticularly, all the materials added to the formula to make it closer to the breast milk should not be harmful (Gökçay, Eren & Devecioğlu, 2012).

The recommended baby formulas during the first 12 months' or '6-12 months' are divided into 4 groups in the market. In the first group, there are number one formulas used to feed babies of '0-6 months'. Marketable Products for the first 6 months are: baby milk, baby food, baby formula, bottle products or known as formula milk. In the second group: Milk number 2 and 3 are used after the 6th month Continuously and can also be named as Continuous formula or follow-on formula as well. While the other group is named as formula spoons. Finally there are baby food and jar foods (Gökçay, Eren & Devecioğlu, 2012).

The formulas are recommended base on age groups: 0-6 month period, 9-12 and over 1 year old formulas are on the market. The daily nutritional requirements of these formulas should be appropriate, the composition must be stable, well tolerated, meet human needs and help the digestive system. It should not cause metabolic disturbance and lack of nutrition when used for a long time (İşler, 2010).

Breast milk and formula milk

Breast milk is very important in newborn nutrition and offers the best physiological nutrition to the newborn. However, formula milk can be substituted for breast milk by mothers when breast milk is not available (Guo, 2020; Henderson, Antony & McGuire, 2007; Scano & et al., 2016).

Looking at the historical development of infant formulas, it is seen that the first articles coincide with the end of the 19th century. However, the evidence for the use of alternative solutions in cases where the mother cannot breastfeed her own baby dates back to 2000 BC. These solutions were breastfeeding the baby by another woman or giving the milk of a mammal (cow, goat, pig, horse and camel) to the baby. It is seen that the first commercial baby food was developed

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in 1865 (Castilho & Barros; 2010; Osborn 1979a, 1979b; Schuma, 2003; Stevens, Patric & Pickler, 2009; Wickes 1953a, 1953b, 1953c, 1953d,1953e).

Although breast milk contains less nutrients than formula milk, it can provide significant non-nutritive advantages over formula milk for preterm and low birth weight infants (Henderson, Antony & McGuire, 2007).

Infant formulas are available in 3 forms: powder, liquid concentrate, ready-to-feed. While liquid and powder forms are mixed with water before feeding; formulas that are ready to be fed do not require any processing. Mixing with water is the first step when preparing powder and liquid concentrated formulas. Vegetable oils, vitamins, minerals and iron-added cow's milk are added to formula milks during preparation. In addition, soy-based and specialized formulas are also available in the market. As a result, some ingredients can be added to formula milk to mimic the composition of breast milk. However, the suitability of the newly added compounds for infant feeding needs to be proven (Hernell, 2011).

In a study, powdered and ready-to-feed formulas were compared with breast-fed infants as models, and it was concluded that infants fed with ready-to-feed formula showed better physiological growth than standard formula prepared from powder form. However, since these findings are important in terms of infant nutrition, these results should be repeated using other formulas (Lucas, Locton & Davies, 1992).

Breastfeeding mothers and healthcare professionals will need evidence of the advantages of formula milk before opting for formula over breast milk when feeding premature and low birth weight infants (Henderson, Antony & McGuire, 2007).

In a study by Scano et al., when the metabolites of formula milk were compared with breast milk, breast milk showed a higher amino acid content; higher amounts of malic acid, sugars (glucose, fructose and galactose) and mannitol were observed in formula milk (Scano et al., 2016).

Dessi et al. investigated urine metabolites in newborns in terms of breastfeeding and formula feeding. The results proved that there is a significant difference in infant metabolism as a result of feeding with breast milk and formula milk in the first week of life (Dessi et al., 2016).

Further research is needed as the results observed in formula-fed infants do not match those observed in breast-fed infants (Dessi et al., 2016).

Table 1: Comparison of Mother's Milk and Formula Contents (İşler, 2010)

Contents	**Mother'smilk** **100 ml**	**Formula** **100 ml**
Protein (g)	1.3	2.6
Fat (g)	4.2	3.6
Carbohydrate (g)	7	9.8
Energy (kcal)	71	85
Calcium (mg)	35	72
Phosphorus (mg)	15	48

Basic composition of ready-to-eat baby formulas

The values are valid for the products prepared according to the instructions given by the manufacturer or offered for sale directly ready for use (Anonim, 2008).

Energy

The formula should contain at least 60 kcal / 100 mL and up to70 kcal/100mL of energy.

Protein

Baby formulas made from cow's milk proteins

The formula should contain at least 1,8 g/100 kcal and up to3 g/100 kcal of protein.

Baby formulas made from protein hydrolysates

The formula should contain at least 1,8 g/100 kcal and up to3 g/100 kcal of protein.

The L-carnitine contents hould be at least1.2 mg / 100 kcal.

Infant formula sproduced solely from soy protein isolates or a mixture of soy protein isolates and cow milk proteins:

The formula should contain at least 2,25 g/100 kcal and up to3 g/100 kcal of protein.

The L-carnitine content should be at least1.2 mg / 100 kcal.

Cholin

The formula should contain at least 7 mg/100 kcal and up to50 mg/100 kcal of cholin.

Minerals

Table 2: Infant Formulas Produced From Cow's Milk Proteins Or Protein Hydrolysates: Annex 1

	At 100 kcal	
Minerals	**Least**	**Most**
Sodium(mg)	20	60
Potassium (mg)	60	160
Chlorine (mg)	50	160
Calcium (mg)	50	140
Phosphorus(mg)	25	90
Magnesium (mg)	5	15
Iron (mg)	0,3	1,3
Zinc (mg)	0,5	1,5
Copper (mcg)	35	100
Iodine (mcg)	10	50
Selenium (mcg)	1	9
Manganese (mcg)	1	100
Fluoride (mcg)	-	100

Calcium / phosphorus ratio should not be less than 1 and not more than 2.

Table 3: Baby Formula Made From Soya Protein İsolates Single Or Mixed With Cow's Milk Proteins

	At 100 kcal	
Vitamins	**Least**	**Most**
Vitamin A (mcg-RE) (1)	60	180
Vitamin D (mcg) (2)	1	2,5
Tiamin (mcg)	60	300
Riboflavin (mcg)	80	400
Niasin (mcg) (3)	300	1500
Pantothenicacid(mcg)	400	2000
Vitamin B6 (mcg)	35	175
Biotin (mcg)	1,5	7,5
Folicacid(mcg)	10	50
Vitamin B12 (mcg)	0,1	0,5
Vitamin C (mg)	10	30
Vitamin K (mcg)	4	25
Vitamin E (mg α-TE)(4)	0,5	5

(1) RE = all trans retinol equivalent

(2) In the form of cholecalciferol, 10 mcg = 400 I.U. Vitamin D

(3) Niasine trans formed forms

(4) α-TE = d-α-tocopherol equivalent

Table 4: Recommended Daily Energy and Nutrient Intake Levels for Turkey (2)

	0-6 months	**7-12 months**
Energy (kcal)	545	710
Protein (g)	9.12	14.9-18.0
Vitamin A (mcg RE)	400	500
VitaminD (mcg)	10	10
VitaminE (mg)	4	5
VitaminK (mcg)	2	2.5
VitaminC (mg)	40	50
Tiamin (mcg)	200	300
Riboflavin (mcg)	300	300
Niasin (NE mcg)	2000	4000
VitaminB6 (mcg)	100	300

Folate(mcg)	65	80
VitaminB12 (mcg)	0.4	0.5
PantothenicAcid(mcg)	1700	1800
Biotin(mcg)	5	6
Cholin (mg)	125	150
Calcium (mg)	210	600
Phosphorus (mg)	100	270
Iodine(mcg)	110	130
Flor (mcg)	10	500
Magnesium (mg)	30	75

The energy requirement of the individual is 545 kcal in 0-6 month period and 710 kcal in 7-12 month period. In these periods, when the energy need sare met 100% with the formula milk, the ratios of formula milk to nutritional requirements for individual needs shown in table.

Table 5: Ratios Met Of Nutritional Needs Of İndividuals Fed With Formula Milk Which İs 545 Kcal İn 0-6 Month Period And 710 Kcal İn 7-12 Month Period: (%)

	0-6 months		**7-12 months**	
Nutrients	**Least (%)**	**Most (%)**	**Least (%)**	**Most (%)**
Energy (kcal)	100	100	100	100
Protein (g)**	107.52	179.25	71-85.76	118.28-142.9
Protein (g)***	107.52	179.25	71-85.76	118.28-142.9
Protein (g)****	134,45	179.25	88.75-82.29	118.28-142.9
Fiber(g)	-	-	-	-
VitaminA (mcg RE)	81.75	245.25	85.2	255.6
VitaminD (mcg)	54.5	136.25	71	177.5
VitaminE (mg)	68.12	681.25	71	710
VitaminK (mcg)	1090	6812.5	1136	7100
VitaminC (mg)	136.25	408.75	142	426
Tiamin (mcg)	163.5	817.5	142	710
Riboflavin (mcg)	145.29	726.48	189.28	946.43
Niasin (NE mcg)	81.75	408.75	53.25	266.25
VitaminB6 (mcg)	190.75	913.75	82.78	414.14
Folate(mcg)	83.82	419.21	88.75	443.75

VitaminB12 (mcg)	136.25	681.25	142	710
PantothenicAcid(mg)	128.18	641.13	157.76	788.88
Biotin(mcg)	163.5	817.5	177.5	887.5
Cholin (mg)	30.52	218	33.08	236.64
Calcium (mg)	129.71	389.23	147.89	177.5
Phosphorus(mg)	136.25	490.5	65.67	236.64
Phosphorus (mg)*	163.5	545	78.88	262.91
Iron (mg)	605.54	2624.06	19.31	83.85
Iron (mg)*	908.29	437.03	29.03	129.07
Zinc (mg)	136.25	408.75	118.28	355
Iodine(mcg)	49.54	747.7	54.59	273.06
Flor (mcg)	-	5450	-	142
Magnesium (mg)	90.79	272.5	47.28	142
Manganese (mcg)	181.64	18166.48	1.36	118.28
Copper(mcg)	95.37	272.5	112.89	322.69
Selenium(mcg)	36.29	322	35.5	319.5

*Baby formula made from soya protein isolates sigle or mixed with cow's milk proteins

**Baby formulas made from cow's milk proteins

***Baby formulas made from protein hydrolysates

**** Infant formulas produced solely from soy protein isolates or a mixture of soy protein isolates and cow's milk proteins (Anonim, 2008).

Result

Baby's formula which is ready for consumption is prepared according to the produced formula values as a reference while the daily energy and nutritional needs of the baby between 0-6 months and 7-12 months were researched and compared.

The formula milk is produced according to the base reference value to the reference values given in the Communiqué and does not adequately meet the requirements of vitamins A, D, E, Niacin, Folate, Choline, Iodine, Magnesium, Copper and Selenium during the 0-6 month period while It has been determined too that the needs of protein, vitamins A, D, E, Niacin, B6, Folate, Choline, Phosphorus, Iron, Iodine, Magnesium, Manganese and Selenium are not enough in 7-12 month period.

The formula milk produced according to the ceiling reference values of the Communiqué is found to meet all the requirements in 0-6 month period and not enough to meet Iron needed in 7-12 month period adequately.

As a result, since formula milk can not meet the baby needs at the equivalent level of mother's milk, breastfeeding should be supported during 0-12 month period.

References

Anonim, 2008. Bebek Formülleri Tebliği. No: 2008/52, R.Gazete: 04.09.2008-26987.

Baysal, A., 2009. Beslenme. Hacettepe Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, 12. Baskı, Ankara, Sayfa 403-404.

Castilho, S.D., Barros Filho, A.A., 2010. The history of infant nutrition. J. Pediatr. 86 (3), 179–188.

Dessì, A., Murgia, A., Agostino, R., Pattumelli, M. G., Schirru, A., Scano, P., ... & Caboni, P. (2016). Exploring the role of different neonatal nutrition regimens during the first week of life by urinary GC-MS metabolomics. International journal of molecular sciences, 17(2), 265.

Gökçay, G., Eren, T., Devecioğlu, E., 2012. Bebek Mamalarındaki Katkı Maddeleri. Çocuk Dergisi Sayfa 60-65.

Guo, M. (Ed.). (2020). Human milk biochemistry and infant formula manufacturing technology.

Happe, R. P., & Gambelli, L. (2015). Infant formula. In Specialty oils and fats in food and nutrition (pp. 285-315). Woodhead Publishing.

Henderson, G., Anthony, M. Y., & McGuire, W. (2007). Formula milk versus maternal breast milk for feeding preterm or low birth weight infants. Cochrane database of systematic reviews.

Hernell, O. (2011). Human milk vs. cow's milk and the evolution of infant formulas. Milk and milk products in human nutrition, 67, 17-28.

İşler. A., 2010. Prematüre Bebeklerde Enteral Beslenme ve Hemşirelik Yaklaşımı. Enteral Feeding in Preterm Infants and Nursing Approach, Ege Pediatri Bülteni, Cilt:17, Sayı:2, Sayfa 79.

Köksal, G., Gökmen, H., 2000. Çocuk Hastalıklarında Beslenme Tedavisi. Ankara, Sayfa 31.

Lucas, A., Lockton, S., & Davies, P. S. (1992). Randomised trial of a ready-to-feed compared with powdered formula. Archives of disease in childhood, 67(7), 935-939.

Osborn, M.S., 1979a. The rent breast: a brief history of wet-nursing. Midwife Health Visit. Community Nurse 15 (8), 302–306.

Osborn, M.S., 1979b. The rent breast. Part II. Midwife Health Visit. Community Nurse 15 (9), 347–348.

Scano, P., Murgia, A., Demuru, M., Consonni, R., & Caboni, P. (2016). Metabolite profiles of formula milk compared to breast milk. Food Research International, 87, 76-82.

Schuman, A.J., 2003. A concise history of infant formula (twist and turns included). Contemp. Pediatr. 20, 91–103.

Stevens, E.E., Patrick, T.E., Pickler, R., 2009. A history of infant feeding. J. Perinat. Educ. 18 (2), 32–39.

Wickes, I.G., 1953a. A history of infant feeding. Part I. Primitive peoples: ancient works: renais¬sance writers. Arch. Dis. Child. 28, 151–158.

Wickes, I.G., 1953b. A history of infant feeding. Part II. Seventeenth and eighteenth centuries. Arch. Dis. Child. 28, 232–240.

Wickes, I.G., 1953c. A history of infant feeding. Part III. Eighteenth and nineteenth century writers. Arch. Dis. Child. 28, 332–340.

Wickes, I.G., 1953d. A history of infant feeding. Part IV. Nineteenth century continued. Arch. Dis. Child. 28, 416–422.

Wickes, I.G., 1953e. A history of infant feeding. Part V. Nineteenth century concluded and twentieth century. Arch. Dis. Child. 28, 495–502.

Urethral Strictures

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A.Yüksel

Urethral stricture is a disease that causes voiding dysfunction by narrowing the urethral lumen due to fibrotic scar formation.

The age of the patient and the location of the stricture play an important role in the etiology of urethral stricture. In general, urethral stricture develops due to inflammatory, traumatic, iatrogenic, and idiopathic causes (Mathur et al. 2011). Urethral stricture causes serious complications when left untreated. Urinary tract infection, prostatitis, bladder stone, epididymitis, orchitis, hydronephrosis, periurethral abscess, sepsis, and kidney failure are the most important complications due to chronic retention.

Histology of the urethra:

While the prostatic urethra and membranous urethra are lined with transitional epithelium, the bulbar and penile urethra are lined by pseudostratified epithelium except for the distal part. In the distal part, the epithelium is the squamous epithelium and continuous with the penile skin. The inner epithelium of the female urethra is lined with squamous epithelium in the distal part, while it is transitional in the rest.

Anatomy of the urethra:

The male urethra is approximately 18 cm long, starting from the internal orifice in the bladder neck and extending to the external meatus in the penis. The male urethra is divided into anterior and posterior urethra by the urogenital diaphragm. The anterior urethra is distal to the urogenital diaphragm which consists of three parts fossa navicularis, penile and bulbar urethra. Posterior urethra extending from urogenital diaphragm to bladder neck. It consists of two parts, the membranous urethra, and the prostatic urethra. The narrowest part of the urethra is the external meatus, and the second narrowest part is the membranous urethra. Since the membranous urethra is not surrounded by a spongy tissue, it is the most affected part in external traumas. The wall of the membranous urethra is formed by an inner thin layer of smooth muscle and an outer layer of striated muscle. The outer skeletal muscle fibers form the external sphincter. The widest region of the urethra is the prostatic urethra**.** The bulbous urethra is the segment of the anterior urethra between the suspensory ligament and the membranous urethra. It is surrounded by a thick cancellous tissue and the cancellous tissue is surrounded by a bulbospongiosus muscle. The penile urethra is located between the fossa navicularis and the suspensory ligament. It is surrounded by the corpus spongiosum. It is located in the free-moving penis and is the longest part of the urethra. The fossa navicularis is surrounded by spongy tissue and is the second widest part of the penile urethra. (Latini et.al 2014)

Pathogenesis of urethral stricture

Studies have shown that the incidence of UD is 0.2-1.2%, and it is more common especially in men over 55 years of age. The pathogenesis of urethral stricture is due to damage to the urethra

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and followed by normal mucosal epithelium of the urethra is replaced by squamous metaplasia, and urine leaks into the small slits in the epithelium, causing fibrotic reactions by including the corpus spongiosum. These repetitive openings and reactions cause the development of stenosis by increasing the underlying spongiofibrosis over time. However, some extracellular cellular changes are also observed in the histopathology of urethral strictures, such as the displacement of the connective tissue from fibroblasts with dense fiber tissue (Latini, 2014).

Etiology of urethral stricture

Urethral strictures are classified into 4 main groups etiologically. These are idiopathic, iatrogenic, traumatic, and inflammatory groups. While the most common causes are iatrogenic and inflammatory in penile urethral strictures, idiopathic and iatrogenic causes are common in bulbar urethral strictures (Table 1).

Table 1: Etiology of urethral strictures

Penile Urethra Strictures	**Bulbar Urethra Strictures**
Iatrogenic, 40%	Idiopathic, 40%
Inflammation – Infection, 40%	Iatrogenic, 35%
Idiopathic, 15%	Traumatic, 15%
Traumatic, 5%	Inflammation, 10%

While idiopathic causes are mostly attributed to unnoticed traumas in the bulbar urethra or the problems of the junctional areas during embryological development, decreased blood flow and ischemia are mostly blamed on the elderly. In iatrogenic urethral strictures, cystoscopic interventions, transurethral prostate resections, and urethral catheterizations play a role in the development of penile urethra and bulbous urethral stricture. Urethral strictures with a rate of 10-40% develop mostly due to recurrent infections. Balanitis xerotica obliterans is the cause of inflammatory stenosis in young men. It causes the narrowing of the urethral meatus. Usually, urethral stricture in the bulbous urethra caused due to trauma by falling in a straddle style.

Diagnosis of urethral stricture

Male patients with urethral stricture usually apply to the outpatient clinic with complaints of decreased maximum flow rate, intermittent urination, and a residual feeling. In addition, they may apply with complications such as epididymo-orchitis, recurrent urinary tract infection, prostatitis, hematuria, or bladder stones due to urethral strictures. (Mundy & Andrich 2014)

Urine flow measurements (uroflowmetry), urine culture, urinary ultrasonography (USG), and residual urine determination are the first-line tests in the evaluation of patients who present with these complaints and are suspected of having urethral stenosis (Verla et.al, 2019). The patient urinates with a low flow in the urine flow measurement test. The presence of postvoid residual urine on USG is also important for diagnosis. USG results such as bladder wall thickening and increased trabeculation on the urinary system support the diagnosis of urethral stricture (Buckley et al.**.** Hydronephrosis, one of the complications of urethral stricture, can also be detected by the USG of the urinary system. Dynamic retrograde urethrography is another choice performed on the patient for diagnosis (Figure 1). The sensitivity of this process is 75-100% and the specificity is between 72-97%. While retrograde urethrography only detects the distal stenosis area, it is possible

to determine the proximal stenosis and the length of the stricture combined with antegrade urinary urethrography. (Mundy & Andrich 2014) Cystourethroscopy is a valuable procedure with high sensitivity and specificity in diagnosing urethral stricture. However, there is still no consensus on the best method for the diagnosis of urethral strictures.

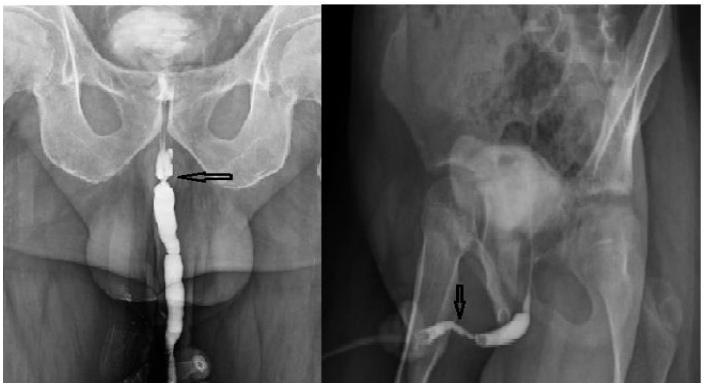

Figure 1: Representation of stricture by retrograde urethrography.

If urethral stricture cannot be detected by these diagnostic tests, cystoscopy can be performed as a next evaluation. The location, length, and severity of the stricture can be obtained with the cystoscopy procedure. Also, It allows endoscopic treatment at the same time.

Treatment of urethral stricture:

In general, three basic information including the location, length of the stricture, and the associated pathology are necessary for the treatment of urethral stricture. The treatment of urethral stricture is performed with two main methods, endoscopic and open surgical methods (GD W, 2014). The treatment methods should be discussed with the patient in terms of the severity of the disease, success rate, and recurrence rate.

Endoscopic treatment methods

Urethral dilation:

It is the simplest treatment method used only in patients with epithelial stenosis in which spongiotic tissue is not involved. This treatment aims to stretch the scar tissue and open the stenosis area before spongiofibrosis develops. In the beginning, the procedure is performed with the thinnest bougie dilator and is continued with increasing diameters to prevent traumatic false passage during dilatation. The urethral dilatation method provides an advantage in terms of local anesthesia, especially in patients who cannot receive anesthesia. The stenosis has a high probability of recurrence (Jordan, 2002).

Internal urethrotomy :

Internal urethrotomy was first described by Sachse in 1973, based on the principle of opening the urethral stricture by transurethral entry and cutting with a cold urethrotome knife at the position of 12 o'clock (Sachse 1973). After the procedure, the epithelium in the stenosis area is left for secondary wound healing. The criteria for success are healing of the stenotic area without narrowing the lumen and providing high-flow urine.

The ideal criteria for effective internal urethrotomy are localization to the bulbar urethra, the single, primary stricture <1 cm and not accompanied by spongiofibrosis. On the other hand, recurrent stricture, the size of stricture >2 cm and multiple, the presence of urinary tract infection, localization in the penile or membranous urethra, and accompanying diffuse spongiofibrosis cause the stricture to recur (Dubey, 2011).

The most common complication of internal urethrotomy is the recurrence of stenosis with a rate of 30-50%. Perineal hematoma, urethral bleeding, fistula development, and erectile dysfunction are other rare complications. The most advantageous features of this process are that it is easy to apply, cheap and effective (Heyns, 2008).

Laser urethrotomy :

It is a method that removes the scar tissue by cutting the stenotic area until it reaches the normal tissue by using a laser probe instead of a cold knife. Lasers such as Nd: YAG, Ho: YAG, argon, and excimer are used for this application. Each laser application has different levels of burning and tissue penetration at different depths. It is more successful in short and shallow stenosis. Hematuria, dysuria, urinary tract infection, urinary retention, urinary extravasation, incontinence, and fistula formation are rare complications.

Urethral stent:

It is a method used to prevent re-narrowing of the stricture by placing a stent with a urethroscope in patients with recurrent strictures. The most common complications are stent displacement and petrification, the development of spongiofibrosis, recurrent urinary tract infections, urinary incontinence, urinary retention, hematuria, and erectile dysfunction.

Open surgical reconstruction

Treatment of urethral stricture depends on the etiology of the stricture, the number of stenotic areas, the location and length of the stricture, the proximity to the sphincter, and the accompanying complications such as false passage or diverticulum. Therefore, there is no single surgical option. Surgical methods can be performed alone or in combination with each other**.** While the success rate is 30% in urethrotomy, it can be up to 90 % in urethroplasty. Although urethroplasty is considered the most effective treatment method, endoscopic treatment methods are still preferred as the first-line treatment method based on cost-effectiveness (Eltahawy et al 2007).

Anastomotic repairs

End-to-end anastomotic urethroplasty is a treatment method applied in bulbar urethral strictures <2 cm. Both ends of the stricture are removed by excision, leaving a margin of 1 cm, and the remaining urethral ends are spatulated and a tension-free end-to-end anastomosis is applied. Success rates are over 90%. In this surgery, the success rate increases if the stenosis area is completely removed and the anastomosis is made wide and without tension.

In case of urethral stenosis > 2 cm where flap or graft urethroplasty cannot be applied end-to-end anastomosis can be performed with the mobilization of the urethra by separating the two corpus cavernosum tissues to prevent tension. During this procedure, shortening of the penis, penile curvature, and temporary erectile dysfunction may develop. Because of these complications, flap or graft urethroplasty should be preferred, especially in patients with stenosis > 2 cm.

Graft urethroplasty

Graft urethroplasty is used in patients longer than 2 cm, in whom anastomotic urethroplasty cannot be performed and tension may occur during anastomosis. Buccal oral mucosa, penile skin, scrotal skin, or bladder mucosa can be used as graft material (Lumen et.al, 2012). Oral mucosa grafts are the most commonly used grafts in terms of being easy to obtain, not containing hair, being durable, and having high success rates. Complications such as intraoperative oral bleeding, pain, temporary difficulty in opening the mouth, infection, and salivary gland duct injury may occur in graft urethroplasty performed with the oral mucosa. Grafts can be placed on the ventral or dorsal side (Horiguchi,2017).

Ventral Onlay graft urethroplasty

It is especially applied in strictures with long midbulbar or proximal urethra. It was first described by Morey and McAninch in 1996 (Morey&McAninch 1996). Since the buccal graft is dependent on spongious tissue for blood supply, ventral Onlay urethroplasty is especially preferred in patients with spongious tissue > 1 cm. This is because the proximal and midbulbar urethra has thicker spongious tissue than the distal bulbar urethra. In this technique, the corpus spongiosum is opened along its ventral surface over the stenosis area. The oral mucosa graft is sutured to the edge of the urethral mucosa layer and the corpus spongiosum is closed on the graft to increase blood supply (Kellner et.al, 2004).

Dorsal Onlay graft urethroplasty

The technique used in this surgical application can be performed by circumferential mobilization of the urethra as stated by Barbagli or by unilateral dissection applied by Kulkarni (Barbagli 2003). In the dorsal Onlay urethroplasty technique described by Barbagli, the urethra is fully mobilized and rotated 180°, after urethrotomy is performed on the dorsal side of the stricture segment. the oral mucosa graft is harvested and fixed to the corpus cavernosum to cover the stricture area completely.

Unilateral dorso-lateral urethroplasty technique

The unilateral dorso-lateral urethroplasty technique was first described by Kulkarni. The urethra is mobilized unilaterally and opened only along the dorsolateral line to eliminate the complete peripheral mobilization, to keep circulation to the dorsal part, and to protect the nerve structure of the urethra. After the oral mucosa graft is sutured to the albuginea, the right edge of the oral graft is sutured to the left edge of the urethral opening. With this technique, the bulbospongiosus muscle, the central tendon of the perineum, and the neurovascular structures of the urethra can be preserved.

Although both ventral and dorsal Onlay graft techniques have advantages and disadvantages compared to each other, it is stated that there is not much difference between them. Studies have shown that ventral grafting is technically easier and more advantageous in terms of ischemia due to the more limited application of dissection. It has been shown that dorsal grafting reduces the risk of the diverticulum. In recent years, the dorsal Onlay technique is ideal for distal bulbar urethral strictures and the ventral graft urethroplasty technique is preferred for mid and proximal bulbar urethral strictures.

Bidirectional graft urethroplasty (Palminteri Technique)

It is among the alternative surgical treatment options in the treatment of strictures with severe obstruction. In this technique, only the mucosal tissue of the urethra is opened throughout the stenosis and the graft material is sutured to the damaged mucosal edges, and the spongious tissue is tried to be preserved to a large extent.

Asopa dorsal inlay graft urethroplasty

It is a surgical method especially applied in urethral strictures with complicated, immobilized due to previous operations, and severe stenosis. In this technique, the urethra is incised proximally and distally up to the healthy urethra, ventrally, and the scarred tissue on the dorsal face is excised, and the graft is sutured to the edges of the urethral layer. The urethral tissue on the ventral face is also retubulized through the urethral catheter.

Flap Urethroplasty

After defining flap urethroplasty with the repair of stenosis by reversing penile skin tissue for the first time by Orandi, Quartey developed this technique by using distal penile or preputial

tissue. McAninch further developed the technique by using Buck's fascia as a flap. In flap urethroplasty, penile skin with sufficient width and length is incised and mobilized to cover the urethral stenosis.

After an injury to the urethra, the stratified columnar epithelium of the urethra replaces to squamous metaplasia. Urine leakage into small slits in this epithelium causes fibrotic reaction and stenosis. While the amount of type 3 collagen and smooth muscle decreases, type 1 collagen increases in the stenotic area.

Urethral dilatation, internal urethrotomy, laser urethrotomy, urethral stenting and open surgery are the main treatment modalities in the treatment of urethral stricture (Martins et. Al 2019). In the long term, the success rate of methods such as endoscopic urethrotomy is between 20-30%, while the success rates of open surgery operations are as high as 85-90%. Despite the high success rates of open surgeries, endoscopic methods are still the first choice in the treatment of urethral stenosis recently, due to the high cost and the difficulty of the operation (Smith, 2016).

References

Andrich DE, Mundy AR. What's new in urethroplasty? Current opinion in urology. 2011;21 (6):455-60.

Atan A, Tuncel A, Balcı M, Aslan Y, Köseoğlu E, Erkan A. Penile fasciocutaneous flap urethroplasty in long segment urethral stricture. Ulus Journal of Trauma Emergency Surgery. 2014 ;20 (6):427-31.

Barbagli G, Guazzoni G, Lazzeri M. One-stage bulbar urethroplasty: Retrospective outcome analysis in a series of 375 patients. The Journal of urology. 2008 ;4 (179):261.

5-Morey AF, McAninch JW. When and how to use buccal mucosal grafts in adult bulbar urethroplasty. Urology. 1996 Aug;48(2):194-8.

6-Sachse, H.: Die transurethrale scharfe Schlitzung der Harnrohrenstriktur mit einem Sichturethrotom. Verhandl. Deutsches Gesell. Urol., 25: 143, 1973

Barbagli G, Palminteri E, Guazzoni G, Cavalcanti A. Bulbar urethroplasty using the dorsal approach: current techniques. International braz jurol. 2003 ;29 (2):155-61.

Buckley JC, Wu AK, McAninch JW. Impact of urethral ultrasonography on decision-making in anterior urethroplasty. BJU international. 2012 ;109 (3):438-42.

Dubey D. The current role of direct vision internal urethrotomy and self-catheterization for anterior urethral strictures. Indian Journal of Urology: IJU: Journal of the Urological Society of India. 2011 ;27 (3):392.

Eltahawy EA, Virasoro R, Schlossberg SM, McCammon KA, Jordan GH. Long-term followup for excision and primary anastomosis for anterior urethral strictures. The Journal of urology. 2007 ;177 (5):1803-6.

GD W, NV W. Strictures of the male urethra in Adult and Pediatric Surgery. 4th ed. Hampson LA, McAninch JW, Breyer BN. Male urethral strictures and their management. Nature reviews Urology. 2014 ;11 (1):43-50.

Mundy AR, Andrich DE. Urethral strictures. BJU Int. 2011 Jan;107(1):6-26.

Heyns CF. Urethrotomy and other minimally invasive interventions for urethral stricture. Urethral Reconstructive Surgery: Springer; 2008. p. 63-83.

Horiguchi A. Substitution urethroplasty using oral mucosa graft for male anterior urethral stricture disease: current topics and reviews. International Journal of Urology. 2017 ;24 (7):493-503.

Jordan GH, Steven M, Schlossbery SM. Surgery of the penis and urethra. In: Walsh PC, Retik AB, Vaughan ED, editors. Campbell's Urology, 8th ed. Philadelphia2002. p. 3886-954.

Kellner DS, Fracchia JA, Armenakas NA. Ventral onlay buccal mucosal grafts for anterior urethral strictures: long-term followup. The Journal of urology. 2004 ;171 (2):726-9.

Latini JM, McAninch JW, Brandes SB, Chung JY, Rosenstein D. SIU/ICUD Consultation On Urethral Strictures: Epidemiology, etiology, anatomy, and nomenclature of urethral stenoses, strictures, and pelvic fracture urethral disruption injuries. urology. 2014 ;83 (3 Suppl):S1-7.

Lumen N, Oosterlinck W, Hoebeke P. Urethral reconstruction using buccal mucosa or penile skin grafts: systematic review and meta-analysis. Urology internationalis. 2012 ;89 (4):387-94.

Martins FE, de Oliveira PS, Martins NM. Historical Perspective and Innovations in Penile Urethroplasty.

Mathur R, Aggarwal G, Satsangi B, Khan F, Odiya S. Comprehensive analysis of etiology on the prognosis of urethral strictures. Int Braz J Urol. 2011 May-Jun;37(3):362-9; discussion 369-70.

Mundy AR, Andrich DE. Urethral strictures. BJU Int. 2011 Jan;107(1):6-26.

Lower Urinary Tract Dysfunction-From Evidence to Clinical Practice: IntechOpen; 2019.

Smith TG 3rd. Current management of urethral stricture disease. IndianJUrol. 2016 Jan-Mar ;32 (1):27-33.

Verla W, Oosterlinck W, Spinoit AF, Waterloos M. A Comprehensive Review Emphasizing Anatomy, Etiology, Diagnosis, and Treatment of Male Urethral Stricture Disease. Biomed Res Int. 2019 Apr 18 ;2019:9046430

Home Care in Chronıc Obstructıve Pulmonary Disease

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INTRODUCTION

In the Global Initiative for Chronic Obstructive Pulmonary Disease(GOLD) 2022 report, COPD is defined as "persistent respiratory symptoms due to abnormalities in the airway and/or alveoli, influenced by individual host characteristics, caused by significant exposure to noxious particles or gases. and airflow limitation is a common, preventable, and treatable disease (GOLD, 2022; Bartziokas et al., 2022). The airflow limitation is usually progressive and related to the inflammatory response in the lungs to harmful particles or dust. In COPD, there is the presence of either chronic bronchitis and emphysema or a combination of both (Kocabaş, 2015).

Chronic obstructive pulmonary disease (COPD) is known as the third most common cause of death worldwide, and it is thought that one out of every 10 adults has COPD (Stanaway et al., 2018). It is estimated that COPD causes approximately 3.2 million deaths and affects more than 380 million people worldwide each year (Iheanacho et al., 2020; Park et al., 2020). There is not enough data at the national level on the prevalence and incidence of COPD, mortality rates, risk factors, and the burden of disease caused by COPD in Turkey (Akyıl et al., 2018). According to TUIK, respiratory system diseases are the third cause of death (12.5%) and 61.5% of these deaths are caused by COPD (TUIK, 2017). It is estimated that in 2030, more than 4.5 million deaths per year will be associated with COPD (Park et al., 2020).

The World Health Organization has created two different assessment criteria to assess the burden of disease in chronic diseases: Years of healthy life lost due to disability (YLD) and Disability-adjusted life years = DALY. According to the statistical data of the World Health Organization for 2017; COPD ranks 6th in the DALY ranking (WHO, 2017). It is suggested that COPD will be 7th in the world in the DALY ranking by 2030 (Mathers et al., 2006). It is estimated that the prevalence of COPD and the burden of the disease will continue to increase worldwide in parallel with the aging of the population and the increase in continuous exposure to risk factors (occupational exposure, indoor and outdoor air pollution, tobacco smoke) (Kocabaş et al., 2014).

The most important known genetic risk factor for COPD is hereditary alpha-1 antitrypsin deficiency (Enç, 2021). Smoking, indoor air pollution, environmental or occupational exposure to various gases and dust, and outdoor air pollution are other factors that play a role in the development of COPD (Enç, 2021). The most important known and preventable risk factor for COPD is tobacco use (Enç, 2021). Looking at global risks, it is seen that exposure to air pollution is as important as tobacco use (Stanaway et al., 2018). The contribution of risk factors to the development of COPD varies from country to country. While smoking is the most contributing factor to the burden of COPD in countries with a high sociodemographic development index (SDI); environmental (biomass exposure) and occupational risks are more dominant in countries with low-moderate or low sociodemographic development index (Criner et al., 2019). Infection, malnutrition, or environmental pollutants occurring in at least half of the patients, especially in the

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prenatal and postnatal periods, are thought to adversely affect lung development, and as a result, COPD develops later in life (Stanaway et al., 2018).

Chronic and progressive dyspnea, chronic cough, and sputum production are the most common symptoms of COPD (Gundry, 2019). Wheezing and chest tightness may accompany these symptoms (GOLD, 2022). Although the symptoms of COPD can vary during the day and even from day to day; It is felt more during the day, especially in the morning (TTD, 2019).

With the aggravation of the disease; Symptoms and findings such as activity intolerance, exhalation by pursing the lips during expiration, fatigue, weight loss, sleep disorders, psychological disorders, and an increase in total lung capacity (barrel chest) can also be seen (Karadakovan and Eti Arslan, 2011).

According to the Global Initiative for Chronic Obstructive Pulmonary Disease(GOLD-2022), a diagnosis of COPD should be considered in any patient with symptoms of dyspnea, chronic cough or sputum production, and a history of risk factors (Lange et al., 2016; GOLD, 2022).). A preliminary diagnosis of COPD can be made based on the history and physical examination findings, but the diagnosis should be confirmed by evaluating airflow changes in the airways with spirometry (Gentry and Gentry, 2017; GOLD, 2022) (Figure 1). Chest radiography is used to exclude other lung diseases of the individual or to evaluate the presence of additional diseases and complications such as bronchiectasis, tuberculosis, lung cancer, and heart failure. In addition, arterial blood gases and exercise tests are other methods used in the diagnosis of COPD (Kocabaş et al., 2014).

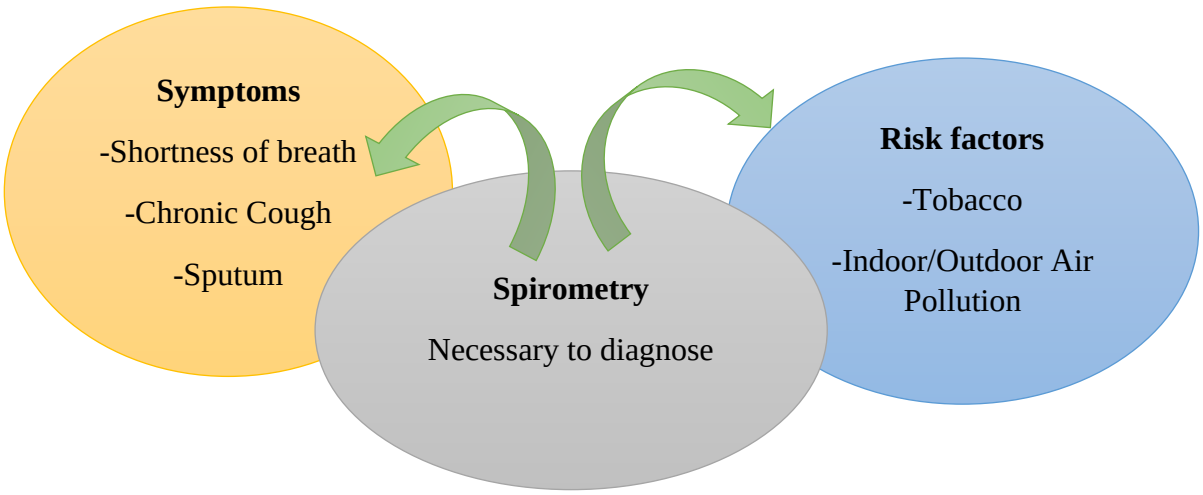


Figure 1. COPD Diagnostic Management Diagram

COPD treatment consists of pharmacological and non-pharmacological approaches. In the pharmacological treatment of COPD; inhalers, bronchodilators, mucolytics, combined drugs, and antibiotics are the main drug groups used (Kocabaş, 2010). In the non-pharmacological treatment of COPD; patient education (risk factors, diet, exercise, etc.), supporting the self-management of the patient, providing regular nutrition, increasing physical activity, and encouraging vaccination (influenza, pneumonia, etc.). In addition, oxygen therapy, noninvasive and invasive ventilators, and respiratory support, mechanical ventilation are also used when necessary (GOLD, 2022).

In the prevention and treatment of COPD, it is extremely important to identify and reduce the risk factors that are exposed and to avoid exposure to risk factors at every stage of the disease. The most effective practice to prevent the progression of the disease in COPD is to quit smoking, which is the most important risk factor (Satman et al., 2020; GOLD, 2022). These practices for

risk factors are used to reduce COPD symptoms and exacerbations; slowing the prognosis of the disease; it is more effective than pharmacological treatment in reducing hospitalizations and mortality rates (Satman et al., 2020; GOLD, 2022).

COPD, with rapidly increasing mortality and morbidity, is a disease that causes serious incapacity and economic burden (Özer, 2019). Depending on the physiopathological changes that occur in COPD, respiratory function is severely affected, leading to life-threatening complications and causing the individual to experience significant limitations while performing daily living activities (Williams et al., 2019). Home care plays an active role in reducing the complications associated with COPD and improving the patient's quality of life.

HOME CARE IN COPD

Today, social and cultural changes, the increase in the elderly population together with the changing population demographics have caused health services to be reviewed and reshaped (Aslan et al., 2018; Cayir, 2020). Situations such as the developments in medicine and technology enabling most of the health services to be provided at home have increased the importance of home care services (Aslan et al., 2018). In the Regulation on the Delivery of Home Care Services, "Home Care Services; is defined as the provision of health care and health services to patients in line with the recommendations of physicians, in the environment they live with their families, by the health team in a way that meets their medical needs, including rehabilitation, physiotherapy and psychological treatment" (Official Gazette dated 10.03.2005 and Issue 2575; Cayir, 2020).

Among the groups receiving home health services; neurological diseases (38%) are in the first place, while lung and respiratory system diseases (5%) are in fifth place (Table 1). Among lung and respiratory system diseases, COPD constitutes a larger patient group (Aslan et al., 2018; TUIK, 2017). In a study examining the home care needs, quality of life, and the factors affecting the quality of life of individuals with respiratory system disease (COPD, asthma, bronchiectasis); It was determined that the majority of the patients needed home care and not satisfied with the care given by their families at home. It was determined that 50.2% of these patients were diagnosed with COPD (Aksu and Fadıllıoğlu, 2013).

Table 1. Distribution of Diseases Receiving Home Health Care**

Disease Group	**%**
Neurological Diseases	38
Cardiovascular Diseases	24
Orthopedics-Traumatology Diseases	12
Chronic-Endocrine Diseases	10
Lung- Respiratory System Diseases	5
Hematological-Oncological Diseases	4
Psychiatric Diseases	3
TOTAL	100

**Aslan, S., Uyar, S., & Guzel, S. (2018). Turkey in Home Health Services Practice. Journal of Social Research and Management, (1), 45-56.

The purpose of home care of individuals with COPD; is to reduce symptoms, slow down progression, prevent recurrent acute attacks and frequent hospital admissions, reduce the cost of diagnosis, treatment, and care of the disease, and provide psychological and physical comfort to the patient, increase survival time and improve the quality of life of the patient (Calverley and Bellamy, 2000; Sharma et al., 2015; Satman et al., 2020; Figueiredo et al., 2022; Aksu and Fadıllıoğlu, 2013). However, home care facilitates end-of-life care in terminal patients and increases the satisfaction of the patient and family with treatment and care (Calverley and Bellamy, 2000; Sharma et al., 2015; Figueiredo et al., 2022).

Home healthcare practices in COPD can be evaluated in four main groups (Kocabaş et al., 2014):
• Home health service delivery in COPD exacerbations (Hospital practice at home)
• Accelerating early discharge in COPD exacerbations (Early discharge)
• Home health service delivery after discharge in hospitalizations due to COPD (Assisted discharge)
• Home health care delivery in end-stage COPD

Home hospital application model; instead of hospitalization of the patient, it is planned that the treatment and follow-up in his home environment will be carried out by a specialist team (mostly respiratory nurses). In particular, the patient is protected from complications such as infection, depression, and anxiety that may develop due to hospitalization. Randomized controlled studies have shown that this method is a successful, safe, and cost-effective method, except for very severe exacerbations (Davies et al., 2000; Skwarska et al., 2000).

Early discharge model; It includes the discharge of the patient after a short-term follow-up (<48 hours) in the emergency room or service due to exacerbation, the continuation of the treatment at home with close monitoring by the home care team, and the creation of treatment and follow-up programs in line with the needs of the patient. In this model, it is stated that there is no significant difference in terms of treatment success between home health care teams and hospitalization, and there is no increase in the rate of re-admissions to the hospital in patients followed up at home (ATS, 2005; Cotton et al., 2000).

Assisted discharge model; It is a model that is mostly used to carry out pulmonary rehabilitation applications after an attack at home or to provide home treatment and follow-up of ventilator-dependent patients. This model contributes to increasing the activity levels of the patients and reducing the symptoms by providing regular health care at home after discharge to COPD patients (Hermiz et al., 2002).

The home health care model in recent COPD; is a model that enables the patient to prevent suffering, control his symptoms, increase the quality of life, inform the patient and their families about the process, and provide psychological and social support (NCP, 2004).

Home care practices in COPD make it easier to access pulmonary rehabilitation, enable better patient follow-up, and enable early recognition of acute exacerbations (Figueiredo et al., 2022). Therefore, home care must be well-planned, especially in patients with a higher risk of exacerbation (Figueiredo et al., 2022). Although the scope of home health care in COPD varies according to the model applied, it consists of five basic components (Table 2).

Table 2. Components of home care in COPD*

1. Administration of the prescribed treatment
   - Drug treatments
   - Oxygen therapy
   - Non-invasive mechanical ventilation
2. Pulmonary rehabilitation at home
   - Exercise training
   - Providing nutritional support
   - Providing psychosocial support
3. Quitting smoking
4. Patient and caregiver education
5. Other Operations (Prescribing, renewing the decisions of the health board, taking samples for examinations, analyzing)

*Ergun, P. (2015). Pulmonary Rehabilitation and At Home. Arseven, O (Ed). Basic Lung Health and Diseases (373-375). Istanbul: Nobel Medicine Bookstores.

Administration of Prescribed Treatment

It consists of three parts: drug treatments, oxygen therapy, and mechanical ventilation. Drug Treatments: Drug treatment in COPD; is used to reduce symptoms, exacerbation frequency and severity, to improve exercise capacity and general health status, and thus to increase the quality of life (Kocabaş et al., 2014; Figueiredo et al., 2022). In addition, drug therapy in home care contributes to reducing mortality and hospitalization rates for COPD exacerbations and improving prognosis (Figueiredo et al., 2022). Pharmacological treatment education in home care; includes information about drugs used in treatment, things to be considered during the use of drugs (correct dose, right route, right time), storage and preservation of drugs, discarding expired drugs, common side effects and what to do in case of undesirable or unexpected effects. and Asti, 2002). Patients using nebulizers and their caregivers should be informed about the correct use of devices, doses of nebulizers, and device cleaning. The factors that cause the patient's non-adherence to treatment should be determined and controlled, the patient's compliance with inhaled drugs should be monitored, and the accuracy of the inhaler drug administration technique should be evaluated (ESHM, 2011).

Oxygen Therapy: According to the Chronic Obstructive Pulmonary Disease Global Initiative (GOLD) report, oxygen therapy is indicated for stable patients with arterial hypoxemia (PaO2 of 55 mmHg or less, or SaO2 of 88% or less). In patients with pulmonary hypertension, peripheral edema suggestive of congestive heart failure, or polycythemia (hematocrit > 55%) and PaO2 55-60 mmHg or SaO2 88%, long-term oxygen therapy is recommended (GOLD, 2022). Oxygen levels should be monitored after 2 or 3 months to determine whether the need for long-term oxygen therapy remains (Figueiredo et al., 2022). Oxygen levels can vary significantly at night and during daily activities. Continuous SpO2 measurements can be made with wearable finger pulse oximeters to monitor SpO2 fluctuations in COPD patients with unexplained shortness of breath or severe exercise intolerance (Buekers et al., 2019). In oxygen therapy; The patient and caregiver should be informed about the duration of treatment, humidification of oxygen, the dangers of changes in oxygen therapy flow rate and flow rate, no smoking near the oxygen source, that the oxygen source should not be near a fire, hygiene of the nasal cannula and humidifier during use, and care. Asti, 2002). As a result of controlled and low-flow oxygen therapy; increase in the patient's exercise tolerance, a decrease in the risk of pulmonary hypertension and cor pulmonale, an improvement in chronic hypoxia, and an increase in the patient's perception of well-being. At the same time, the patient becomes more independent in choosing activities and an increase in the patient's quality of life can be achieved (Smeltzer and Bare, 2000).

Non-Invasive Mechanical Ventilation: Non-invasive mechanical ventilation (NIMV) balances the positive pressure (PEEP) formed in the alveoli at the end of expiration in patients with COPD and reduces the workload of respiratory muscles (Keenan et al., 2011). Since many chronic hypercapnic patients benefit from this treatment, the number of home mechanical ventilation users has gradually increased and this has led to the widespread use of NIMV in-home care (Struik et al., 2013). Non-invasive mechanical ventilation at home is widely used to correct CO2 levels in stable hypercapnia, reduce dyspnea, exacerbations and hospitalizations, improve prognosis, and improve quality of life (Lun et al., 2015; Crimi et al., 2016; Ergan et al., 2019). ; Figueiredo et al., 2022). Before discharge, the patient and caregiver who are planning to use NIMV at home should be trained on the selection of the appropriate ventilator, mask and mode, and ventilator settings. In patients receiving NIMV treatment at home, it is recommended that the first check-up be done within the first 1-2 months after discharge (ESHM, 2011).

Pulmonary Rehabilitation at Home

Exercise training consists of three parts: providing nutritional support and providing psychosocial support. Home care contributes to providing equal access to pulmonary rehabilitation, increasing the compliance of patients with reduced mobility and advanced stages of the disease to pulmonary rehabilitation (Figueiredo et al., 2022; Sebio-García, 2020).

Exercise Training: Exercise training improves exercise capacity and skeletal muscle strength in people with COPD. Accordingly, it causes a decrease in the symptom burden and an increase in the quality of life (Chapman, 2017). Exercise training; In terms of endurance, it should be planned as aerobics for large muscle groups, strengthening exercises to strengthen smaller muscle groups, and breathing exercises for the coordination of breathing and regaining normal breathing patterns (Erdinç & Gülmez, 2019). The purpose of respiratory exercises recommended to patients with COPD; is to reduce hyperventilation, alleviate symptoms and help eliminate negative physiological effects, increase gas exchange and exercise tolerance, and improve daily living activities (Saad and Desoky, 2018). Aerobic breathing exercise applied in home care alleviates the effects of hyperinflation by improving inspiratory capacity and respiratory muscle function (Figueiredo et al., 2022; Lu et al., 2020). On the other hand, diaphragmatic breathing and pursed lip breathing are the most commonly used breathing techniques. Performing these exercises regularly facilitates secretion excretion and reduces the patient's dyspnea (Özkaptan & Kapucu, 2013).

Providing Nutritional Support: Approximately 20-35% of individuals with COPD have nutritional problems and these problems are usually accompanied by weight and muscle loss (Akıncı, 2008). It provides correction of nutritional status, prevention of weakness, increases the strength and endurance of respiratory muscles, and strengthens the immune system in individuals with COPD. Nutrition therapy is more effective when used together with exercise (Ferreira et al., 2000). Patients with COPD need more protein and calories than normal. Therefore, a high-calorie and high-protein diet should be recommended. However, since carbohydrates cause carbon dioxide accumulation, foods containing high levels of carbohydrates should be avoided (Ovayolu and Ovayolu, 2016). Providing nutritional support to home care patients contributes to an increase in body weight, a decrease in airflow limitation, and an increase in quality of life (Kara and Aşti, 2002).

Providing Psychosocial Support: Anxiety, depression, social isolation, addiction, and denial are conditions that often accompany COPD. The home care team needs to evaluate the patient for the presence of these conditions. To effectively manage these problems, it is recommended to include psychosocial behavioral therapies, stress management, progressive muscle relaxation techniques, panic attack control, and training for the existing problem in the pulmonary rehabilitation program (Paz-Diaz et al., 2007).

Smoking Cessation

Smoking cessation in patients with COPD slows the loss of respiratory functions and reduces the symptoms of the disease (Enç, 2021). In addition, it will reduce the development of other tobacco-related diseases such as lung cancer and cardiovascular diseases, and the secondary mortality associated with them (Kocabaş, 2015). Quitting smoking is the most effective and cost-effective risk-reduction method (Enç, 2021). It is aimed to gain behavioral change with the smoking cessation process. Home care team members must understand the behavioral stages and willingness to bring about change in smokers so that they can provide effective support for successful smoking cessation. Since the physical dependence on nicotine and the psychological dependence on smoking habits differ from individual to individual, quit attempts should be planned in a way that is specific to the individual (Fadıllıoğlu et al., 2013). In-home care contributes to the assessment of smoking motivation and the implementation of an individualized comprehensive approach that includes behavioral interventions for smoking cessation by allowing the environment in which smoking occurs, the resources available to quit, and individual preferences to be taken into account (Figueiredo et al., 2022).

Patient and Caregiver Education

For COPD treatment to be successful, patient education about smoking cessation, breathing exercises, protection from risk factors, correct use of drugs, regular check-ups, and home care are very important (Cazzola et al., 2007). The best results can be obtained in the treatment by the healthcare professionals evaluating the patients holistically and providing the appropriate conditions for the environments in which the patients live with their caregivers. Necessary

interventions should be made to evaluate the patient's home environment and make it suitable for the patient (Durna and Akyıl, 2018).

Other Processes

In the Regulation on the Provision of Home Health Services, it is stated that the health services provided in-home care include the examination and examination in the home environment of the patient, taking sample samples for the desired analysis, and providing treatment, care, and rehabilitation services. In addition, without prejudice to the special regulations in prescribing drugs, it is necessary to assist in prescribing drugs documented with a health report due to long-term use, and issuing reports on the use of medical devices and materials (Kunter et al., 2015).

During the delivery of home care services, caregivers, as a result of the lack of social support and long-term care; are faced with problems such as not being able to deal with their health problems, experiencing stress, being depressed, restricting their freedom, and feeling inadequate in providing care (Tekin, 2018). In addition, there are not enough personnel trained in-home care, lack of a multidisciplinary approach, service standards are not clearly defined, insufficient coordination between health institutions, insufficient education is given to patients and their relatives, transportation problems, financial inadequacies, and inadequate home care services. Problems such as not being able to control at a high level negatively affect the provision and maintenance of home care (Satman et al., 2020).

REFERENCES

Akinci, A.C. (2008). The Effect of Pulmonary Rehabilitation Applied to Patients with COPD on Physical and Psychological Parameters. Doctoral Thesis. Istanbul: Marmara University.

Aksu, T., & Fadıloğlu, C. (2013). Investigation of Home Care Needs and Quality of Life of Individuals with Respiratory System Disease. Journal of Ege University Faculty of Nursing, 29(2), 1-12.

Akyıl R.Ç., Uçan, E.S, Durna, Z, Olgun, N. (2018). Chest Diseases Nursing. Ankara: Scientific Medicine Publishing House.

American Thoracic Society (ATS). (2005). Statement On Home Care For Patients With Respiratory Disorders. American Journal Of Respiratory And Critical Care Medicine, 171(12), 1443.

Aslan, S., Uyar, S., & Guzel, S. (2018). Turkey in Home Health Services Practice. Journal of Social Research and Management, (1), 45-56.

Bartziokas, K., Papaporfyriou, A., Hillas, G., Papaioannou, A. I., & Loukides, S. (2022). Global Initiative for Chronic Obstructive Pulmonary Disease(GOLD) Recommendations: Strengths And Concerns For Future Needs. Postgraduate Medicine, 1-7.

Prime Ministry Legislation Development and Publication General Directorate. Regulation on the Delivery of Home Care Services. Ankara, 10.03.2005; Official Gazette: Issue 2575 https://www.resmigazete.gov.tr/eskiler/2008/10/20081017-6.htm (E.T.: 01.07.2022)

Buekers, J., Theunis, J., De Boever, P., Vaes, A. W., Koopman, M., Janssen, E. V., ... & Aerts, J. M. (2019). Wearable Finger Pulse Oximetry For Continuous Oxygen Saturation Measurements During Daily Home Routines Of Patients With Chronic Obstructive Pulmonary Disease (Copd) Over One Week: Observational Study. JMIR mHealth and uHealth, 7(6), e12866.

Calverley, P., & Bellamy, D. (2000). The Challenge Of Providing Better Care For Patients With Chronic Obstructive Pulmonary Disease: The Poor Relation Of Airways Obstruction?. Thorax, 55(1), 78-82.

Cazzola, M., Donner, C. F., & Hanania, N. A. (2007). One Hundred Years Of Chronic Obstructive Pulmonary Disease (Copd). Respiratory Medicine, 101(6), 1049-1065.

Chapman, S. (2017). Pulmonary Rehabilitation For People With Chronic Obstructive Pulmonary Disease: An Evidence Review. British Journal of Community Nursing, 22(12), 608-610.

Cotton, M. M., Bucknall, C. E., Dagg, K. D., Johnson, M. K., MacGregor, G., Stewart, C., & Stevenson, R. D. (2000). Early Discharge For Patients With Exacerbations Of Chronic Obstructive Pulmonary Disease: A Randomized Controlled Trial. Thorax, 55(11), 902-906.

Crimi, C., Noto, A., Princi, P., Cuvelier, A., Masa, J. F., Simonds, A., ... & Nava, S. (2016). Domiciliary Non-Invasive Ventilation In COPD: An International Survey Of Indications And Practices. COPD: Journal of Chronic Obstructive Pulmonary Disease, 13(4), 483-490.

Criner, G. J., Martinez, F. J., Aaron, S., Agusti, A., Anzueto, A., Bafadhel, M., ... & Celli, B. R. (2019). Current Controversies In Chronic Obstructive Pulmonary Disease. A report from the global initiative for chronic obstructive pulmonary disease scientific committee. Annals of the American Thoracic Society, 16(1), 29-39.

Meadow, Y. (2020). HomeHealthcare. Turkish Journal of Family Medicine and Primary Care, 14(1), 147-152.

Davies, L., Wilkinson, M., Bonner, S., Calverley, P. M. A., & Angus, R. M. (2000). "Hospital At Home" Versus Hospital Care In Patients With Exacerbations Of Chronic Obstructive Pulmonary Disease: Prospective Randomized Controlled Trial. BMJ, 321(7271), 1265-1268.

Durna, Z., Akyil, RC. (2018). Home Care for Individuals with Chronic Obstructive Pulmonary Disease. Turkiye Klinikleri J Intern Med Nurs-Special Topics, 4, 16-22.

Uysal, H. (2021). Chronic Obstructive Pulmonary Diseases. In N. Enç (Editor). Internal Medicine Nursing (189-204). Istanbul: Nobel Medicine Bookstores.

Erdinç, M., Gulmez, I. (2019). Respiratory Rehabilitation. Ankara: Scientific Medical Publishing House

Ergan, B., Oczkowski, S., Rochwerg, B., Carlucci, A., Chatwin, M., Clini, E., ... & Windisch, W. (2019). European Respiratory Society Guidelines On Long-Term Home Non-Invasive Ventilation For Management Of COPD. European respiratory journal, 54(3).
Ergun, P. (2015). Pulmonary Rehabilitation and At Home. In O. Arseven (Editor). Basic Lung Health and Diseases (373-375). Istanbul: Nobel Medicine Bookstores.
ESHM. T.R. Ministry of Health, General Directorate of Primary Health Care Services, Turkey Chronic Airway Diseases Prevention and Control Program, Home Health Services in Chest Diseases, 2011.
Fadıllıoğlu, Ç., Ertem, G., Aykar, F. (2013). Health and care at home. Istanbul: Göktuğ Publishing House.
Ferreira, I. M., Brooks, D., Lacasse, Y., & Goldstein, R. S. (2000). Nutritional Support For Individuals With COPD. Chest, 117(3), 672-678.
Figueiredo, R. G., Laudano, C., Muniz, J., & de Bessa Jr, J. (2022). Home-Based Respiratory Care for COPD Patients. Sinusitis, 6(2), 49-55.
Gentry, S., & Gentry, B. (2017). Chronic Obstructive Pulmonary Disease: Diagnosis And Management. American Family Physician, 95(7), 433-441.
GOLD. (2022) Global Strategy For The Diagnosis, Management, And Prevention Of Chronic Obstructive Pulmonary Disease https://goldcopd.org/2021-gold-reports (E.t.: 01.07.2022)
Gundry, S. (2019). COPD 1: Pathophysiology, Diagnosis And Prognosis. Nursing Times, 116, 4-27.
Hermiz, O., Comino, E., Marks, G., Daffurn, K., Wilson, S., & Harris, M. (2002). Randomized Controlled Trial Of Home Based Care Of Patients With Chronic Obstructive Pulmonary Disease. BMJ, 325(7370), 938.
Iheanacho, I., Zhang, S., King, D., Rizzo, M., & Ismaila, A. S. (2020). Economic Burden Of Chronic Obstructive Pulmonary Disease (COPD): A Systematic Literature Review. International Journal Of Chronic Obstructive Pulmonary Disease, 15, 439.
Kara, M., & Asti, T. (2002). Home Care of Chronic Obstructive Pulmonary Disease. Ataturk University Medical Journal, 34(4), 75-81.
Karadakovan, A., & Aslan, F. E. (2011). Care in Internal and Surgical Diseases. Istanbul: Nobel Bookstore. s. 404-410.
Keenan, S. P., Sinuff, T., Burns, K. E., Muscedere, J., Kutsogiannis, J., Mehta, S., ... & Canadian Critical Care Trials Group/Canadian Critical Care Society Noninvasive Ventilation Guidelines Group. (2011). Clinical Practice Guidelines For The Use Of Noninvasive Positive-Pressure Ventilation And Noninvasive Continuous Positive Airway Pressure In The Acute Care Setting. CMAJ, 183(3), E195-E214.
Kocabas, A. (2010). Chronic Obstructive Pulmonary Disease Epidemiology and Risk Factors. TTD Bulletin of Thoracic Surgery, 1(2), 105-113.
Kocabas, A. (2015). Chronic obstructive pulmonary disease. In O. Arseven (Editor). Basic Lung Health and Diseases (135-138). Istanbul: Nobel Medicine Bookstore.
Kocabaş, A., Atış, S., Garbage, L., Erdinç, E., Ergan, B., Gürgün, A., ... & Yıldırım, N. (2014). Chronic Obstructive Pulmonary Disease (COPD) Prevention, Diagnosis and Treatment Report 2014. Journal Of The Turkish Thoracic Society, 15(2), 1-72.
Kunter, E., Kıraklı, C., Aydoğdu, M. (2015). Home Care in Chronic Respiratory Diseases. TÜSAD Educational Books Series. Ankara: Renkform Printing House. pp.235-259.
Lange, P., Halpin, D. M., O'Donnell, D. E., & MacNee, W. (2016). Diagnosis, Assessment, And Phenotyping Of COPD: Beyond FEV1. International Journal Of Chronic Obstructive Pulmonary Disease, 11(Spec Iss), 3.
Lu, Y., Li, P., Li, N., Wang, Z., Li, J., Liu, X., & Wu, W. (2020). Effects Of Home-Based Breathing Exercises In Subjects With COPD. Respiratory Care, 65(3), 377-387.
Lun, C. T., Tsui, M. S., Cheng, S. L., Chan, V. L., Leung, W. S., Cheung, A. P., & Chu, C. M. (2016). Differences In Baseline Factors And Survival Between Normocapnia, Compensated Respiratory

Acidosis And Decompensated Respiratory Acidosis In COPD Exacerbation: A Pilot Study. Respirology, 21(1), 128-136.
Mathers, C. D., & Loncar, D. (2006). Projections Of Global Mortality And Burden Of Disease From 2002 to 2030. PLoS Medicine, 3(11), e442.
NCP. (2004). National Consensus Project For Quality Palliative Care: Clinical Practice Guidelines For Quality Palliative Care, Executive Summary. Journal of Palliative Medicine, 7(5), 611-627.
Ovayolu, N., Ovayolu, Ö. (2016). Basic Internal Medicine Nursing and Chronic Diseases with Different Dimensions. Ankara: Nobel Medicine Bookstore. p.167-172.
Ozer, S. (2019). Internal Medicine Nursing with Case Scenarios. Istanbul: Istanbul Medical Bookstores.
Özkaptan, B.B., & Kapucu, S. (2015). The Importance of Home Care in Improving Self-Efficacy in Individuals with COPD. Cumhuriyet Nursing Journal, 4: 74-80.
Park, H. Y., Kang, D., Lee, H., Shin, S. H., Kang, M., Kong, S., ... & Yoo, K. H. (2020). Impact Of Chronic Obstructive Pulmonary Disease On Mortality: A Large National Cohort Study. Respirology, 25(7), 726-734.
Paz-Díaz, H., De Jan, M. M., López, J. M., & Celli, B. R. (2007). Pulmonary Rehabilitation Improves Depression, Anxiety, Dyspnea And Health Status In Patients With COPD. American Journal Of Physical Medicine & Rehabilitation, 86(1), 30-36.
Saad, A., & Desoky, G. (2018). The Effect Of Breathing Exercises On The Degree Of Dyspnea And Activities Of Daily Living For Patients With Chronic Obstructive Pulmonary Disease. IOSR Journal of Nursing and Health Science, 7(5), 01-16.
Satman, İ., Erkan, F. et al. (2020). Management of Chronic Obstructive Pulmonary Disease in Turkey: Guide to Prevention, Diagnosis and Treatment Standards. Istanbul: Turkish Institute of Public Health and Chronic Diseases.
Sebio-Garcia, R. (2020). Pulmonary Rehabilitation: Time For An Upgrade. Journal of Clinical Medicine, 9(9), 2742.
Sharma, G., Meena, R., Goodwin, J. S., Zhang, W., Kuo, Y. F., & Duarte, A. G. (2015, April). Burn Injury Associated With Home Oxygen Use In Patients With Chronic Obstructive Pulmonary Disease. In Mayo Clinic Proceedings (Vol. 90, No. 4, pp. 492-499). elsevier
Skwarska, E., Cohen, G., Skwarski, K. M., Lamb, C., Bushell, D., Parker, S., & MacNee, W. (2000). Randomized Controlled Trial Of Supported Discharge In Patients With Exacerbations Of Chronic Obstructive Pulmonary Disease. Thorax, 55(11), 907-912.
Smeltzer, S.C., Bare, B.G. (2000). Brunner and Suddarth's Textbook of Medical Surgical Nursing. 9th ed., Philadelphia: Lippincott Williams & Wilkins. s. 446-460
Stanaway, J., Afshin, A., Gakidou, E., et al. (2018). GBD 2017 Risk Factor Collaborators. Global, Regional, And National Comparative Risk Assessment Of 84 Behavioural, Environmental And Occupational, And Metabolic Risks Or Clusters Of Risks For 195 Countries And Territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study. Lancet, 392(10159), 1923-94.
Struik, F. M., Lacasse, Y., Goldstein, R., Kerstjens, H. A., & Wijkstra, P. J. (2013). Nocturnal Non-Invasive Positive Pressure Ventilation For Stable Chronic Obstructive Pulmonary Disease. Cochrane Database of Systematic Reviews, (6).
Tekin, C. (2018). Developments in the Field of Home Patient Care Services. FU Health Sciences Medical Journal, 32(2), 115-117.
Turkish Thoracic Society (TTD). (2019). Chronic Obstructive Pulmonary Disease (COPD). MİKİ Printing Industry and Trade. Ltd. Sti., Ankara. https://toraks.org.tr/site/ downloads/ ry4RQRb5iqQny6rY (Access date: 01.07.2022)
Turkish Statistical Institute (TUIK). (2017). Cause of Death Statistics 2017. https://data.tuik.gov.tr/Bulten/Index?p=Olum-Nedeni-Istatistikleri-2017-27620 (E.T.: 01.07.2022)

WHO. (2017). Findings from the Global Burden of Disease Study 2017. https://www.healthdata.org/ sites/ default/files/files/policy_report/2019/ GBD_2017_Booklet.pdf (Accessed 01.07.2022)

Williams, V., Price, J., Hardinge, M., Tarassenko, L., & Farmer, A. (2014). Using A Mobile Health Application To Support Self-Management In COPD: A Qualitative Study. British Journal of General Practice, 64(624), e392-e400.

Blood Biochemistry: Blood Cells and Their Functions

Hatice Esra DURAN

Introduction

Blood is a liquid that is carried to the whole body through the circulatory system, plays a very important role in providing and maintaining homeostasis, and contains various cells and biomolecules. 1/13 of body weight is blood. For example, a person weighing 70 kg has an average of 5 liters of blood. The blood volume consists of 50-60% of the liquid part and 40-50% of the cells (Cooper, 2016).

The functions of blood can be examined in 3 main groups as transport, regulation and protection.

a. Transport Function:

- It carries the oxygen required for metabolism from the lungs to the tissues and the carbon dioxide formed as a result of metabolism from the tissues to the lungs.
- It carries the nutrients absorbed from the digestive tract.
- It provides the transport of metabolic end products to the liver, kidney, lung, skin and intestines.
- It carries substances such as enzymes, hormones and vitamins that are involved in body functions.

b. Regulation Function:

- Responsible for balancing body temperature
- Responsible for regulating the body's electrolyte, water and acid-base balance

c. Protection Function:

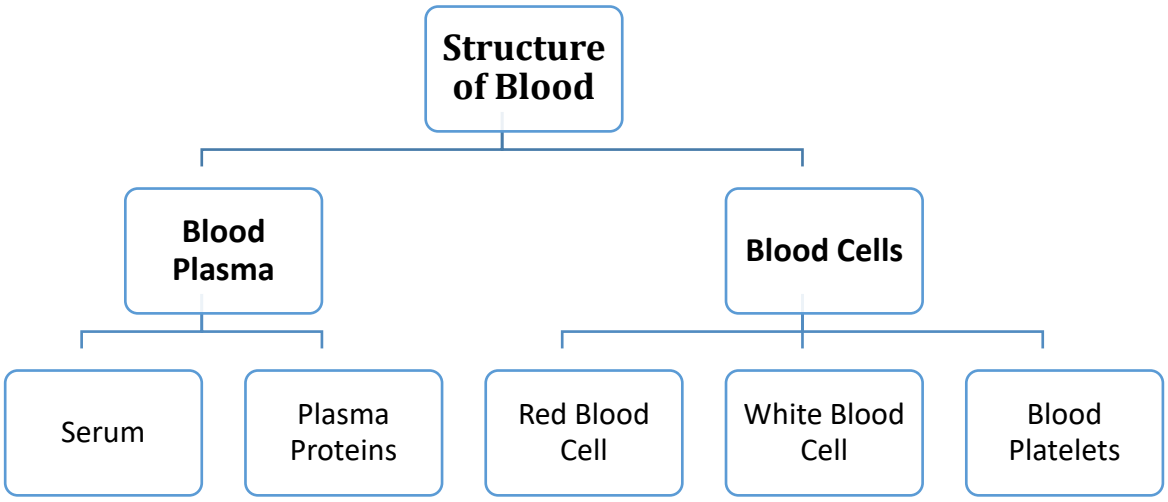
- Provides protection of the organism against microorganisms through leukocytes and antibodies
- To maintain hemostasis by preventing blood loss with coagulation factors (Gürdol, 2019).

The viscosity of blood is 5 times that of water when compared to water. The water content of the plasma, the amount of protein and the number of erythrocytes (red blood cells) affect the blood viscosity. The viscosity of the blood increases when the number of erythrocytes increases, the amount of protein increases and the proportion of water in the plasma decreases. Otherwise, it will decrease (Elert, 2012).

Blood is composed of blood cells and plasma, which is the intercellular substance. The liquid part obtained by precipitating the cells by centrifugation from the blood sample with anticoagulant is called 'plasma'. 90% of plasma is water, the rest is dissolved proteins, glucose, products of digestion/absorption, lipoproteins, coagulation factors, blood cells, hormones, vitamins, antibodies, salt, and gases. There is also a very small amount of CO_2 and O_2 in dissolved form. Cells float in the blood plasma and circulate throughout the body through the vessels (Ageyama et al. 2001).

The cellular elements of the blood are leukocytes, erythrocytes and platelets. All cell types differentiate from hematopoietic stem cells. Erythrocytes, platelets, granulocytes, and monocytes differentiate from common myeloid progenitors while lymphocytes differentiate from lymphoid progenitor. At each stage, stem cell factor, thrombopoietin, various interleukins, erythropoietin and transcription factors act as regulators (Ageyama et al. 2001).

Table 1. Structure of Blood



1. Blood Plasma

It is an intermediate of blood. About 55% of the blood is blood plasma. 90-92% of blood plasma is water, 7-8% is protein and the rest is inorganic substances. Plasma is yellow in color due to the proteins dissolved in it (Krause, 2005).

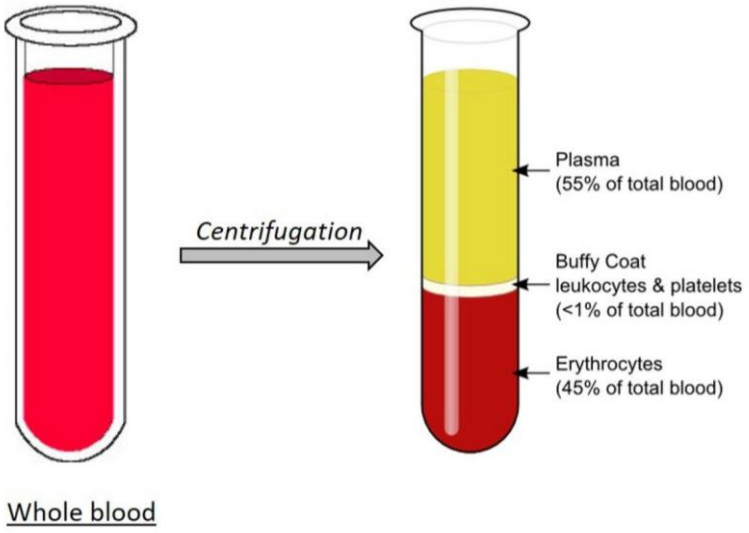


Figure 1. Obtaining plasma from whole blood

When the blood is taken into a tube without adding anticoagulant and left to coagulate, a yellow liquid is observed to separate from the clot formed. This liquid is called serum. There are amino acids, simple carbohydrates, lipids, vitamins, antibodies, hormones, enzymes, mineral salts, nitrogenous wastes, oxygen and carbon dioxide in serum. Plasma and serum are identical in

structure, except for one important difference. The most important difference between them is the absence of some coagulation factors or proteins, especially fibrinogen, which are involved in blood coagulation. For this reason, serum is also called fibrinogen-free plasma (Krause, 2005).

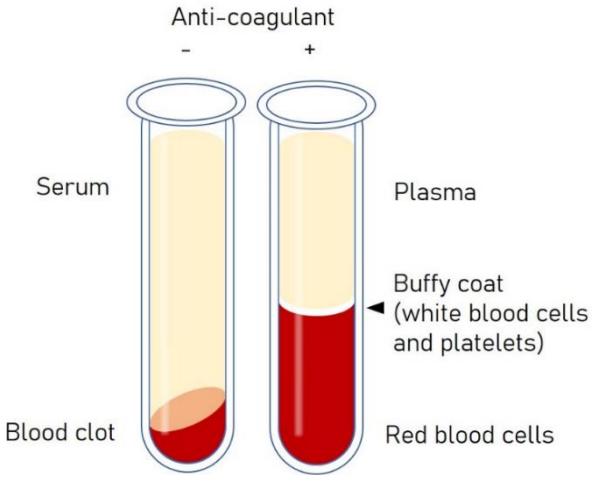


Figure 2. Difference between serum and plasma

Blood proteins are albumin, globulin and fibrinogen. Most of these are large-molecule proteins and cannot go out of the blood vessels and create the osmotic pressure of the blood (Krause, 2005).

Plasma proteins have very important functions and they can be listed as follows:

- The osmotic power created by plasma proteins is called 'colloid osmotic pressure'. This osmotic power is the most important force that keeps the water in the plasma and prevents the water in the plasma from escaping out of the blood vessels. The protein responsible for 70% of this osmotic power is albumin. Insufficient production of albumin or loss of albumin for any reason causes water to escape from the blood vessels and accumulate between tissues, in other words, edema.
- Proteins are an important buffer system involved in the regulation of blood pH.
- Many substances such as hormones, drugs and metals are transported in the blood by binding to proteins.
- They regulate the sedimentation of erythrocytes (the accumulation of erythrocytes on each other by forming a roll form) during the circulation of blood in the vascular system.
- They affect blood viscosity (Anderson & Anderson, 1977)

2. Blood Cells

Blood cells are erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets).

a. <u>Red Blood Cells (Erythrocytes):</u> They are formed from cells in the red bone marrow. In addition to being made in the spleen, they are also made in the liver during the embryonic period. Erythrocytes are cells that are studied intensively because they are easily obtained, have high functional importance and play a role in many diseases (Higgins, 2014).

Figure 3. Red blood cells (erythrocytes)

The main task of erythrocytes is to transport oxygen and carbon dioxide and maintain blood pH. The mature erythrocytes in the circulation are one of the few cells in the body whose life span has been determined with certainty. After developing and maturing from progenitor cells in the bone marrow, the life span of erythrocytes released into the circulation is approximately 120 days (Higgins, 2014).

Erythrocytes are highly specialized cells. They contain hemoglobin, which gives blood its red color, and is an iron-containing protein. In their cytoplasm, which covers 34% of hemoglobin, there are no nucleus, mitochondria, lysosomes, ribosomes, endoplasmic reticulum and golgi complex. No nucleic acid and protein synthesis take place in a mature erythrocyte. Alongside the limited lipid metabolism is carbohydrate metabolism, which is largely dedicated to the maintenance of ion pumps in the plasma membrane, hemoglobin, and intracellular functions. Most of the metabolic pathway enzymes that function in precursor erythrocyte cells are also found in mature erythrocytes. However, they are often metabolically interactive for the remainder of cell life (D'Alessandro, 2017).

When erythrocytes age, they are removed from the circulation by RES cells and are degraded together with their hemoglobin. Globin is hydrolyzed to its amino acids for reuse in protein synthesis. Heme iron is transported to the bone marrow and used for re-synthesis of heme. The remaining porphyrin skeleton is degraded in the RES and liver. The released pigments are excreted into the intestinal lumen via the bile (Berg et al. 2012).

The human erythrocyte is seedless and has the shape of a biconcave disc. The average human red blood cell is 6-8 μm in diameter. The height of the disc decreases towards the center from 2.5 μm to 1 μm (Figure 4). The erythrocyte membrane is 6 nm thick. Its structure consists of 49% protein, 44% lipid, 7% carbohydrate (Berg et al. 2012).

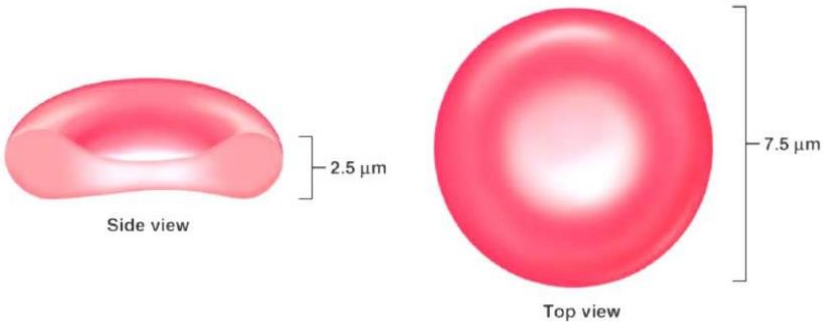


Figure 4. Dimensions of the erythrocyte (top and side view)

A single human red blood cell contains approximately 270 million hemoglobin molecules and each hemoglobin molecule contains four heme groups. It is a heme group that binds oxygen: each heme binds one oxygen molecule, so each hemoglobin molecule can bind four oxygen molecules. The hemoglobin that binds four oxygen molecules is completely saturated and is called oxyhemoglobin. Oxyhemoglobin is bright red in color. If oxyhemoglobin loses one or more of the 4 oxygen molecules it binds, it is called deoxyhemoglobin. Deoxyhemoglobin is dark red in color. There is more deoxyhemoglobin in the blood in the veins (venous blood); therefore, the blood in the veins is darker than the blood in the arteries (arterial blood) (Krause, 2005).

The cell membrane of red blood cells is of oligosaccharide structure. Because of these proteins, human blood is divided into blood groups called ABO.

Blood Group Antigens: It is known that there are 30 genetically independent blood group systems in humans and they contain more than 100 different blood group antigens. Among these, the best defined are the ABO, Rh, and MN systems. Knowing these antigens is important in the fields of blood transfusion, forensic medicine, genetic research, immunology and anthropology. In addition to erythrocytes, blood group markers are found in most biological fluids such as saliva, milk, gastric juice, seminal fluid, urine and ovarian cyst fluid (Farhud & Zarif, 2013).

The ABO system is related to the three blood group substances found on erythrocytes. AB and O group determinants have a complex oligosaccharide structure. Various antigenic markers are found at the non-reducing end of the oligosaccharide. The difference between group A and B antigenic determinants from group O is that there is a galactose (Gal) or N-acetylgalactosamine (GalNAc) linked to galactose to which fucose is bound by an α-1,3 bond. The presence or absence of genes encoding glycosyl transferases involved in the synthesis of A, B and O antigens is the main factor that makes the difference between blood groups (Farhud & Zarif, 2013).

People with A antigen (A blood group) can form a GalNAc α1→3 Gal sequence with N-acetylgalactosaminyltransferase (type A enzyme), but cannot form a Gal α1→3 Gal sequence because there is no galactosyltransferase. People with B blood group antigen (B blood group) have galactosyltransferase (type B enzyme) but not N-acetylgalactosaminyltransferase. In people with O blood type, the H antigen cannot be modified because both enzymes are not present. The ABO group, which is a rare group, is the case in which the 'H' antigen is not made from the ABO precursor molecule (Farhud & Zarif, 2013).

Since antibodies are synthesized against foreign antigens in the organism, individuals belonging to O blood group have antibodies against both A and B antigens. Group B individuals have antibodies against group A antigens, and group A individuals have antibodies against group B antigens. Group AB individuals do not have antibodies against groups A and B (Farhud & Zarif, 2013).

In addition to the A and B systems in blood groups, another antigenic structure found in the erythrocyte membranes is the Rh factor. If a person carries the Rh antigen on his erythrocytes, it is Rh (+), if not, it is Rh (-). 80% of people are Rh (+). The most important difference of Rh antigen from A and B is that it does not have natural antibodies. The formation of antibodies against the Rh antigen is seen in the blood of the recipient after a period of time is given to a person who does not have the Rh antigen (Rh (-)) and a person who has the Rh antigen in his erythrocytes (Rh (+)) (Dean, 2005).

Blood groups are very important in blood transfusions (blood transfusion). When blood transfusion is made from unsuitable groups, hemolytic transfusion reactions occur with hemolysis of erythrocytes. The most important point to be considered in blood transfusions is the agglutinogens in the blood of the donor. A reaction occurs if agglutinin is present in the recipient's blood against agglutinogens in the donor's erythrocytes. For example, if group B blood is given to a person in group A, agglutination and hemolysis develop as a result of the reaction of B agglutinogen in the donor's erythrocytes and anti-B agglutinin in the recipient's plasma. Depending on the severity of the hemolysis, jaundice may be observed. O group blood, which does not carry A and B agglutinogens, is defined as the general donor blood group and can donate blood to other groups in limited quantities and under controlled conditions, but only receives blood from its own group. O group blood, which does not carry A and B agglutinogens, is defined as the general donor blood group and can donate blood to other groups in limited quantities and under controlled conditions, but only receives blood from its own group (Dean, 2005).

AB group, on the other hand, cannot donate blood to any group because it carries both agglutinogens, but can receive blood from all groups, provided that it is limited. For this reason, the AB group is called the general buyer (Dean 2005).

Table 2. Red blood cell compatibility chart

ACCEPTOR	DONOR							
	0-	0+	A-	A+	B-	B+	AB-	AB+
0-	✓	✗	✗	✗	✗	✗	✗	✗
0+	✓	✓	✗	✗	✗	✗	✗	✗
A-	✓	✗	✓	✗	✗	✗	✗	✗
A+	✓	✓	✓	✓	✗	✗	✗	✗
B-	✓	✗	✗	✗	✓	✗	✗	✗
B+	✓	✓	✗	✗	✓	✓	✗	✗
AB-	✓	✗	✓	✗	✓	✗	✓	✗
AB+	✓	✓	✓	✓	✓	✓	✓	✓

Although the table is generally correct, it is obligatory to give the same blood group to people who require long-term blood transfusion (Dean, 2005)

Clinical significance of erythrocytes: Some of the blood diseases associated with red blood cells are:

- *Anemia:* Not having enough red blood cells or hemoglobin in the body. Anemia may also develop in cases where the red blood cells or hemoglobin are abnormal, either due to hereditary or acquired causes. Some types or conditions of anemia include: Anemias are characterized by a low oxygen carrying capacity of the blood due to a low red cell count or some abnormality of red blood cells or hemoglobin.
- *Iron deficiency anemia:* It is the most common anemia. It occurs when dietary iron is insufficient, absorption of iron is insufficient, or iron-containing hemoglobin cannot be formed.
- *Sickle cell anemia:* A genetic disease that results in abnormal hemoglobin molecules. These become insoluble when they release the oxygen charge in the tissues. The result is incorrectly shaped red blood cells. These sickle-shaped red cells are less deformable and less viscoelastic, meaning they can become solid and cause blood vessel occlusion, pain, strokes, and other tissue damage.
- *Thalassemia:* A genetic disease that results in an abnormal production of hemoglobin subunits. One subtype is Beta Thalassemia, also known as Mediterranean anemia or Mediterranean anemia.
- *Spherocytosis:* Hereditary spherocytosis syndromes are a group of inherited disorders that occur due to defects in the cell membrane of the red blood cell, causing the cells to be small spherical and fragile instead of flexible ring-shaped. These abnormal red blood cells are destroyed by the spleen.
- *Pernicious anemia:* An autoimmune disease in which the body lacks the intrinsic factor needed to absorb vitamin B12 from food. Vitamin B12 is required for the production of hemoglobin.
- *Aplastic anemia:* It is caused by the bone marrow not producing blood cells.
- *Hemolytic anemia:* Anemia that occurs by hemolysis is called. Hemolysis is the general term for the excessive breakdown of red blood cells (An & Mohandas, 2008).

The precursor cells of mature erythrocytes are metabolically very active. They can synthesize nucleic acids, complex lipids, carbohydrates and proteins, and produce energy by oxidative phosphorylation. In addition, the mature erythrocyte lacks organelles and therefore the enzymes necessary for these processes. Like all other cells, erythrocytes require energy to perform their functions, but the energy required is less than many cells (Pierigè et al. 2008).

In erythrocytes, the main metabolic pathways maintained after loss of other metabolic pathways are glycolysis and pentose-phosphate pathway. These two pathways meet the energy requirement and reducing power capacity of the erythrocyte (Pierigè et al. 2008).

b. <u>White Blood Cells (Leukocytes):</u> Leukocytes, whose main function is to protect the body against foreign microorganisms, are produced by the bone marrow, lymph nodes and spleen, as well as by lymphoid organs such as the thymus and tonsil. Leukocytes, which are the active elements of the organism's defense system, defend the organism against bacteria, viruses, parasites and tumors. The lifespan of leukocytes in the blood, which are stored in the bone marrow and given to the circulation when needed, varies between 1-2 hours and 100-200 days on average. These cells can be distinguished from erythrocytes by being larger, nucleated, and colorless. Normal blood contains 5,000-10,000/μL leukocytes (Monga et al. 2022)

Figure 5. White blood cells (leukocytes)

Actively mobile leukocytes can pass from the vascular endothelium to the connective tissue. Leakage of blood cells from the vessel wall into the tissue is called 'diapedesis'. The action of cells against a chemical stimulus or biomolecule is called 'chemotaxis'. Leukocytes migrate to the site of infection first by selectins, then by integrins and chemokines. Chemokines are a family of low molecular weight proteins that are structurally similar to proinflammatory cytokines (Orkin & Zon 2008).

Leukocytes are classified according to the presence of granules in their cytoplasm as granulocytes (neutrophils, basophils, and eisonophiles) and agranulocytes (monocytes and lymphocytes). Granulocytes differentiate from myeloid cells in the bone marrow. Since their nucleus are fragmented, they are also called polymorph 'nuclear leukocytes'. The diameter of the cell is about 12-20 μm. Granulocytes, which can survive in anaerobic environments, remove microorganisms from inflamed areas (Orkin & Zon 2008).

- ***<u>Granular white blood cells:</u>***

- ***<u>Neutrophil:</u>*** They constitute 55-65% of circulating granulocytic cells. Neutrophils are innate immune system cells that play an important role in the destruction of pathogens by phagocytosis in acute inflammation. Their diameters usually vary between 10-15 μm. Nucleus are multi-part, lobes may overlap or bend. The number of lobes may increase with cell age (Nelson & Cox, 2017).

Figure 6. Neutrophil

Neutrophils are the most abundant type of leukocytes in the blood. It is the basic cellular element involved in acute inflammation. Phagocytosis abilities are developed. The task of neutrophils is to destroy foreign substances, microbes and cells that have lost their function by

phagocytosis. It envelops the bacteria with its pseudo-feet and digests it with its lysosomes (Nelson & Cox, 2017).

The cytoplasm of neutrophils, which has an active metabolism, contains small amounts of mitochondria, granular endoplasmic reticulum and ribosomes. The Golgi apparatus is not well developed. There are integrins effective in adhesion on the membrane surface. There are many granules in the cytoplasm. These are membrane-bound organelles. They contain proteins with antimicrobial properties. There are also secretory vesicles that act as membrane/cytokine store (Bishop et al. 2000) .

Factors that provide chemotaxis in acute inflammation interact with G-proteins in neutrophils and activate phospholipase C. The resulting second messengers lead to the elevation of intracellular [Ca^{2+}] and the activation of protein kinase C, resulting in activation of various proteins involved in the respiratory burst. Circulating neutrophils migrate along the vessel and adhere to capillary endothelial cells. Integrins on neutrophil surfaces and specific receptor proteins on endothelial cells play a role in this event. The high concentration of chemtactic factors directs the neutrophils towards the bacteria. As a result of phagocytosis of bacteria by neutrophils, superoxide is formed by the NADPH oxidase system. The rapid consumption of oxygen with the formation of superoxide is called respiratory burst. The pathogen in the phagolysosome is destroyed by sequential chemical reactions followed by superoxide formation (Saladin, 2012).

Neutrophils contain enzymes that can hydrolyze other proteins such as elastase, collagenase, gelatinase, cathepsin G, plasminogen activator. The passage of these enzymes into the tissue can cause tissue damage (Saladin, 2012).

- ***Basophil:*** Basophils, which are less than 1% of total leukocytes, are about 10-15 μm in diameter. It secretes heparin, which prevents blood from clotting in the vein, and carries histamine. It causes pain and soreness in injuries and reddening and swelling of the wound. It is an assistant of connective tissue mast cells in allergic conditions. Their number increases with prolonged inflammation (Falcone et al. 2000).

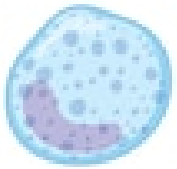


Figure 7. Basophil

They have larger granules in their cytoplasm compared to other granular leukocytes. The granules contain heparin, histamine, serotonin, bradykinin, leukotriene, eosinophil chemotactic factor, proteases and abundant glycogen. There is a receptor for IgE on the membrane surface. Exposure of basophil granules in allergic reactions leads to reactions such as asthma, eczema, and anaphylaxis (Falcone et al. 2000).

- ***Eosinophil:*** Eisonophiles, which make up 1-3% of leukocytes and are similar in size to neutrophils, usually have two lobed nucleus. There are few and large granules in their cytoplasm. Their diameter usually varies between 10-15 μm (Kaushansky, 2010).

Figure 8. Eosinophil

Eisonophiles are defense cells against foreign proteins and parasites. Eosinophils are involved in allergic reactions and parasitic infections. They are found in large amounts at sites of chronic inflammation. Eisonophils are stimulated by chemotactic substances released by some cells, such as T cells, basophils, and mast cells. In the face of this stimulus, eosinophils, which perform their duties by emptying their granules, then release histaminase and arylsulfatase enzymes, inhibiting the secretion of histamine and leukotrienes of mast cells. Eosinophils have complement receptors on their membranes. The eosinfils, which remain in the bloodstream for a short time, complete their life in the connective tissue (Kaushansky, 2010).

- ***Agranular white blood cells:***

- ***Monocyte:*** They are large leukocytes, about 20 m in diameter. They constitute approximately 2-8% of all leukocytes. Their nucleus are slightly concave on one side. As the cell ages, this cavity increases and the nucleus takes the shape of a kidney (Krause, 2005).

Figure 9. Monocyte

Monocytes migrate to tissues and become macrophages. Macrophages have enhanced phagocytosis capabilities. It belongs to the class of professional antigen presenting cells (Krause, 2005).

There are golgi, granular endoplasmic reticulum, free ribosome, glycogen particles and 15-20 homogeneous granules in their cytoplasm. Since these granules show acid phosphatase, arylsulfatase and peroxidase activities, they are called primary lysosomes (Krause, 2005).

Most of the circulating monocytes are located in the vessel wall and migrate to the tissue to become macrophages. These cells have chemotaxis and phagocytosis features very similar to neutrophils. They participate in the destruction of necrotic tissues. They destroy cells and tissues that have expired (Krause, 2005).

- ***Lymphocyte:*** Lymphocytes, which are the basic cells of the adaptive immune response, constitute approximately 20-40% of leukocytes. In these cells with an average diameter of 7 μm, the size of the nucleus varies according to the maturation stage (Kaushansky, 2010).

Figure 10. Lymphocyte

Fragmented nuclei are very rare. Lymphocytes are examined in three groups according to their functions as T, B and natural killer (NK). T lymphocytes originate from the thymus and B lymphocytes originate from the bone marrow. The proliferation and differentiation of both take place in the surrounding lymph organs. NK cells are cells that complete their development in the bone marrow and are activated by interferons. However, in recent studies, the existence of a thymus-derived cell population has been demonstrated. NK cells are considered to be innate immune

response cells and have been reported to be associated with adaptive immune response in recent years (Goljan, 2019).

The adaptive immune response develops within days. T and B lymphocytes and their products neutralize microbes. T lymphocytes are responsible for cellular immunity, B lymphocytes are responsible for humoral immunity. In humoral immunity, B lymphocytes destroy extracellular microbes through the antibodies they secrete. In cellular immunity, T lymphocytes activate macrophages with helper T cells and kill infected cells via cytotoxic T lymphocytes (Bishop et al. 2000).

There are several subgroups of T and B lymphocytes. T cells consist of helper T cells, cytotoxic T cells and regulatory T cells. Helper T cells secrete cytokines (interferon-γ, IL-4, IL-5, IL-13) that play a role in the regulation of humoral immunity. In this way, it helps B lymphocytes. Cytotoxic T cells are effective in cellular immunity by secreting substances that kill infected cells. Regulatory T cells are inhibitory. They secrete TGF (transforming growth factor)-β and IL-10 (Bishop et al. 2000).

Active B lymphocytes are cells that form antibodies in response to pathogens and toxins. When circulating memory B cells encounter the antigen again, they divide rapidly and produce a response (Bishop et al. 2000).

c. <u>Blood Platelets (Thrombocytes):</u> Platelets are small, oval discoid-shaped, 2-4 μm in diameter, non-nucleated, specialized blood cells. Thrombocytes or platelets are the name given to the cell parts involved in the formation of blood clots. Platelets are colorless cell fragments (Krause, 2005).

Platelets are particles of megakaryocytes in the bone marrow and have vital functions in stopping bleeding. It has no nuclei. It contains various granules. When the endothelium is damaged, platelet activation occurs and these granules are released out of the cell. Normally disc-shaped platelets acquire a spherical appearance during activation and produce pseudopods (Krause, 2005).

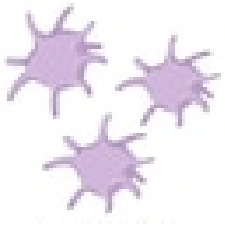


Figure 11. Blood platelets (lthrombocytes)

Platelets are formed from megakaryocytes with a diameter of 35-160 μm in the bone marrow as a result of the fragmentation of the cytoplasm. This formation is stimulated by hematopoietic growth factors. Thrombopoietin (Tpo) is an important growth factor that controls platelet production of megakaryocytes. Tpo is released from parenchymal and endothelial cells of the liver and stroma cells of the bone marrow. Tpo activity is regulated inversely with platelet count and megakaryocyte mass (Nelson & Cox, 2017, Kumar et al. 2015))

High platelet levels often increase the risk of blood clots in the vein, while low platelet levels or function abnormalities can bring bleeding closer. The circulating life of blood platelets is 9-10 days. It is then separated in the spleen. Hyposplenism (decreased or absent spleen function) can cause high platelet counts, while hyperslepnism (abnormal increase in spleen activity) can cause low platelet counts (Nelson & Cox, 2017)

Platelets are activated when they come into contact with collagen. When the endothelium in the vessel is damaged in a way, the collagen (connective tissue) underneath is exposed, and the activated platelets bind to the collagen. Platelets cluster on the damaged area and form a thrombotic plug. As a result, they discharge the contents of the granules they contain into the environment. Due to some substances discharged into the environment, the platelets bind to each other, the

newly arrived platelets are attached to the damaged surface. In addition, the release of serotonin, which occurs when the contents of the granules are discharged into the environment, causes the contraction of the smooth muscles in the vessel wall, preventing blood flow from the damaged part. This is because serotonin is a vasoconstrictor. In addition, myosin and actin filaments, which are high in platelets during aggregation, strengthen the plug formed by contraction. Platelets secrete fibrinogen in addition to fibrinogen found in plasma. As a result, more fibrinogen turns into fibrin during coagulation, forming a fibrous network to which more (platelet and other) blood cells will adhere (Murray et al. 2006).

There are many known platelet activators. Some of them: collagen, thrombin, thromboxane A2, ADP, convulxin. On the other hand, there are also platelet inhibitors. These are: prostacyclin, nitric oxide(Murray et al. 2006).

Clinical significance of thrombocytes: The platelet count in a healthy person is between 250-500 x 109/L of blood.

Thrombocytopenia (low platelet count) and thrombocytosis (high platelet count) can cause coagulation problems. Roughly speaking, a low platelet count increases the risk of bleeding, while a high platelet count increases the risk of thrombosis.

- Some disorders that can cause low platelet count are:
- Thrombocytopenia,
- Gaucher's disease,
- Aplastik anemi.
- Some disorders that can cause platelet dysfunction (decreased or absent in function) or low count are:
- HELLP syndrome,
- Hemolytic-uremic syndrome,
- Chemotherapy.
- One disorders that can cause a high platelet count is:
- Thrombocytosis
- Some of the platelet adhesion (attachment-adhesion) or aggregation (clustering) disorders are:
- Bernard-Soulier syndrome
- Glanzmann thrombasthenia
- Scott syndrome
- von Willebrand disease
- One of the platelet metabolism disorder is:
  - Low cyclooxygenase activity (Marshall & Bangert, 2004)

KAYNAKÇA

Ageyama, N., Shibata, H., Narita, H., Hanari, K., Kohno, A., Ono, F., Yoshikawa, Y., Terao, K. (2001) Specific Gravity of Whole Blood in Cynomolgus Monkeys, Squirrel Monkeys, and Tamarins. *Contemporary Topics in Laboratory Animal Science, 40 (3).*

An X, Mohandas N. (2008) Disorders of red cell membrane. *British Journal of Haematology. 141 (3):* 367-75.

Anderson, N. L., Anderson N. G. (1977) High Resolution Two-Dimensional Electrophoresis of Human Plasma Proteins. *Proceedings of the National Academy of Sciences. 74 (12),* 5421–5425.

Berg, J. M., Tymoczko, J. L.; Stryer, L. (2012). *Biochemistry* (7th ed.). New York: W.H. Freeman.

Bishop, M. L., Duben-Engelkirk, J. L., Fody, E. P. (2000). *Clinical Chemistry.* (4th edit). Lippincott Williams & Wilkins.

Cooper, C. E. (2016). *Blood : a very short introduction* (First edit). Oxford. ISBN 978-0199581450.

D'Alessandro, A. (2017) Red blood cell proteomics update: is there more to discover? *Blood Transfusion. 15 (2):* 182–87.

Dean, L. (2005). *Blood Groups and Red Cell Antigens, a guide to the differences in our blood types that complicate blood transfusions and pregnancy.* Bethesda MD: National Center for Biotechnology Information.

Elert, G. (2012). Volume of Blood in a Human. The Physics Factbook. (15.12.2022 tarihinde http://hypertextbook.com/facts/1998/LanNaLee.shtml adresinden ulaşılmıştır.)

Falcone, F. H., Haas, H., Gibbs, B. F. (2000) The human basophil: a new appreciation of its role in immune responses. *Blood. 96 (13),* 4028–38.

Farhud, D. D., Zarif Y. M. (2013) A brief history of human blood groups. *Iranian Journal of Public Health, 42 (1),* 1–6.

Goljan, E. F. (2019). *Rapid Review Pathology.* (5th edit) Philadelphia:Elsevier,

Gürdol, F. (2019). *Tıbbi Biyokimya.* (4th edit). İstanbul: Nobel Kitapevi

Higgins, J. (2014) Red Blood Cell Population Dynamics. *Clinics in Laboratory Medicine. 35 (1),* 43–57.

Kaushansky K. (2010). *Williams hematology.* (8th edit). New York: McGraw-Hill Medical.

Krause, W. J. (2005). *Krause's Essential Human Histology for Medical Students* (3rd ed.). Universal-Publishers.

Kumar, V., Abbas, A.K., Aster. J.C. (2015) *Robbins and Cotran Pathologic Basis of Disease.* (9th edit) Philadelphia:Elsevier.

Marshall, W. J., Bangert, S. K. (2004). *Clinical Biochemistry.* (5th edit). London: Elsevier Publising.

Monga, I., Kaur, K., Dhanda, S. (2022) Revisiting hematopoiesis: applications of the bulk and single-cell transcriptomics dissecting transcriptional heterogeneity in hematopoietic stem cells. *Briefings in Functional Genomics. 21 (3),* 159–176. doi:10.1093/bfgp/elac002

Murray, R. K., Granner, D. K., Mayes, P.A., Rodwell, V. W. (2006). *Harper's Biochemistry* (27.th edit). McGraw-Hill Companies.

Nelson, D. L. & Cox, M. M. (2017). *Lehninger Principles of Biochemistry.* (7th edit). New York: W.H. Freeman

Orkin, S.H., Zon, L.I. (2008) SnapShot: hematopoiesis. *Cell. 132 (4): 712.e1–712.e2*

Pierigè F, Serafini S, Rossi L, Magnani M (2008) Cell-based drug delivery. *Advanced Drug Delivery Reviews. 60 (2),* 286-95.

Saladin, K (2012). *Anatomy and Physiology: the Unit of Form and Function* (6th edit.). New York: McGraw Hill.

Taste Sense Physiology

Gönül GUROL CIFTCI[1]

It is known that the sense of taste, which can be stimulated by chemicalsubstances in low concentrations, has five different components, namely sweet, salty, bitter, umami, and sour, which is defined as the taste ofsome amino acids such as L-glutamate and 5'-ribonucleotides (Ninomiya, K, 2015). Taste perception is provided by three morphologically different taste buds, which contain taste receptors (Figure 1). Taste buds vary between 3000-10000 in adults and arelocated in the tongue, palate, tonsil, epiglottis, and proximal esophagus (Kurtuldu & al., 2018). Responses to bitter, sweet, and umami stimuli are obtained from Type II taste cells (Figure 1), while Type III taste cells respond to sour and salty stimuli (Cooper & al., 2020, Koyama & al., 2021). Taste buds are on 4 different types of papillae called Circumvallat papilla, Fungiform papilla, Filiform papilla, and Foliate papilla on the tongue (Kurtuldu & al., 2018).

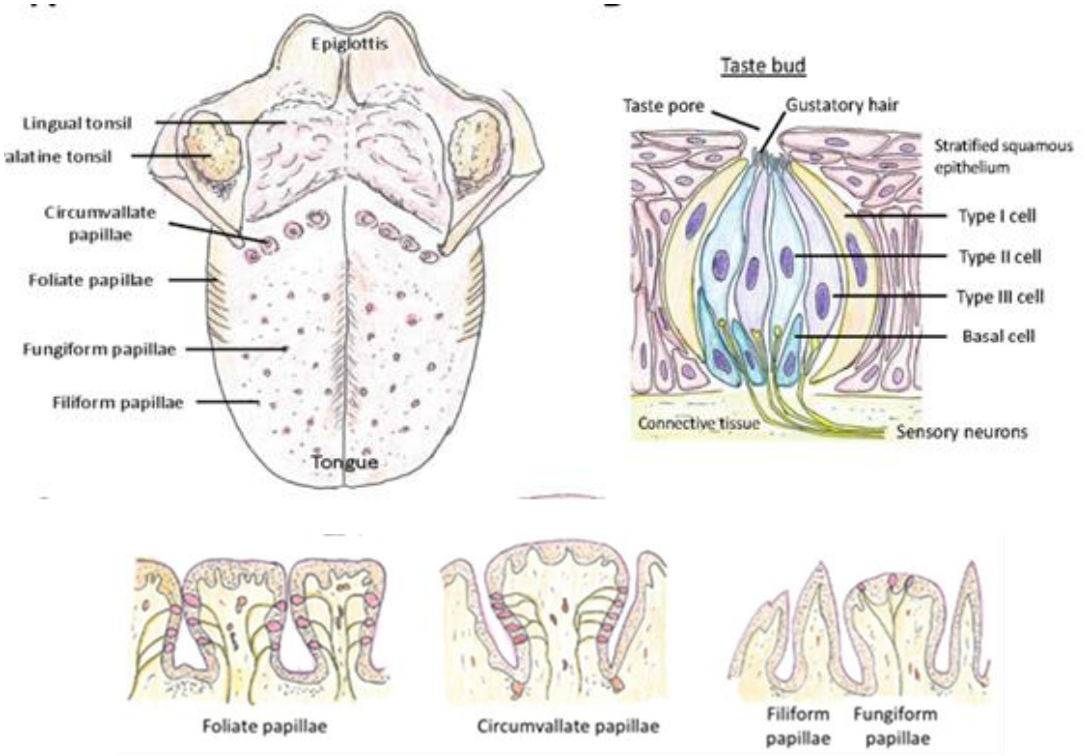


Figure 1*. Localization of four different papillae and type I, II and III cells in the tongue (Koyama et al., 2021)*

In the perception of pure tastes, information such as smell, texture, and taste quality is essential. It is also expressed in surprise flavors in new studies. It is stated that the sixth sense of taste is defined as lipids, kokumi taste, and calcium taste, which describe the feeling of food in the mouth, which means fullness, and density in the mouth and expressed as flavor in Japanese, maybe in people (Besnard, Passilly-Degrace & Khan, 2016, Shibata & al. ., 2017, Alwani, Makki, & Robbins, 2021). Miso and soy sauce, widely consumed worldwide, especially in Japanese culture,

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are produced by fermentation techniques. The food that gives umami flavor to traditional dishes in Japanese culture is soybean soup made with dried algae or mushrooms consumed with soybeans. The ramen added to these soups increases the flavor, and the soy milk and the cooking technique in casseroles leave a taste with a feeling of fullness in the mouth, so it is called kokumi. Kokumi flavor is found in green tea, beer, onion, garlic, gouda cheese, yeast extract, and parmesan cheese. γ-glutamyl peptide derivatives consist of sulfur-containing amino acids, such as γ-glutamyl-S-alk(en)yl-cysteine (γ-ES-alk(en)yl-C), formed by alkylation. A, γ-glutamyl peptide derivatives consist of sulfur-containing amino acids, such as γ-glutamyl-S-alk(en)yl-cysteine (γ-E-S-alk(en)yl-C), which are formed via alkylation in the presence of glutathione (GSH) and the corresponding alk(en)yl source to generate GSH S-conjugates (GS-X) that are catalyzed by GSH-S-transferase, (Figure 2) followed by the removal of the glycyl moiety (Ueda & al., 1994, Hughes & al., 2005, Toelstede & al., 2009, Dunkel & al., 2007; Kuroda & al., 2012, Miyamura &al., 2015, Amino & al., 2018, Kurod et al., 2020, Toelstede S & Hofman, T, 2021; Wang et al., 2022)

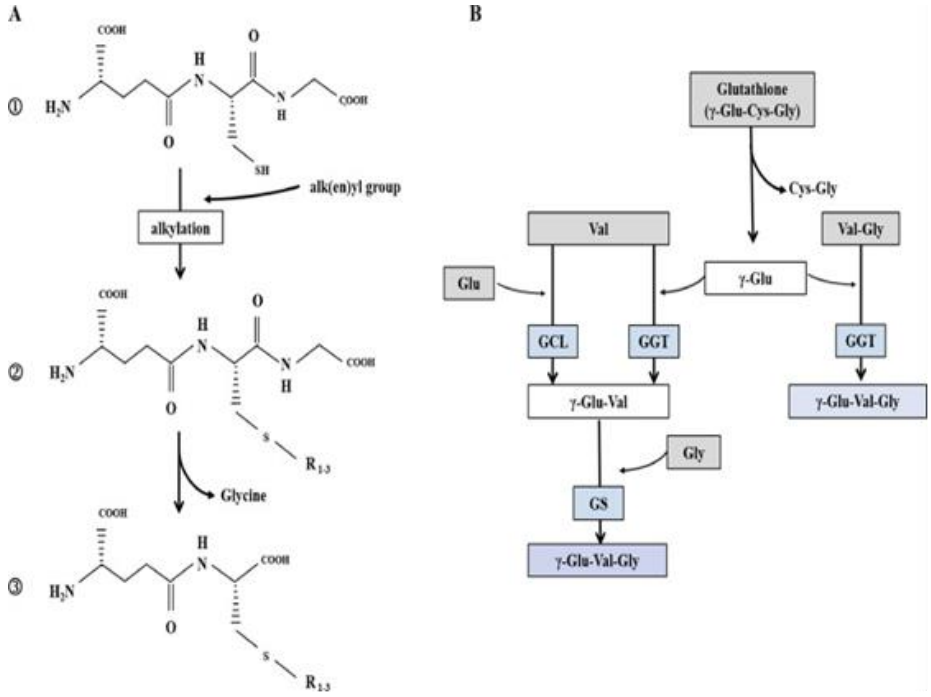

Figure 2 *The synthesis of γ-glutamyl-S-alk(en)yl-cysteine (A) and γ-glutamyl-valyl-glycine (γ-Glu-Val-Gly) (B). (Wang & al., 2022)*

①, γ-glutamyl-cysteinyl-glycine (glutathione); ②,γ-glutamyl-S-alk(en)yl-cysteinyl-glycine; ③,γ-glutamyl-S-alk(en)yl-cysteine. R1, methyl; R2, allyl; R3, 1-propenyl. GCL, glutamate-cysteine ligase; GGT, γ-glutamyl transferase; GS, glutathione synthetase.

Food engineering focuses on both long-term storage and flavoring of foods with food processing techniques. The effects of these processes on aromatization, which makes us feel the flavor of foods more dominantly, are also significant and complex. It has been shown in studies that Kokumi taste affects salt and umami taste by modulating taste cell receptor cells (TRC) (Katsumata & al., 2008, Rhyu & al., 2020; Rhyu & Lyall, 2021). Perception of bitter, sweet, and umami taste is via Type 1 and Type 2 taste receptors and type G-protein coupled receptors (GPCRs). Results of a study investigating the effects of bitter taste-forming compounds that can

interact with at least 25 subtypes of the T2R family of GPCRsinHEK293T cells expressing T2R16 (Figure 3a) show that the foods that make up the umami taste suppress the bitter taste by directly binding to the bitter taste receptors, but in humans. reveals that its effect on T1R2 or T1R3 receptors that detect sweet taste is different (Figure 3b). When added to foods (Figure 3c), Kokumi taste peptides reveal more of a salty taste (Rhyu & Lyall, 2021).

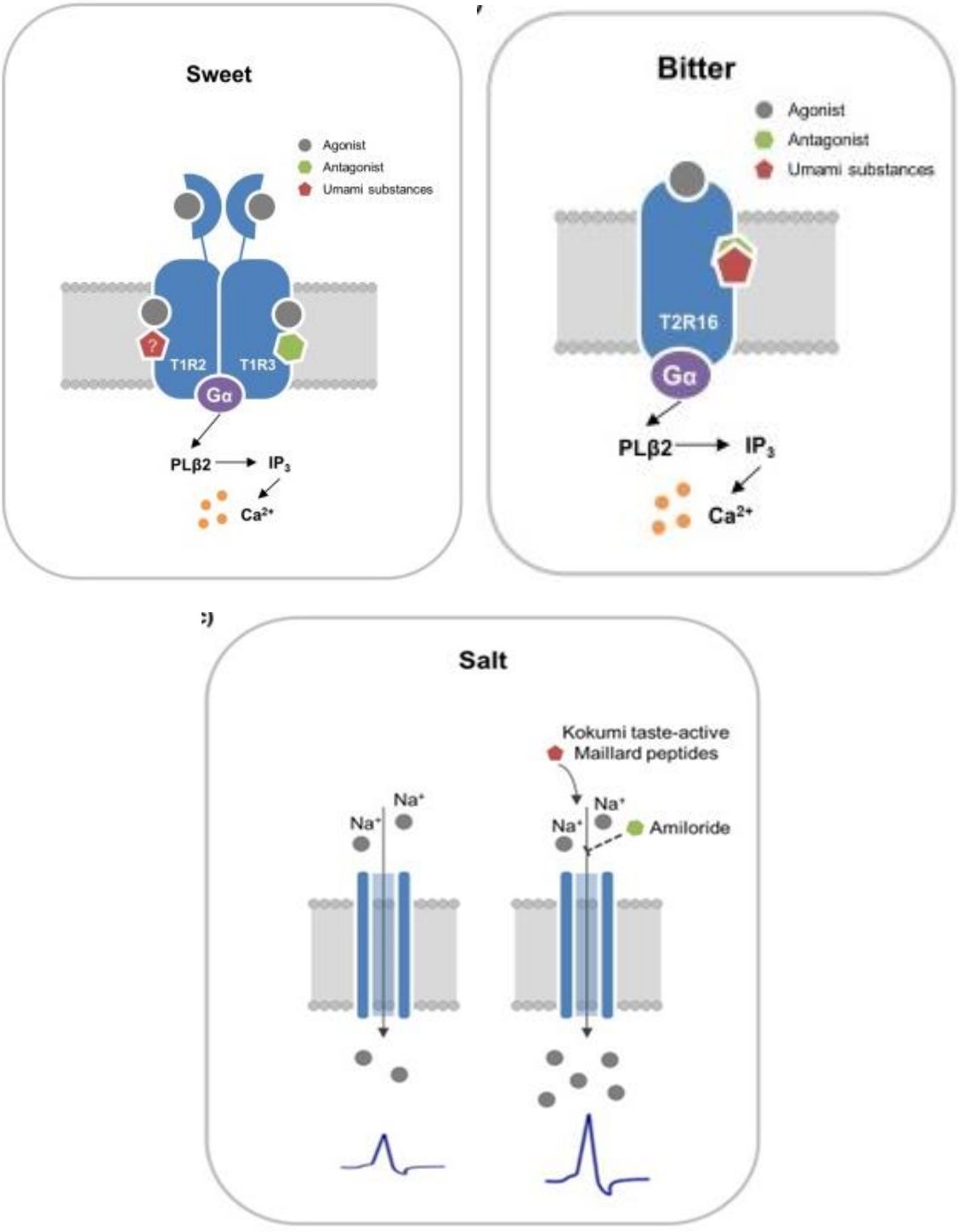

Figure 3. *Interaction of kokumi taste with sweet (a), bitter (b), and salt (c) receptors (Rhyu & Lyall, 2021).*

The settings of the γ-glutamyl peptides in the literature to date and the threshold heights for the kokumi taste were obtained using many methods, such as the sensing receptor test (CaSR) (Figure 4). These uses of γ-glutamyl peptides, human sensory evaluation methods, and CaSR activity assays are retained in Kokumi research (Ohsu & al., 2010, Wang & al., 2022). Kuroda & al. examined the effects of two kinds of cheese in 2020 from the difference in flavor saturation between the sheep and cow aspects, and as a result, much literature has been gained since the long-term flavor restriction γ-Glu-Val-Gly includes more in comparison to the cow's characteristics. CaSR agonists such as calcium can increase eating by enjoying kokumi, showing these contributions by directly affecting CaSR. The information in the taste cells in these interaction units regulates the receptor in the brain through the central nervous system.

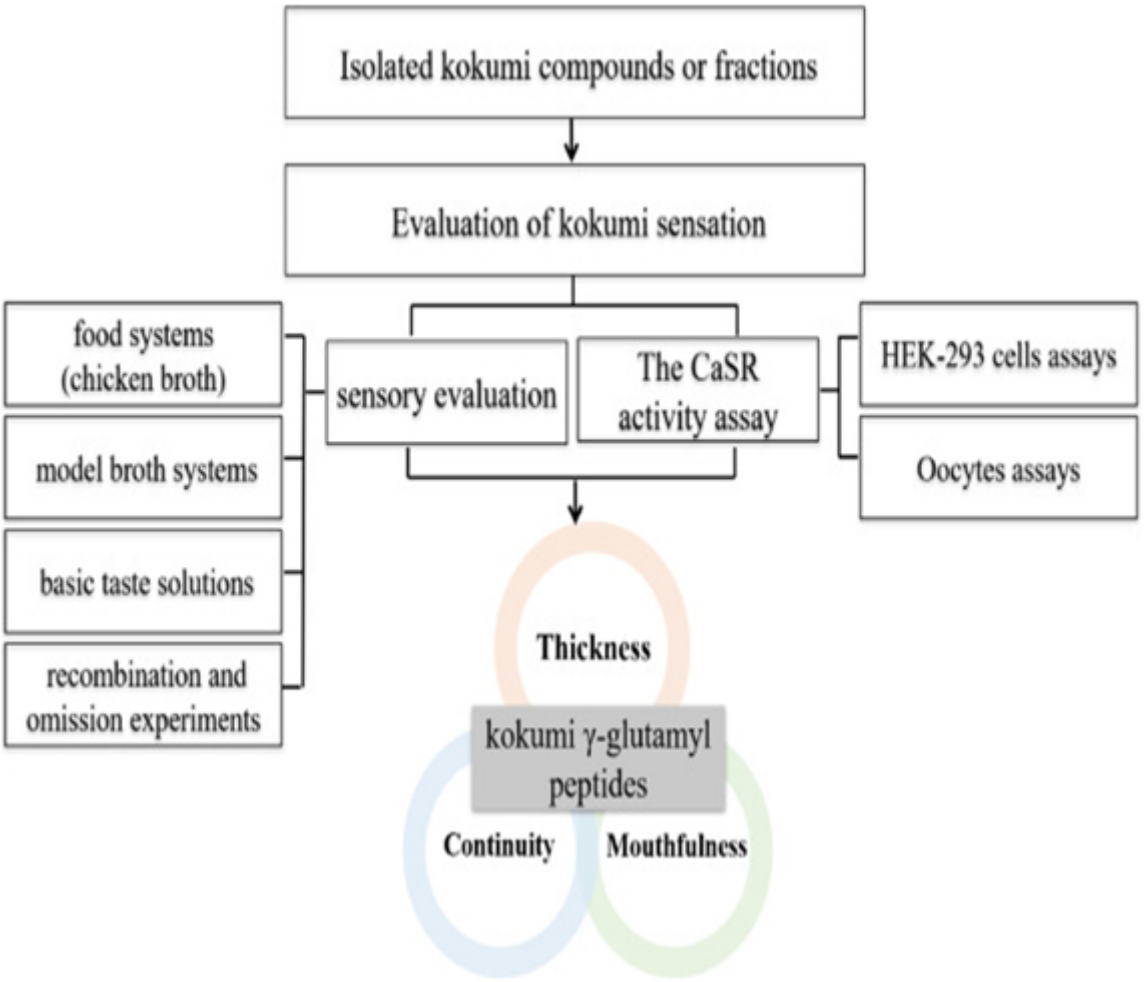

Figure 4. *The research methodology of kokumi γ-glutamyl peptides (Wang & al., 2022).*

The first rule of detecting food is to take it orally. The process begins with the activation of the following three extension sensory systems. This is somesthesia, which includes mechanical perception, thermal sensation and nociception, retronasal olfaction with the detection of chemicals in the place or the inhabitants of the mouth, and taste by detecting the chemical surface by resolving the chemical surface through taste buds. Taste information is transmitted to the brain by three nerves. These are the chorda tympani,glossopharyngeal, and vagus nerves. (Besnard, Passilly-Degrace & Khan, 2016, Alwani, Makki & Robbins, 2021, Meunier & al., 2021).

In summary, the tastes that cause the taste warning in humans and create this perception are usually salty and sweet. On the other hand, flavors such as umami and kokumi balance and regulate the flavor, giving a higher taste sensation, and using excessive salt and sugar consumption

in a center and supports people to live their health in a way. Much of the newfound presence of these flavors will be unmatched by new food classes and new foods, as well as discovering anew field of study. For this, it is necessary to fully usethe structuring of the provisions and to determine the cell boundaries as soon as possible.

REFERENCES

Alwani, M.K.M., Makki, F.M., Robbins, K.T. (2021) Physiology of the Oral Cavity. *Cummings Otolaryngology: Head and Neck Surgery*, 86, 1213-1229.e5.

Amino, Y., Wakabayashi, H., Akashi, S., Ishiwatari, Y. (2018) Structural analysis and taste evaluation of -glutamyl peptides comprising sulfur-containing amino acids. *Bioscience Biotechnology and Biochemistry,* 82 (3), 383-394,

Besnard, P., Passilly-Degrace, P., Khan, N.A. (2016) Taste of Fat: A Sixth Taste Modality? *The American Physiological Society* .

Cooper, K., W., Brann, D.H., Farruggia, M.C., Bhutani, S., Pellegrino, R., Tsukahara, T., Weinreb, C., Joseph, P.V., Larson, E.D., Parma, V., Albers, M.W., Barlow, L.A., Datta, S. R., Pizio, A.D. (2020). COVID-19 and the Chemical Senses: Supporting Players Take Center Stage, *Neuron*, 107; 2, 219-233.

Hughes, J., Tregova, A., Tomsett, A.B., Jones, M.G., Cosstick, R., Collin, R.A. (2005). Synthesis of the flavour precursor, alliin, in garlic tissue cultures. *Phytochemistry,* 66 (2), 187-194.

Katsumata, T., Nakakuki, H., Tokunaga, C., Fujii, N., Egi, M., Phan, T.H., Mummalaneni, S., DeSimone, J.A., Lyall, V. (2008). Effect of Maillard reacted peptides on human salt taste and the amiloride-insensitive salt taste receptor (TRPV1t). *Chem Senses,* 33 (2008), 665-680.

Koyama, S., Kondo, K., Ueha, R., Kashiwadani, H., Heinbockel, T. (2021). Possible Use of Phytochemicals for Recovery from COVID-19-Induced Anosmia and Ageusia. *Int. J. Mol. Sci.* 2021, 22, 8912.

Kurtuldu, E., Miloğlu, Ö., Derindağ, G., Özdoğan, A. (2018). The Overvıew To Taste Dısorders. *Journal of Dent Fac Atatürk Uni,* 28:2, 277-283.

Kuroda, M., Sasaki, K., Yamazaki, J., Kato, Y., Mizukoshi, T. (2020). Quantification of the kokumi peptide, γ-glutamyl-valyl-glycine, in cheese: Comparison between cheese made from cow and ewe milk, *Journal of Dairy Science*, 13;9, 7801-7807.

Meunier, N., Briand, L., Jacquin-Piques, A., Brondel, L., & Pénicaud, L. (2021). COVID 19-induced smell and taste impairments: putative impact on physiology. *Frontiers in physiology*, 11, 625110.

Ninomiya, K., (2015). Science of umami taste: adaptation to gastronomic culture. *Flavour* 4, 13.

Ohsu, T., Amino, Y., Nagasaki, H., Maruyama, Y., Miyamura, N., Eto, Y. (2010). Involvement of the Calcium-sensing Receptor in Human Taste Perception. *Mechanısms Of Sıgnal Transductıon*, 285;2,1016-1022.

Rhyu, M.R., Song, A.Y., Kim, E.Y., Son, H.J., Kim, Y., Mummalaneni, S., Qian, J., Grider, J.R., Lyall, V. (2020). Kokumi taste active peptides modulate salt and umami taste. *Nutrients,* 12, p. 1198.

Rhyu, M.R., Lyall, V., (2021). Interaction of taste-active nutrients with taste receptors, *Current Opinion in Physiology*, 20, 64-69.

Shibata, M., Hirotsuka, M., Mizutani, Y., Takahashi, H., Kawada, T., Matsumiya, K., Hayashi, Y., Matsumura, Y. (2017). Isolation and characterization of key contributors to the "kokumi" taste in soybean seeds, *Bioscience, Biotechnology, and Biochemistry*, 81:11, 2168-2177.

Toelstede, S., Hofmann, T. (2009). Kokumi-Active Glutamyl Peptides in Cheeses and Their Biogeneration by Penicillium roquefortii. *Journal of agricultural and food chemistry*. 57.

Wang, H., Suo, R., Liu, X., Wang, Y., Sun, J., Liu, Y., Wang, W., Wang, J. (2022). Kokumi γ-glutamyl peptides: Some insight into their evaluation and detection, biosynthetic pathways, contribution and changes in food processing. *Food Chemistry Advances*,1;100061.

Thermoregulation

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It has long been known that the brain plays avital role in regulating body temperature, mediated by heat-responsive neurons, often called heat-sensitive neurons, and cold-responsive neurons called cold-sensitive neurons (Rothhaas & Chung, 2021). Changes in temperature in the human body are considered a clinical finding and have been accepted as a symptom of many diseases, especially injury and infection, for more than two thousand years (Grodzinsky & Levander, 2020; Rzechorzek & al., 2022).

In temperature measurements, mainly two units are used throughout the world. In one, the temperature is measured in degrees Celsius (°C), while in the other, it is used in Fahrenheit (°F). Both units can be converted to each other with the formulas (Celsius = [(°Fahrenheit - 32) × 5]/9, Fahrenheit = (9 × °Celsius/5) + 32) (Kim & al., 2021). In a study he conducted in 1861, German doctor Karl RA Wunderlich found that the human body temperature was 37.0 °C, which was equal to 98.6 °F and wrote his name in the literature by providing the application of the thermometer in the clinic. Standard temperature ranges (normal temperature range 35.5° to 37.0°C for underarm, 33.2° to 38.2°C for oral, 34.4° to 37.8°C rectal and tympanic temperature ranges currently known in routine) 35.4° to 37.8°C) was done by Sund-Levander, Forsberg & Wahren, (2002). The concept expressed by the term thermoregulation is keeping the normal body temperature determined by Larl RA Wunderlich constant within the limits of ±0.2°C, despite changing environmental conditions.

The ability to tolerate core (or central) body temperature in this way gives humans the characteristics of a homeothermic organism (Engorn & al., 2022). However, it should be noted that this value doesnot precisely express the internal temperature of the brain. Due to the necessity of risky invasive methods for measuring brain temperature, the clinic ignores this situation, and it is assumed that this temperature reflects core body temperature (Rzechorzek & al., 2022). The situation that determines the limits of homeothermic is the survival process. This is because the enzymatic reactions that determine the functions of cells are affected by temperature. In various situations where high temperature is effective (>42 °C), proteins are denatured, while at low temperatures (<36.1 °C), the metabolism slows down and the reaction rate decreases (Figure 1), so functions may come to a standstill (Kuht & Farmery, 2014). The amount of extreme conditions compatible with life isreported in the literature as approximately 13.7°C and 44°C. As can be understood from this value,people have amuch greater tolerance for cold (Gilbert & al., 2000; Engorn & al., 2022).

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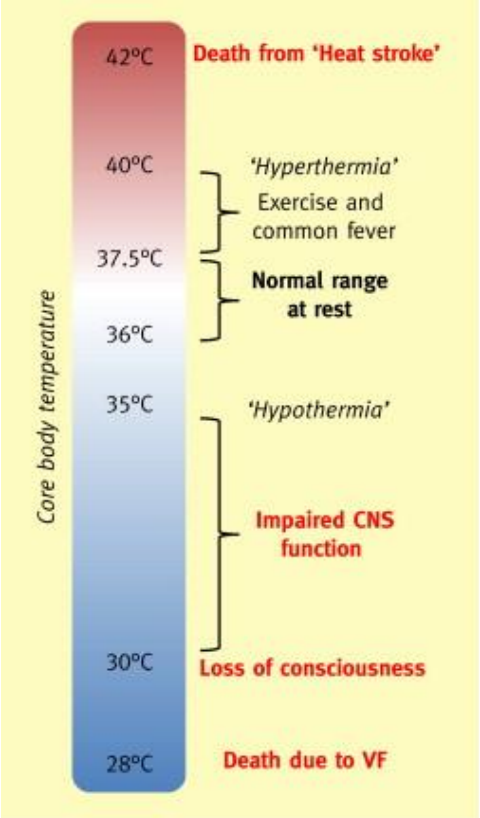

Figure 1. Core (Central) Change intervals of body temperature and consequences of extreme conditions (Kuht & Farmery., 2014).

Heat and cold-sensitive neurons in the brain(Figure 2) play animportant role in maintaining body temperature(Rothhaas & Chung, 2021). The preoptic area (POA), the subfornical organ (SFO), and the circulatory organ of the lamina terminalis are divided into three (Figure 2) parts: the ventrolateral preoptic area (VLPO), the medial preoptic area (MPA), the dorsal and ventral median preoptic area (MnPO). Acquisition of close with (VOLT) (Yu & al., 2018).

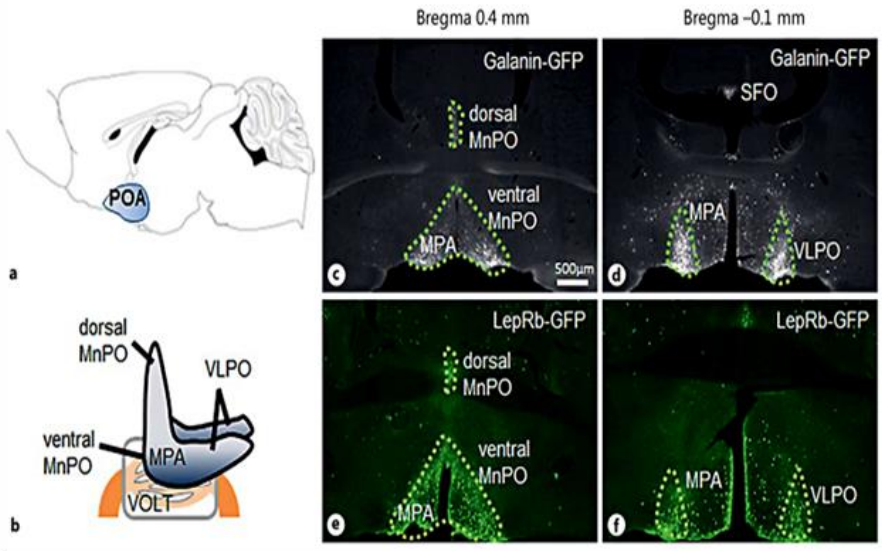

Figure 2. Sagittal preoptic area (POA) from mouse brain (Yu & al., 2018)

Vascular organ of the lamina terminalis (VOLT), subfornical organ (SFO), galanin- or leptin receptor (LepRb), dorsal and ventral median preoptic area (MnPO), medial preoptic area (MPA), ventrolateral preoptic area (VLPO).

Adaptation of both peripheral thermoreceptors locatedin the skin and thermoreceptors found in internal organs, information responses to POA through afferent pathways are seen in structures such as adipose tissue,blood vessels in the skin, and muscles(Yu & al., 2018). Thus, the POA is the basic structure responsible for central and peripheral integration. A possible heat increase in POA elicits behavioral responses and aspects that trigger heat loss, such as diffuse vasodilation. Similarly, counter-reactions such as vasoconstriction and stretching (Figure 3) are also observed at cooling temperatures in POA (Nkurikiyezu, K, 2019, Rothhaas & Chung, 2021).

The main reasonfor this period and behavioral responses observed inthe nuclei are the responses they receive by systems that balance heat production and loss. In these frames observed in the peripheral compartments in this center, after the analysis of theskin, which serves as protection from the environment, the known responses obtained from the buffering of the brain, heart, organs suchas the brains and the muscle executive system, which show richness in terms of vascularity. But in this buffering, the most sensitive and powerful regulation center is decided. However, this arrangement isnot always successful at extreme values in ambient conditions (Engorn et al., 2022).

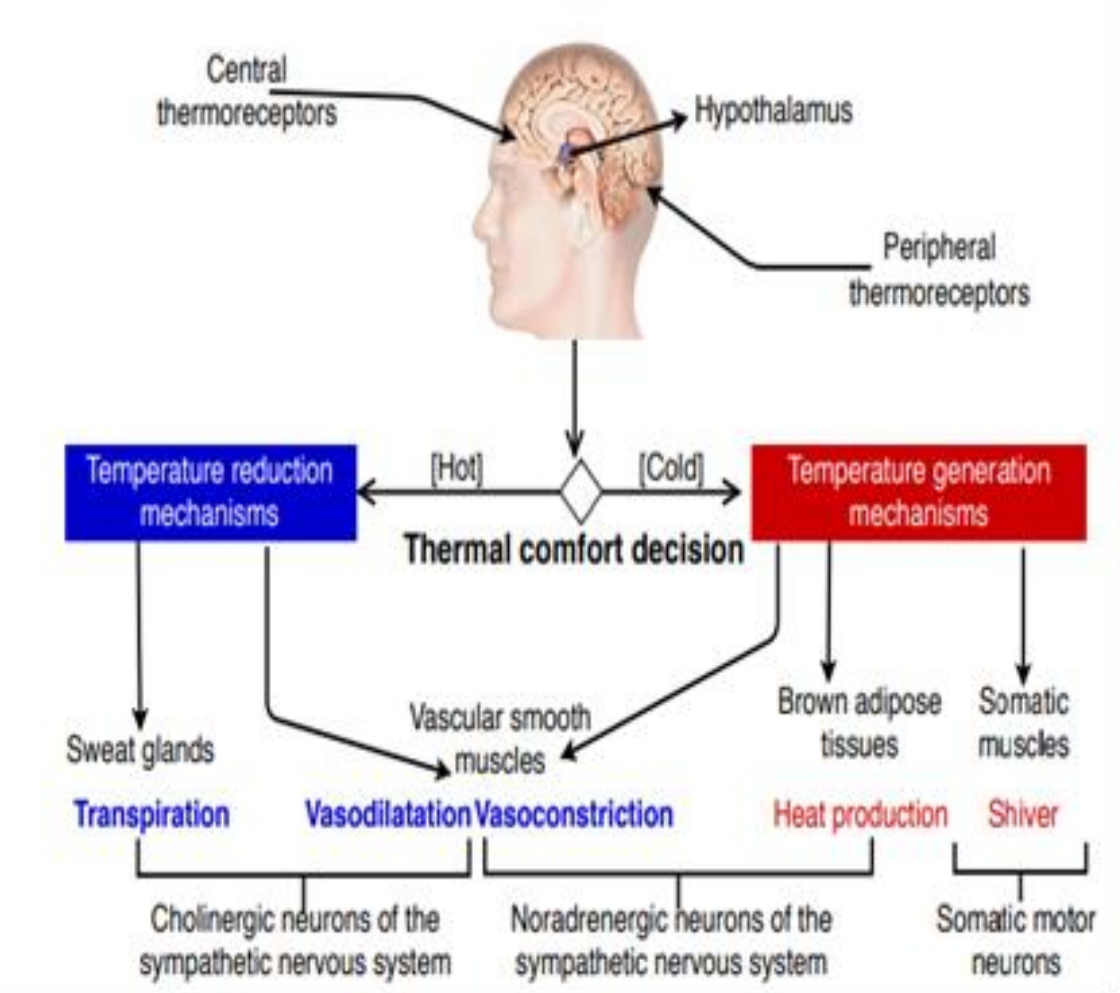

Figure 3. Summary of human thermoregulation (Nkurikiyeyezu, K, 2019).

Heat transfer takes place in the human body in four ways (Figure 4): convection (15%), evaporation (20%), conduction (5%), and radiation (60%). Radiation is the transfer ofbody heat to a more relaxed place with infrared rays, just as we are warmed by the sun's rays (Kuht & Farmery., 2014). Evaporation is the loss of liquid water in our body by becoming gas. Convection is the transfer of liquid by its movement across the surface. Conduction is how molecules in contact transfer heat to each other (Kuht & Farmery., 2014, Zaza & Hopf, 2019).

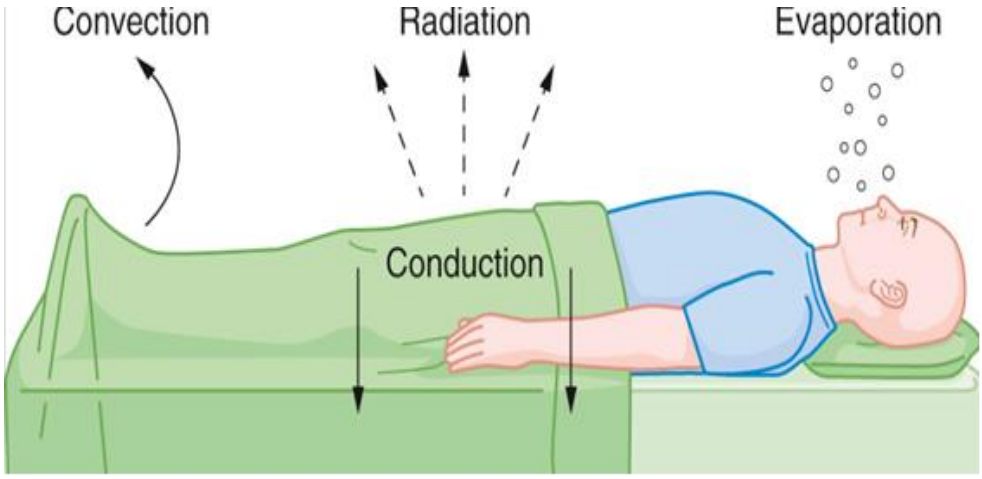

Figure 4. Heat transfer mechanisms (Zaza & Hopf, 2019).

Afferent and efferent projections in the POA of the hypothalamus,located in the anterior part of the hypothalamus, diffuse (Figure 5), are detected by the primary sensory ganglia at the temperature and come to the dorsal horn of the spinal cord, the lateral parabrachial nucleus (LPB) in the pons, and the POA. Markers of POA neurons activated in response to warming are pituitary adenylate cyclase-activating polypeptides (PACAP) and brain-derived neurotrophic factor (BDNF), Neuronal nitric oxide synthase (NOS1) and leptin receptor (LepRb). It is a local sensor in the POA, the transient receiving data is the M2 (TRPM2) ion channel. Thermoregulatory neurons in the POA project to the dorsomedial hypothalamus (DMH) and rostral raphe pallidus (rRPa) (Rothhaas & Chung, 2021).

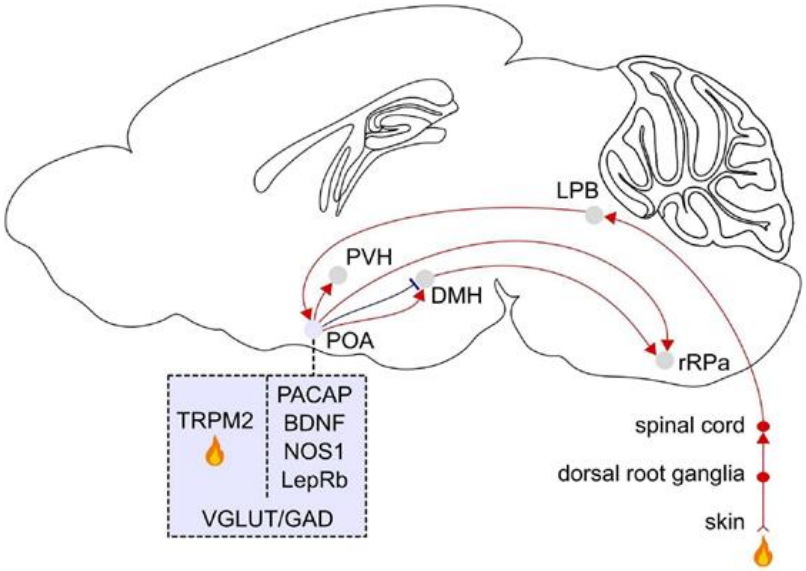

Figure 5. Afferent and efferent projections of heat-sensitive neurons in POA (Rothhaas & Chung, 2021).

The fact that heat-sensitive neurons are also involvedin the sleep mechanism and that thermoregulation changes with the circadian rhythm reveal that these processes are closely related to metabolism. This results from the control offood intake in the hypothalamus being influenced by neurons in the POA.

REFERENCES

Grodzinsky, E., Levander, M.S. (2020) History of the thermometer. In: Grodzinsky E, Sund Levander M, eds. Understanding fever and body temperature. *Palgrave Macmillan*:23–25.

Engorn, B., Harvey, H., Davis, P.J., Luginbuehl, I., Bissonnette, B. (2022). Thermoregulation. Smith's Anesthesia for Infants and Children, 7, 158-177.e8.

Kim, K., Jung, J., Schollaert, C., Spector, JT. (2021) A Comparative Assessment of Cooling Center Preparedness across Twenty-Five U.S. Cities. *International Journal of Environmental Research and Public Health.* 18(9):4801.

Kuht, J., Farmery, A.D. (2014). Body temperature and its regulation, *Anaesthesia & Intensive Care Medicine*, 15;6, 273-278.

Nkurikiyeyezu, K. (2019). An efficient thermal comfort delivery in workplaces. In 2019 IEEE International Conference on Pervasive Computing and Communications Workshops (*PerCom Workshops*) (pp. 427-428). IEEE.

Rzechorzek, N.M., Thrippleton, M.J., Chappell, F.M., Mair, G., Ercole. A., Cabeleira. M. (2022), The CENTER-TBI High Resolution ICU (HR ICU) Sub-Study Participants and Investigators, Jonathan Rhodes, Ian Marshall, John S O'Neill, A daily temperature rhythm in the human brain predicts survival after brain injury, *Brain*, 145, Issue 6, 2031–2048.

Rothhaas, R., Chung, S. (2021). Role of the Preoptic Area in Sleep and Thermoregulation. *Frontiers in Neuroscience* (15).

Sund-Levander, M., Forsberg, C., Wahren, L. K. (2002) Normal oral, rectal, tympanic and axillary body temperature in adult men and women. *Scandinavian Journal of Caring Sciences* 2002; 16: pp. 122.

Tongfei A.W., Chin F.T., Malin Å., Chao C., Marena T.F., Vanille J.G., Aaron D., Michael T.M., Yuh, N.J., Lily, Y. (2019). Thermoregulation via Temperature-Dependent PGD2 Production in Mouse Preoptic Area, *Neuron,* 103; 2, 309-322.e7.

Zaza, K.J, Hopf, H.W., (2019). Thermoregulation: Normal Physiology, Anesthetic Effects, and Perioperative Considerations. *Pharmacology and Physiology for Anesthesia*, 15, 300-310

Yu, S., François, M., Huesing, C., Münzberg, H. (2018) The Hypothalamic Preoptic Area and Body Weight Control. *Neuroendocrinology*,106:187-194.

The Relationship Between Vitamin Deficiency and Mental Diseases in Gastrointestinal System Diseases

Ebru TAŞ[1]

Introduction

Nutrition is of vital importance in terms of getting the nutrients necessary for the body and maintaining their lives. Thanks to nutrition, the body ensures the continuation of a healthy and balanced life, and nutrition is also a process that closely affects the quality of life of individuals, in which the living environment and socio-cultural characteristics are also decisive. Scientific studies and journals focusing on nutrition, the number of which is increasing day by day, include studies that examine different aspects and dimensions of nutrition. The common point of these studies is the fact that gastrointestinal system diseases are of vital importance in the nutrition process.

The gastrointestinal system is the system that starts with the mouth, ends with the anus, and includes all the organs in between. Therefore, the gastrointestinal system refers to the area where the nutrients, vitamins and minerals required by the body are taken, the raw material of energy is selected to be transferred to other systems in the body, and the processes in this area. The gastrointestinal system includes processes that are vital for the body, occurring in the chewing, swallowing, digestion, absorption, excretion and digestive system organs that occur in the space between the mouth and anus.

The phenomenon of eating and drinking is a process in societies from the past to the present, not only for meeting individual needs, but also for individuals to enjoy in their lives, and for this reason, eating and drinking culture, gastronomic tourism and culinary arts are also developed. Gastrointestinal system and its related parts/diseases are at the forefront of the fields that are most medically related to eating and drinking. Gastrointestinal system diseases can be seen as a phenomenon that affects not only the intake of nutrients in the daily lives of individuals and for this reason the protection of physical health, but also mental health.

In gastrointestinal system diseases, disorders related to the nutritional habits of individuals are diseases that affect not only the intake of basic nutrients, but also the intake of nutrients that perform the basic functions of the body, such as vitamins and minerals. For this reason, external supplements are given to ensure the balance of vitamins and minerals taken by individuals in gastrointestinal diseases as in nutritional disorders. However, all these supplements and external interventions do not replace the tendency and process of taking vitamins and minerals through the body's own system.

Another issue that is as important and prominent as gastrointestinal diseases today, and whose incidence and prevalence is increasing day by day, is mental diseases. Although it is still unclear whether the incidence of these diseases has increased in the past and there is a significant difference between the current incidence, or whether the already existing frequency has come to the fore with the increase in diagnosis and diagnosis opportunities, the most important finding is mental diseases. In terms of individual and public health, the situation is worse than it was known in the past. In this process, studies were also carried out on the causes of mental illnesses, which diseases they are

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associated with, what their effects are on the lives of individuals, and ways to increase treatment and quality of life studies in the short and long term. However, the way forward in the studies is progressing much more slowly than the progression or emergence of the disease.

Theoretically, it can be stated that nutrition and food intake have a fundamental and decisive effect in almost every disease. It is possible to define the body as a system where food enters and energy exits. Within this function, there should be no problem in the entry of energy or in other words, raw materials into the body in order to perform the output, that is, life and daily metabolism and functions. With this approach, it is possible to state that gastrointestinal diseases are related to almost all medical fields and systems. This relationship is also valid for mental illnesses.

There are many studies on the basic causes of mental diseases, and in these studies, mental diseases are generally divided into two groups as congenital and acquired. In congenital or genetic/hereditary mental diseases, although studies directly related to nutrition are not sufficient, there are studies reporting that nutrition, sugar intake and use of wrong foods during motherhood affect brain development in the fetus. The common point of these studies indicates that maternal nutrition and food intake affect mental health as well as infant health. At this stage, the nutrients taken by the mother directly have an important effect on the development of the brain.

In the case of acquired mental diseases, current studies are not yet sufficient to establish this relationship directly. However, today, studies are carried out that reveal the direct effects of heavy metal intake in mild autistic spectrum disorders, especially Down syndrome. In these studies, it is reported that important and remarkable developments in mental diseases are experienced with the control or reduction of heavy metal accumulation in the brain. This situation directly reveals the relationship between nutrition and therefore gastrointestinal system diseases and mental diseases.

Although studies revealing the relationship between nutrition and vitamin intake and mental diseases have started to emerge today, there has not been enough study among them that examines the relationship between vitamins and mental diseases through gastrointestinal system diseases. For this reason, in this section, it is aimed to examine the relationship between vitamin deficiency and mental diseases in gastrointestinal diseases in order to contribute positively to the development of field applications and treatment processes in this field.

Gastrointestinal System Diseases and Vitamins

The gastrointestinal tract is of great importance as it is the channel through which solid and liquid foods in the body are separated into small building blocks where carbohydrates, proteins and fats can be absorbed and mixed with water, vitamins and minerals into the blood circulation. in the gastrointestinal tract during their progression. Adequate and balanced nutrition; It is the supply of nutrients necessary for living things to grow, develop, maintain their lives and perform their life activities in the best way possible. A healthy digestive system is as important as adequate and balanced nutrition to maintain human health and life. Nutrition and treatment principles also differ depending on the type, region and individual factors of many diseases that occur in the digestive system from the mouth to the anus (Barlin ve Ercan, 2020).

Diseases associated with the gastrointestinal tract are divided into two groups: those arising from organic pathologies and those that arise functionally. Functional diseases are more common in the gastrointestinal tract than in other organ systems. The fact that the gut-brain axes are closely related to each other has revealed that the emergence of many functional gastrointestinal disorders can be triggered by the effect of psychosocial factors. An international panel of experts met in Rome in certain years and developed guidelines for the diagnosis and treatment of functional gastrointestinal diseases, known as the Rome criteria. Although functional gastrointestinal

symptoms have been described for centuries, the concept of functional gastrointestinal disease has emerged in recent years (İliaz ve Kaymakoğlu, 2016).

Vitamins are families of chemically unrelated organic compounds that are essential for normal metabolism in limited quantities as vital nutrients. These vitamins, other than vitamin D, cannot be synthesized in the human body and therefore must be obtained from food. In the last decade, a significant amount of research has been devoted to the role of vitamins in a variety of diseases, including their potential use in the prevention or treatment of various malignancies, inflammatory diseases of the gastrointestinal tract, and hepatobiliary diseases. Most of these trials are observational, but a significant number of well-designed interventional trials have emerged recently (Masri et al, 2015).

Some vitamins have been shown to beneficially modulate the gut microbiome when taken in high doses or administered to the large intestine, by increasing putative commensals such as vitamins A, B2, D, E and beta-carotene, increasing or maintaining microbial diversity. Richness such as vitamins A, B2, B3, C, K and vitamin D, increased production of short-chain fatty acids such as vitamin C or increased production of short-chain fatty acids such as vitamins B2, E. It indirectly affects gastrointestinal health or the microbiome by modulating barrier function (Pham et al, 2021).

In recent years, a wealth of research data has been published regarding the role of various vitamins in various gastrointestinal diseases. For example, most vitamins in observational trials have shown an inverse association with the risk of colorectal carcinoma as well as other malignancies such as gastric and esophageal cancer, but intervention trials have failed to show a clear beneficial preventive role. On the other hand, stronger evidence has been obtained from high-quality studies regarding the role of certain vitamins in certain issues. Examples of this include the therapeutic role of vitamin E in patients with nonalcoholic steatohepatitis, the adjunctive role of vitamins B12 and D to standard therapy for chronic hepatitis C virus, the role of vitamin C in reducing the risk of gallstones, a positive outcome. Beneficial effects of vitamin B12 in patients with aphthous stomatitis and vitamins D and B1 in patients with inflammatory bowel disease were reported. Other potential applications such as celiac disease, pancreatic cancer, pancreatitis, cholestasis and other potential areas are yet to be developed. Over the next few years, data from several ongoing intervention studies are expected to add to current knowledge (Masri et al, 2015).

The Relationship between Vitamin Deficiency and Mental Diseases

The main benefit of vitamins is for physical health. Vitamin insufficiency results in physiological abnormalities and ill health at low intake levels, but once at the moderate range, further quantities are not helpful. Similar to how the absence or nearly total absence of an environment's primary feature can cause unfavorable psychological states, its continued existence does not enhance these circumstances. Additionally, when moderate levels of specific vitamins are exceeded, the connection between greater vitamin intake and physical health changes from positive to negative. This pattern may also apply to several environmental factors, especially when happiness is considered in its entirety as opposed to in a more constrained sense. Many parts of life are emotionally upbeat until very high levels are attained (Warr, 2016).

Past research shows the importance of dietary nutrients for mental health. Some of the earliest research on nutrients related to mental illness identified irritability and mood problems in people with known B vitamin deficiencies and reported positive improvements in mental illness when treated with nutrients such as manganese and nicotinic acid; regardless of whether or not patients are deficient. Although interest in such research has declined since the advent of psychiatric drugs in the 1950s, recent studies on folic acid or vitamin B9 suggest that low levels may be associated

with depressive symptoms and poor response to antidepressants (Cornish ve Mehl-Madrona, 2008).

Although the relationship between vitamin deficiency and mental diseases has been the subject of attention in the past, the number of studies in this field has been increasing in recent years. People with depression, alcohol abuse, eating disorders, obsessive-compulsive disorder, or schizophrenia may neglect their self-care or adhere to certain eating patterns. Deficiency is more common in elderly patients and those with medical conditions. Because dietary patterns are associated with risk of psychiatric disorders, nutritional research often identifies several modifiable risk factors, such as folic acid, vitamin B12, and vitamin D intake. Nutritional counseling offers clinicians an intervention with minimal risk and ability for side effects. Psychiatrists should evaluate patients' diets and vitamin status (Ramsey and Muskin, 2020).

Relationships of Vitamins with diseases were shown in the Table 1.

Table 1. Relationship of Vitamins with diseases

Deficiency	Insufficiency	Symptoms
B1 (thiamine): Glycolysis, tricarboxylic acid cycle		
Rare; 7% in heart failure patients	5% total, 12% of older women	Wernicke-Korsakoff syndrome, memory impairment, confusion, lack of coordination, paralysis
B2 (riboflavin): FMN, FAD cofactors in glycolysis and oxidative pathways. B6, folate, and glutathione synthesis		
10% to 27% of older adults	<3%; 95% of adolescent girls (measured by EGRAC)	Fatigue, cracked lips, sore throat, bloodshot eyes
B6 (pyridoxal): Methylation cycle		
11% to 24% (<5 ng/mL); 38% of heart failure patients	14% total, 26% of adults	Dermatitis, glossitis, convulsions, migraine, chronic pain, depression
B9 (folate): Methylation cycle		
0.5% total; up to 50% of depressed patients	16% of adults, 19% of adolescent girls	Loss of appetite, weight loss, weakness, heart palpitations, behavioral disorders
B12 (cobalamin): Methylation cycle (cofactor methionine synthase)		
10% to 15% of older adults	<3% to 9%	Depression, irritability, anemia, fatigue, shortness of breath, high blood pressure
C (ascorbic acid): Antioxidant		
7.1%	31%	Scurvy, fatigue, anemia, joint pain, petechia. Symptoms develop after 1 to 3 months of no dietary intake

Kaynak: Ramsey and Muskin, 2020: 38.

Some meta-analyses, mostly for depression, have shown that vitamin D is crucial for a number of mental health difficulties in addition to physical health problems. Vitamin D supplements may be useful in easing the symptoms of depression, according to meta-analyses. The findings, however, are contradictory because adequate baseline levels of 25(OH)D and the studies under review were characterized by a high risk of bias. Additionally, some meta-analyses have reported results that contradict one another and that vitamin D does not reduce the severity of depressive symptoms. According to a systematic review and meta-analysis, the beneficial effects of vitamin D supplementation were also linked to a decrease in the emergence of negative emotions and an improvement in quality of life (Guzek et al, 2021).

In mental health, vitamin D deficiency has been linked to schizophrenia, depression, anxiety, Alzheimer's, and others. Risk factors for vitamin D deficiency include darker skin, insufficient sun exposure, autoimmune diseases, flu, advanced age, and the use of certain medications such as anticonvulsants. Vitamin D is likely to play an important role in the treatment and prevention of various mental health problems. In particular, adequate vitamin D intake during the perinatal phase is likely to positively affect a person's long-term mental health (Mutsatsa et al, 2013).

The persistent symptoms of a mental disease are brought on by vitamin and mineral deficiencies. In addition to neurological symptoms, thiamine deficiency, which is frequent in alcoholism, can result in disorientation and psychotic symptoms. Patients with anxiety disorders have been successfully treated with thiamine. Depression has been successfully treated with vitamin B6. In addition to inhibiting hypnotic and anticonvulsant action, niacinamide has also been shown to influence spinal cord activity, promote muscular relaxation, and lessen aggressive behavior. Low serum vitamin D levels have been linked in studies to the presence and severity of depression. Numerous other symptoms, such as hallucinations, agitation, anxiety, irritability, disorientation, insomnia, migraines, and delirium, can result from a deficiency of dietary magnesium when it is combined with too much calcium and stress. The study's findings suggested that the pathophysiology of schizophrenia may be influenced by changes in important trace elements like manganese, copper, and iron. Deficits in vitamin B12, folic acid, and ferritin can also cause psychiatric symptoms (Örüm et al, 2018).

The link between vitamin D and depression was furthered by the identification of vitamin D receptors in brain areas associated with depression. Studies on both humans and animals have demonstrated the presence of vitamin D receptors and the 1-hydroxylase enzyme in the brain, as well as the contribution of vitamin D to the health of the central nervous system. Low serum vitamin D levels were found in people with depression and other psychiatric diseases. These findings indicate the potential benefits of vitamin D supplementation in the treatment of depression and rationally explain the link between limited sunshine exposure and an increased risk of seasonal depression (Ersoy ve Ersoy, 2017).

Relationship between Gastrointestinal System Diseases and Mental Diseases

The gastrointestinal tract and brain are closely linked by bidirectional neural, endocrine and immune pathways, often referred to as the gut-brain axis. This complex communication system not only maintains the homeostasis of the gastrointestinal tract but also influences motivation and higher cognitive functions. The gut-brain axis includes the central nervous system, autonomic nervous system, enteric nervous system, and the hypothalamic-pituitary-adrenal axis, as well as signal transduction via gut peptides. The role of the gut-brain axis is to integrate gut functions and connect the brain's emotional and cognitive centers to peripheral gut functions and mechanisms such as appetite, satiety, immune activation, gut permeability, gut reflexes, and enteroendocrine signaling (Söderquist et al, 2020).

According to studies, the brain has a direct effect on the stomach, so the two are closely related. Gastrointestinal diseases usually consist of chronic diarrhea, nausea, vomiting, irritable bowel or abdominal pain, and people suffering from these symptoms often experience some psychological problems and stress associated with it. Therefore, the idea that chronic illness affects mental health is not new; In fact, there are many studies examining the relationship between mental illness and other chronic diseases. The relationship between certain abdominal pains, such as bowel syndrome, and depression or anxiety is receiving a lot of attention. However, several studies have looked at the link between certain gastrointestinal problems and the occurrence of general mental problems such as anxiety or depression (Cantarero-Prieto and Moreno-MenciaI, 2022).

Psychobiotics, or probiotics for mental health, are currently of great interest to academics, physicians, and people with mental problems and stress-prone people. The first concrete proof of the contribution of probiotics on mental well-being comes from studies on animals (Łoniewski et al, 2022).

The digestive tract's breakdown, absorption, and disposal of food are controlled and directed by the enteric nervous system. The intestinal flora, which is now recognized as a distinct organ dependent on its digestive capabilities, has a bidirectional link with the enteric nervous system, which has grown in significance in addition to its digestive functions (Kurt et al, 2014).

It has been established that giving psychobiotics to animals can alter their behavior and the composition of their gut microbiome. Additionally, isolated strains of the probiotics known as psychobiotics have been shown to have an impact on vagus nerve activity and, when used for an extended period of time, can alter the expression of GABA receptors linked to the etiology of anxiety and depressive disorders. Numerous research projects, both experimental and clinical, have demonstrated that various probiotics can lower human cortisol levels (Łoniewski et al, 2022).

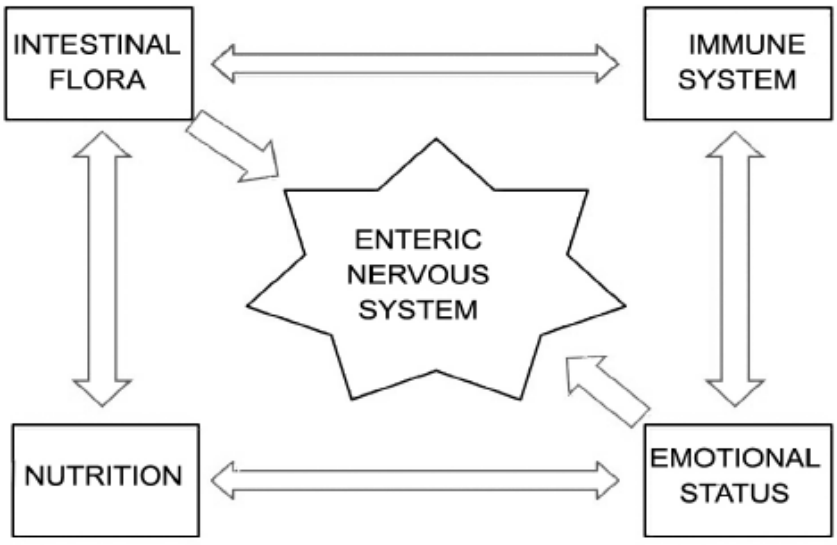
Table 2. Effects of motions on gastrointestinal system

Fright and depression	Anger and resentment
• Pallor of mucosa	• Hyperaemia of mucosa
• Reduced acid secretion	• Accelerated acid secretion
• Reduced gastric motor activity	• Increased motor activity

Source: Wilhelmsen, 2000: 73.

About 100 million nerve cells make up the enteric nervous system, which functions both independently and in concert with the central nervous system. It interacts with numerous neurotransmitters and is linked to a variety of bodily functions and structures, including intestinal flora, mood, the immune system, food efficiency, and a variety of diseases. The link between enteric nervous system, gut flora, and disease-development pathways has been the subject of recent research. Understanding the structure, connections, and operation of the enteric nervous system is crucial in order to comprehend these research and disease mechanisms (Kurt et al, 2014).

Figure 1. Relations of gastrointestinal system with immune system, nutrition and emotional status



Source: Kurt et al, 2014: 32.

It is well known that irritable bowel syndrome and mental illness are often associated conditions. A previous systematic review also found that irritable bowel syndrome is common in patients with chronic fatigue syndrome, pelvic pain, and temporomandibular joint disorder, which is thought to have some psychological component in their presentation. Despite these results, this prospective study has yet to prove that irritable bowel syndrome is actually caused by and not simply related to psychological stressors. Emerging evidence is beginning to characterize potential pathophysiological mechanisms of irritable bowel syndrome, such as post-infectious irritable bowel syndrome or altered gut microbiota that opposes a predominantly psychological underlying cause. Moreover, the role of microinflammation in the intestinal wall in irritable bowel syndrome is becoming clear, which may explain the overlapping irritable bowel syndrome-like symptoms in patients with irritable bowel syndrome (Shah et al, 2014).

Functional gastrointestinal disorders are a group of disorders that affect the gastrointestinal tract, often having a significant impact on health-related quality of life, but without a clear pathogenesis. The most common functional disorder of the gastrointestinal tract is irritable bowel syndrome, which affects the lower gastrointestinal tract. Psychiatric comorbidity is high in patients with functional gastrointestinal disorders; this suggests common or interacting disease mechanisms possibly associated with a hypothetical gut-brain connection in which gut microbiome variation may play an important role. In particular, a recent review points to an alternative pathway for the development of depression from the gut microbiome via the vagus nerve and gut-brain bidirectional communication mechanisms. Another report found a high correlation between anxiety and stress-related mental symptoms, including irritable bowel syndrome, laying the groundwork for further research on the vagus nerve and gut-brain axis. In addition, a pattern is emerging where uncontrolled sympathetic and poor vagal control interact with the microbiome and immune system, predisposing people to mental and gastrointestinal diseases (Söderquist et al, 2020).

Conclusion

As a result, when the relationship between vitamin deficiency and mental diseases in gastrointestinal diseases is examined, the rate of studies revealing the importance of vitamin levels in mental diseases with increasing prevalence and incidence is increasing. The most important finding in these studies is the fact that gastrointestinal diseases can affect not only the related physical diseases and quality of life of individuals, but also mental health issues. The increasing incidence of gastrointestinal diseases in societies and all over the world indicates that these diseases continue to be an important problem of both individuals and public health, despite the advances and successes in treatment processes, and that they are also related to nutrition and vitamin intake.

Today, developments in food processing and production technologies take place in an understanding that highlights certain features among foods and makes processed products, especially functional foods, more common. In this process, the most important indicator of how much the nutritional habits of individuals deteriorate is the studies on obesity and the increasing prevalence of weight-related problems in the society. In addition, changes in physical activity and daily life routines, increasingly sedentary life, cause significant changes in the metabolism of individuals. In this process, a number of problems arise in the intake of vitamins, just like the imbalances in the intake of other foods.

Although the importance of vitamins for our body is undoubtedly accepted in all medical sub-disciplines, it can be stated that studies examining the common or interrelationship of these vitamins in different branches of medicine are not yet sufficient. In fact, nutrition is the process of taking the raw material of energy, which is necessary for the body to perform all its activities, from outside. The type, quality, content and structure of the energy raw material or food taken in this

process directly affects the energy issue, which will be used in all other medical fields and which is the most basic input of all processes. Therefore, gastrointestinal diseases should be subject to more multidisciplinary studies.

The effects of gastrointestinal diseases on the mental health of individuals occur not only in the physical sense through vitamins, but also through nutritional disorders, diet or restrictions in the eating part of individuals' lives. Although nutrition is one of the basic needs of individuals and is a mandatory process, nutrition from the past to the present is also a process in which individuals feel good spiritually and is directly related to their mental health. Therefore, nutrition and therefore gastrointestinal diseases have important effects on mental health in this regard.

There is a mental as well as physical relationship in taking vitamins and maintaining the vitamin-mineral balance in the body. Especially in recent years, the increasing use of daily vitamin and mineral combinations, their transformation into products with high market value in marketing channels, and the encouragement of their use in pharmacies or various health institutions indicate that the issue of vitamins has become a global market rather than just a medical issue. In fact, this situation, which is similar to the rate of increase in mental diseases in the society, indicates that the effect of vitamin and mineral supplements on these diseases may be more than is known.

In general, when the relationship between vitamins and mental health in gastrointestinal diseases is evaluated, it is possible to make significant contributions to understanding the cause of many diseases, the mechanisms of which have not yet been fully explained, to develop solutions and treatment alternatives related to them, and to studies in different disciplines within the framework of preventive medicine.

REFERENCES

Barlin D, Ercan A, (2020). Conditions of use of food and vegetable products in the adults having digestive system problems. *The Turkish Journal of Academic Gastroenterology,* 19(1), 31-37.

Cantarero-Prieto D, Moreno-Mencia P. (2022). The effects of gastrointestinal disturbances on the onset of depression and anxiety. *PLoS ONE,* 17(1): e0262712

Cornish ve Mehl-Madrona, 2008

Ersoy, N. and Ersoy, G. (2017). D Vitamini Yetersizliği ve Depresyon: Ne Yapabiliriz? *H.Ü. Sağlık Bilimleri Fakültesi Dergisi,* 4(3), 1-14.

Guzek, D., Kołota, A., Lachowicz, K., Skolmowska, D., Stachoń, M., & Głąbska, D. (2021). Association between Vitamin D Supplementation and Mental Health in Healthy Adults: A Systematic Review. *Journal of clinical medicine*, *10*(21), 5156.

İliaz, R. ve Kaymakoğlu, S. (2016). Fonksiyonel Gastrointestinal Sistem Hastalıkları ve Otilonyum Bromür-Simetikon kombinasyonu. *Güncel Gastroenteroloji,* 20(4), 409-414.

Kurt, C., Göy, D.F., Özden, G., Barutçu, Ö. ve Bozer, C. (2014). Enteric Nervous System: A Review, *TMSJ,* 31-37.

Łoniewski, I., Skonieczna-Żydecka, K., Sołek-Pastuszka, J., & Marlicz, W. (2022). Probiotics in the Management of Mental and Gastrointestinal Post-COVID Symptomes. *Journal of clinical medicine*, *11*(17), 5155.

Masri, O. A., Chalhoub, J. M., & Sharara, A. I. (2015). Role of vitamins in gastrointestinal diseases. *World journal of gastroenterology*, *21*(17), 5191–5209.

Mutsatsa, S., Mushore, M., Ncube, K. & Currid, T. J. (2013). Vitamin D: the role of the sunshine vitamin. *British Journal of Mental Health Nursing,* 2(4), pp. 182-187.

Örüm, M. H., Kara, M. Z., Eğilmez, O. B. (2018). Determination of vitamin B12, folate, and ferritin levels of inpatients in a psychiatry clinic: A one-year retrospective study. *FNG & Bilim Tıp Dergisi*, 4(2), 71-78.

Pham, V. T., Dold, S., Rehman, A., Bird, J. K., & Steinert, R. E. (2021). Vitamins, the gut microbiome and gastrointestinal health in humans. *Nutrition research (New York, N.Y.)*, *95*, 35–53.

Ramsey, D. and Muskin, P.R. (2020). Vitamin deficiencies and mental health: How are they linked? *Current Psychiatry,* 12(1), 37-43.

Shah, E., Rezaie, A., Riddle, M., & Pimentel, M. (2014). Psychological disorders in gastrointestinal disease: epiphenomenon, cause or consequence?. *Annals of gastroenterology*, *27*(3), 224–230.

Söderquist, F., Syk, M., Just, D., Kurbalija Novicic, Z., Rasmusson, A. J., Hellström, P. M., Ramklint, M., & Cunningham, J. L. (2020). A cross-sectional study of gastrointestinal symptoms, depressive symptoms and trait anxiety in young adults. *BMC psychiatry*, *20*(1), 535.

Warr, P. (2016). Happiness and Mental Health: A Framework of Vitamins in the Environment and Mental Processes in the Person. in J. C. Quick and C. L. Cooper (Eds.), *Handbook of stress and health: A guide to research and practice*. London and New York: Wiley.

Wilhelmsen, I. (2000). The role of psychosocial factors in gastrointestinal disorders. *Gut* (Suppl IV)47, iv73–iv75

Bioinformatic Analysis Of ANGPTL4, Estimated to be Effective in Obesity

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Introduction

Obesity

According to the World Health Organization, obesity is defined as abnormal or excessive fat accumulation that poses a risk to health (World Health Organization, 2022). Obesity occurs from the imbalance between calorie intake and energy expenditure, which triggers the expansion or increase of adipose tissue that provides the storage of nutrients taken into the body (Marcelin et al., 2019). The energy imbalance that occurs because of the decrease in energy expenditure due to inactivity and the increase in energy intake due to the disorders in nutritional routine and consumption of high-calorie foods, has made obesity one of the most common diseases today and it is known that obesity affects more than 2 billion people around the world (Abenavoli et al., 2019).

Obesity can cause Type 2 diabetes and many different cardiovascular diseases like coronary heart disease, atrial fibrillation, heart failure, hypertension, and sudden cardiac death (Koliaki et al., 2019; Malone and Hansen, 2019). Apart from these diseases, it causes dyslipidemia, ischemic stroke, and cancer development, and it is reported that approximately 2.8 million people die annually due to overweight and related diseases (Abenavoli et al., 2019).

Obesity Related Genes

Other than high-calorie food consumption, obesity can be caused by environmental factors, lifestyle, and genetic factors (Abenavoli et al., 2019; Castaner et al., 2018). Research on obesity genetics has shown that the main genetic factors affecting obesity are ADIPOQ, FTO, LEP, LEPR, INSIG2, MC4R, PCSK1, and PPARG (Choquet and Meyre, 2011; Walley et al., 2009).

The PPARG gene encodes a transcription factor responsible for the expression of genes involved in lipid uptake and adipose tissue development (Aprile et al., 2014). This transcription factor is involved in the expression of many genes, especially related to lipid metabolism. One of these genes is the LPL gene, which encodes the lipoprotein lipase enzyme. Lipoprotein lipase (LPL) is the main enzyme responsible for the hydrolysis of triglycerides in chylomicrons and very low-

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density lipoproteins (VLDL) (Bensadoun, 1991). It catalyzes the hydrolysis of triglycerides to fatty acids intravascularly and plays a key role in lipid metabolism (Wang and Eckel 2009). ANGPTL4 is an inhibitory protein that is involved in the control of the hydrolysis activity of the LPL enzyme. Therefore, since ANGPTL4 is a protein that plays a direct role in lipid metabolism in the human body, it has a direct link with obesity (Morris, 2018).

ANGPTL4

Angiopoietin-like protein 4 (ANGPTL4) is also known as fasting-associated fat factor (FIAF). It is one of eight members of the ANGPTL family (ANGPTL1–8) (Santulli, 2014). This protein plays a role in the regulation of blood serum triglyceride clearance (Yin et al., 2009) and lipid metabolism (Lei et al., 2011) by mediating the inactivation of the lipoprotein lipase enzyme. Besides that, it can also play roles in regulating glucose homeostasis and insulin sensitivity (Gusarova et al., 2018), and reduces vascular permeability by inhibiting proliferation, migration, and tubule formation of endothelial cells (Mysling et al., 2016; Kristensen et al., 2018).

ANGPTL4, a glycosylated protein which produced and secreted in human hepatocytes and mouse adipocytes (Santulli, 2014), is encoded by the *ANGPTL4* gene. (O'Leary et al., 2016). The protein produced by induction of the gene's expression by peroxisome proliferation activator receptors (PPAR), functions as a serum hormone that regulates glucose homeostasis and lipid metabolism (La Paglia et al., 2017). PPAR's functions are modulated by the concentration of fatty acids and oxygen levels in the blood and intracellular composition (González-Muniesa et al., 2011). Dietary fats pass from the intestines to the blood as lipoproteins. Triglycerides in triglyceride-rich lipoproteins (VLDL and chylomicrons) are broken down into fatty acids by LPL activity (Lei et al., 2011). When the concentration of fatty acids acting as agonists of PPAR transcription activator increases, ANGPTL4 expression is initiated by the heterodimer transcription activator complex formed by PPAR (Michalik et al., 2006) and RXR transcription factors (Kaddatz et al., 2010). Although the proteins involved in expression differ from tissue to tissue, the mechanism of expression is still not fully elucidated (Dijk and Kersten, 2014). In white adipose tissue (WAT), expression of the gene is regulated in by PPARγ, glucocorticoid receptor (GR), and hypoxia-inducible factor 1α (HIF1α) protein, while in the liver it is induced by PPARα, PPARδ, and GR. There is no LPL expression present in the liver, therefore ANGPTL4 is secreted directly into the bloodstream and is responsible for the inhibition of LPL in peripheral tissues (Dijk and Kersten, 2014; Kaddatz et al., 2010). Since obesity can be defined as widened WAT (Reyes-Farias et al., 2021), the expression mechanism in WAT can be taken as a basis when investigating the relationship of ANGPTL4 with obesity.

This protein demonstrates its biological effects through autocrine/paracrine and endocrine processes, and it has been associated with various diseases such as cancer metastasis, cardiovascular diseases, wound healing, inflammation, diabetes, and obesity (Santulli, 2014).

ANGPTL4 is a fusion protein composed of an N-terminal α-helix and a C-terminal fibrinogen-like domain (Lei et al., 2011). The N-terminal domain is responsible for converting the active form of LPL into an inactive form and for the inhibitory effects on LPL, whereas the C-terminal domain facilitates the protein's antiangiogenic functions (Yin et al., 2009). These two domains can be separated from each other by a short connecting region which can be cleaved upon secretion. This cleavage varies among tissues in humans; the liver secretes the cleaved ANGPTL4 while the adipose tissue secretes the full-length form (Lei et al., 2011).

The physiological importance of ANGPTL4's proteolytic processing is largely unknown. In a study, treatment with a strong PPARα agonist, fenofibrate, significantly increased the levels of cleaved ANGPTL4 in plasma. From this, it has been postulated that the cleaved form of ANGPTL4 could have certain functions (Mandard et al., 2004). There is evidence that the anti-angiogenic activity of the protein is regulated by its interaction with heparan sulfate proteoglycans (HSPGs) (Jin et al., 2005).

The question arises as to how ANGPTL4 modulates lipid metabolism by regulating the activation of the LPL enzyme, and its relationship with weight gain and obesity. In this study, various *in silico* analyses such as protein interactions, three-dimensional structure, and potential effects of mutations on the protein were evaluated and the results obtained from these analyses were intended to serve as a pre-study for future works and contribute to the literature.

Method

In this study, *in silico* analyses were carried out in two separate groups at the gene and protein level. In the gene level analysis, the Gene Multiple Association Network Integration Algorithm (GeneMANIA) database was used to identify genes that had previously been proven to interact with the ANGPTL4 gene and could potentially interact. (Warde-Farley et al., 2010). In the analysis at the protein level, various *in silico* tools were used for various purposes. First, the reference sequence of the protein was obtained in FASTA format from the NCBI protein database (Sayers et al., 2022) by searching for 'angptl4'. To investigate the similarity of human ANGPTL4 with other species, amino acid sequences from 20 different species (including human sequence) obtained from NCBI were loaded into the MEGA11 embedded tool and aligned using the ClustalW method, followed by a phylogenetic analysis (Tamura et al., 2021).

BioGRID (Oughtred et al., 2021) and STRING (Szklarczyk et al., 2021) databases were used to identify the proteins with which ANGPTL4 is or may be interacting Initially, a general overview has been provided regarding ANGPTL4 interactions with amino acid sequences loaded into the STRING database. Subsequently, the sequences of the proteins shown to interact in the BioGRID were acquired from the NCBI and reloaded into the STRING tool along with the sequences of the other proteins shown to interact in the STRING. The data obtained from the databases were then compared.

The estimation of the three-dimensional structure of proteins was made using the Chimera (Pettersen et al., 2004) tools. The amino acid sequences obtained from NCBI were loaded into these tools and the 3D structures were predicted using the default settings of the tools.

At the outset of the mutation analysis, the known mutations of ANGPTL4 were provided from UniProtKB. To determine which sites may be critical, an estimation of the protein's evolutionary conservation was performed using The ConSurf server (Ashkenazy et al., 2010; Ashkenazy et al., 2016; Berezin et al., 2004; Celniker et al., 2013; Sali, 1996; Meier and Söding, 2015). The server was run with just with the loaded amino acid sequence and default settings, and the results were obtained.

Based on the information conducted from The ConSurf server, the PROVEAN (Choi et al., 2012) was employed to predict the effects of potential mutations and their effects on the biological functions and stability of the protein, and the potential for disease formation. After the

tools were provided with the amino acid sequence and the potential mutations at particular positions, the results were obtained.

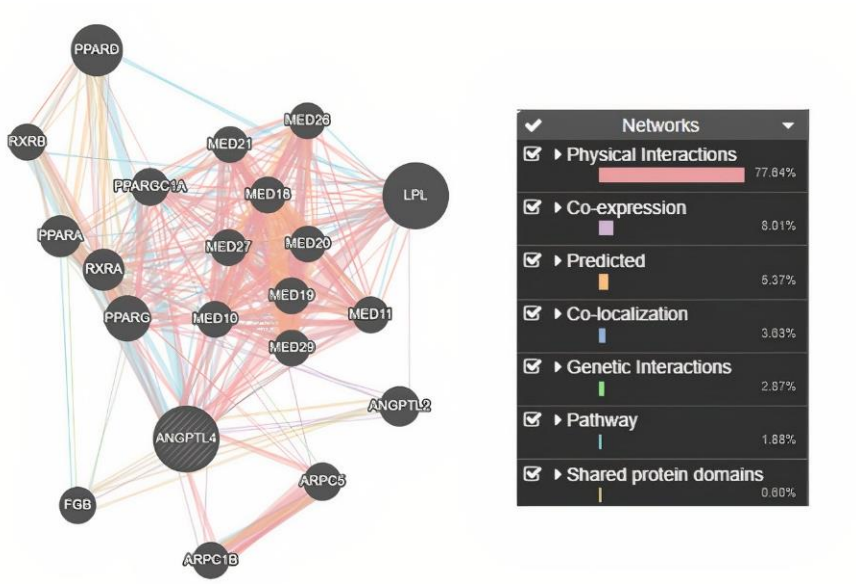
Results and Discussion

In silico Analysis of *ANGPTL4*

The GeneMANIA tool was used to identify the other genes that ANGPTL4 gene might be interacting with. GeneMANIA, when a single gene or list of genes is given, produces an output that contains functionally similar genes using existing genomic and proteomic data (Warde-Farley et al., 2010).

According to the GeneMANIA results, 20 genes that could interact with the ANGPTL4 gene were identified and are shown in Figure 1. GeneMANIA illustrates the degree of connection between a certain gene and other genes by the size of the gene labels. Regarding the results related to ANGPTL4, the genes with which the gene has the highest interaction are LPL, PPARD, PPARG, and ANGPTL2.

Figure 1 – GeneMANIA output.



GeneMANIA classifies the relationships between genes by seven different colors. According to these classifications, the interaction rates of the given genes with ANGPTL4 are 77.64%, the co-expression rates are 8.01%, and the estimated interaction rates are 5.37%. In addition to these, the rates of co-localization, genetic interaction, being on the same pathways, and containing associated protein domains are below 5%.

The results show that the LPL gene has the strongest connection to ANGPTL4. This is in accordance with expectations due to ANGPTL4's role as an inhibitor of LPL. Another interaction outcome is the PPARD and PPARG genes, which code for transcription factors with the same name. Given that these transcription factors serve as proteins that regulate the expression of ANGPTL4 in certain tissues, it is not surprising that the results of the interaction are high.

In silico Analyses of ANGPTL4 Protein

In the next stage of the study, the 406 amino acid length NP_647475.1 ID number sequence is selected among the four reference sequences found in the NCBI protein database. The selected sequence is also accepted as a precursor sequence by UniProt. The FASTA format of the sequence is as follows.

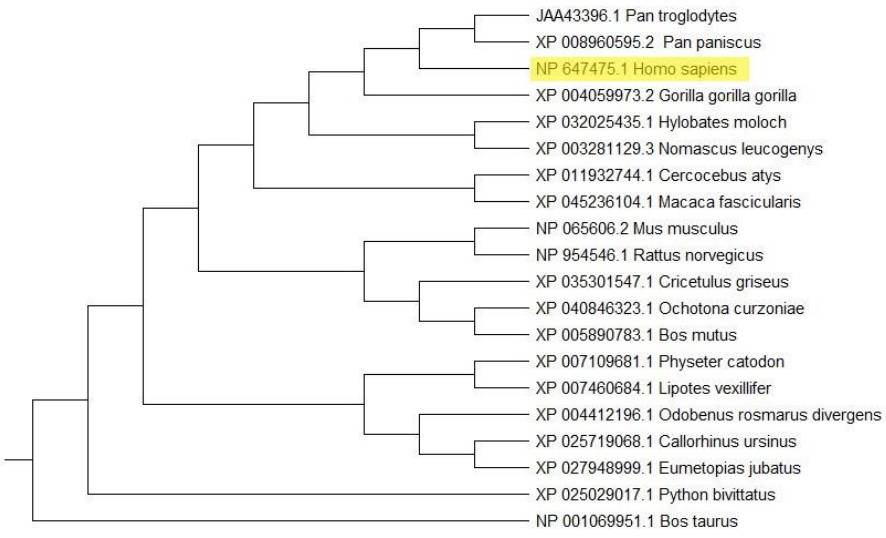
>NP_647475.1 angiopoietin-related protein 4 isoform a precursor [Homo sapiens]
MSGAPTAGAALMLCAATAVLLSAQGGPVQSKSPRFASWDEMNVLAHGLLQLGQGLR
EHAERTRSQLSALERRLSACGSACQGTEGSTDLPLAPESRVDPEVLHSLQTQLKAQNSR
IQQLFHKVAQQQRHLEKQHLRIQHLQSQFGLLDHKHLDHEVAKPARRKRLPEMAQP
VDPAHNVSRLHRLPRDCQELFQVGERQSGLFEIQPQGSPPFLVNCKMTSDGGWTVIQ
RRHDGSVDFNRPWEAYKAGFGDPHGEFWLGLEKVHSITGDRNSRLAVQLRDWDGN
AELLQFSVHLGGEDTAYSLQLTAPVAGQLGATTVPPSGLSVPFSTWDQDHDLRRDKN
CAKSLSGGWWFGTCSHSNLNGQYFRSIPQQRQKLKKGIFWKTWRGRYYPLQATTMLI
QPMAAEAAS

Phylogenetic Analyses

Sequences of amino acids in FASTA format belonging to 20 different animal species, including humans, were obtained from the NCBI protein database and were aligned with the MEGA11 tool to generate a phylogenetic tree of the ANGPTL4 (Figure 2). MEGA11 is a molecular and evolutionary genetic analysis program developed to analyze DNA and protein sequence data from species and populations (Tamura and Kumar, 2011).

Based on the output, the closest two primate species to human (*Homo sapiens*) ANGPTL4 protein are the bonobo (*Pan paniscus*) and chimpanzee (*Pan troglodytes*) species. The proteins from the bonobo and chimpanzee species are the closest and have undergone the most evolutionary changes. In addition to these two species, the protein from the western lowland gorilla (*Gorilla gorilla gorilla*) is following closest protein to the human ANGPTL4.

Figure 2 - Phylogenetic tree of the ANGPTL4.



The most suitable animals for the *in vivo* studies involving ANGPTL4 are bonobos and chimpanzees. When considering production costs and the phylogenetic affinity of their ANGPTL4 proteins with human ANGPTL4, the most suitable organisms for *in vivo* studies would be mice (*Mus musculus*). Studies with mice can give us important enlightenment about ANGPTL4.

Proteins ANGPTL4 May Interact With

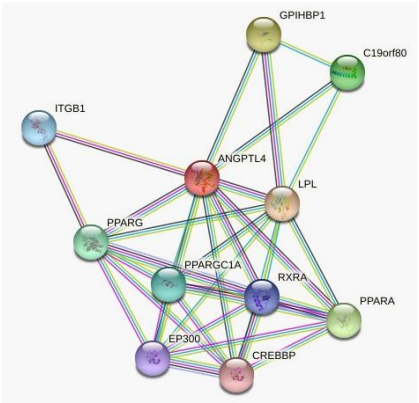
The ANGPTL4 protein is involved in many biological processes such as lipid metabolism, glucose homeostasis, angiogenesis, and metastasis. It is highly relevant to determine the interaction of this protein with other proteins and pathways to better understand its role in these biological processes and to identify other pathways with which it might interact. In this study, the BioGRID database (Oughtred et al., 2021) and the STRING tool (Szklarczyk et al., 2021) were utilized to determine protein-protein interactions.

BioGRID (Biological General Repository for Interaction Datasets) is a public database that archives and disseminates genetic and protein interaction data from model organisms and humans. BioGRID contains over 1,740,000 interactions derived from both high-throughput datasets and individual studies derived from over 70,000 publications in the literature (Oughtred et al., 2021). BioGRID was used to identify proteins and interactions associated with ANGPTL4.

STRING is a database containing known and predicted protein-protein interactions. These interactions originate from direct (physical) and indirect (functional) relationships, information transfer across organisms, and calculations from other databases. The tool evaluates the accumulated data and compares and scores it in seven separate categories and integrates these scores into a final 'combined score'. The combined score is scaled between zero and one and based on all data sources included in the calculation, a decision is made on whether the interaction is biologically meaningful. The closer the combined score is to one, the more reliable the type of interaction is (Szklarczyk et al., 2021).

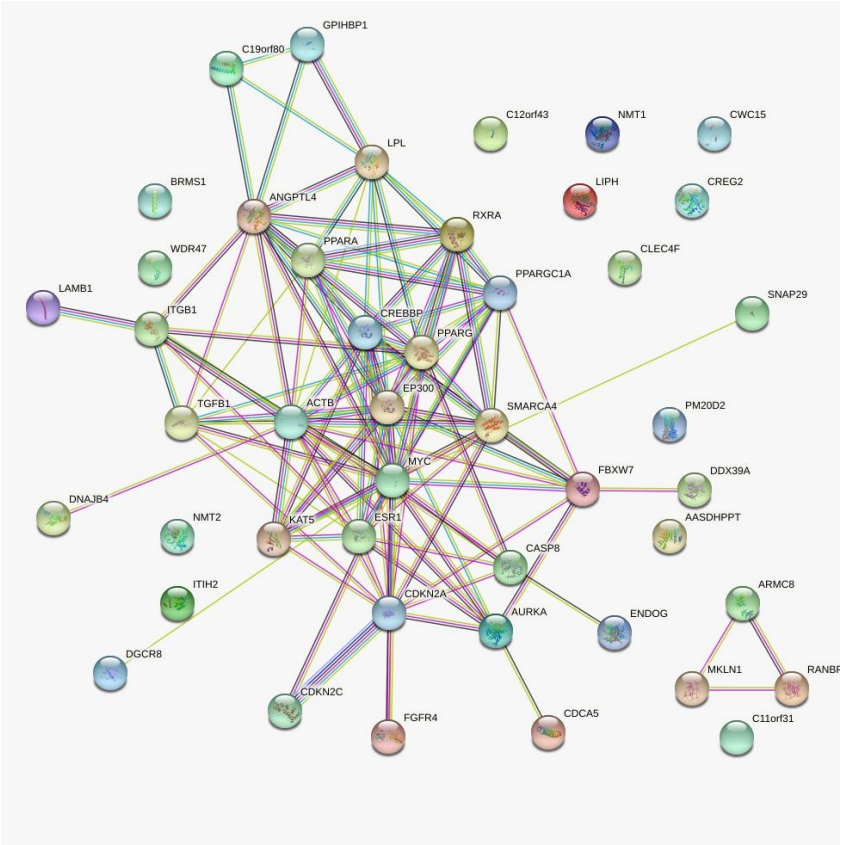
The amino acid sequence of ANGPTL4 was loaded into the STRING database and there were interactions between 10 proteins in the obtained results (Figure 3). The protein with the highest combined score was LPL with 0.991 points followed by GPIHBP1 (0.971), PPARA (0.966) and C19orf80 (0.962) proteins. The combined scores of the other proteins were also higher than 0.900. Having a combined score higher than 0.900 indicates that the proteins have strong interactions with ANGPTL4.

Figure 3 - STRING output obtained using the amino acid sequence of ANGPTL4.



Subsequently, the amino acid sequences of 36 proteins interacting with ANGPTL4 (aquired from BioGRID) obtained in FASTA format from NCBI and uploaded to the STRING database along with the amino acid sequence of ANGPTL4. The given result is shown in

Figure 4 – The STRING output using amino acid sequences of ANGPTL4 and 36 proteins (obtained from BioGRID).



After the results given in Figure 4, the 'more' button was used to find more proteins that could be in interaction with ANGPTL4. Upon effectuating this step, STRING reveals proteins with calculated lower combined scores and fewer interaction possibilities. Even if the interaction scores are low, these proteins could still interact directly with ANGPTL4 or indirectly with other proteins that interact with the protein. For example, in Figure 3, proteins SMARCA4 and SNAP9 are not present in results obtained only using STRING database, but in the results with proteins from BioGRID added (Figure 4), it is seen that these two proteins could interact with each other and with ANGPTL4.

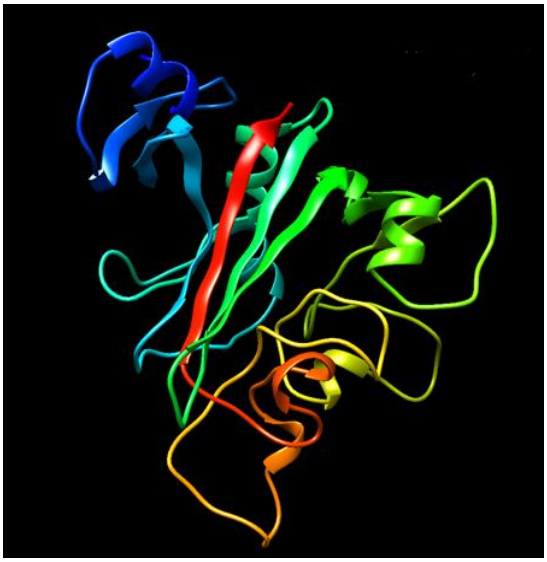
Predicted Protein Structure of ANGPTL4

Because there is no experimentally verified three-dimensional structure data for whole ANGPTL4 protein, the UCSF Chimera tools utilized in this study to predict the three-dimensional structures of the whole protein.

The three-dimensional structure obtained through Chimera program has been depicted in three different ways. The first representation is a ribbon diagram, which denotes the different secondary structures of the protein with different colors (Figure 5). A 3D structure has been

obtained that will be used when investigating the direct or indirect relationship of ANGPTL4 with obesity *in silico*.

Figure 5 - The ribbon diagram of predicted structure of ANGPTL4 (by Chimera).



Predicted Conserved Regions of ANGPTL4 Inhibitor and The Effects of Possible Mutations on Protein

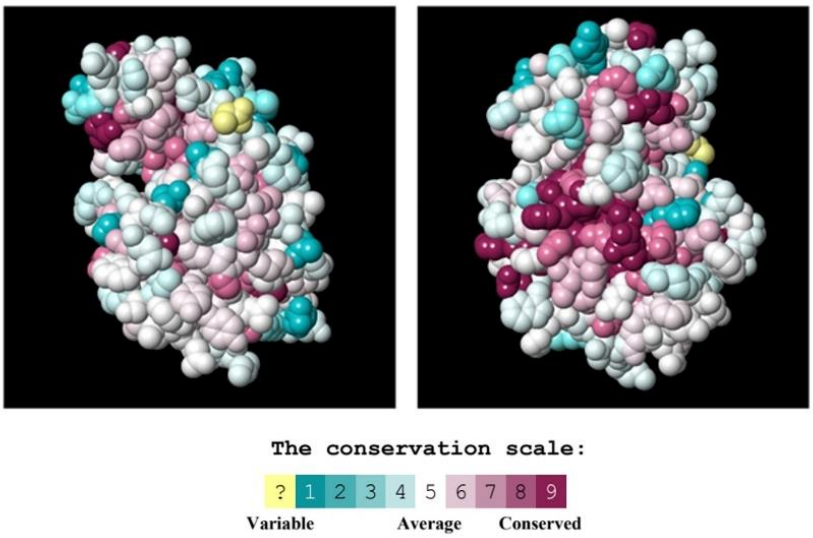
Mutations on proteins occur by changes in DNA or RNA sequences, leading to permanent alterations of the amino acid sequence of the protein. Information regarding known mutations of ANGPTL4 was obtained from UniProtKB (The UniProt Consortium, 2021). According to this information, 8 different mutations are known to occur at 8 positions in the amino acid sequence of ANGPTL4 (Yin et al., 2009; Lei et al., 2011; Biterova et al., 2018). In this study, potential mutations at positions 161-164, which are among known mutation locations, were investigated and distinct mutations capable of affecting protein functions were found. Based on the information acquired from UniProtKB, subsequent sections of this study have been conducted on potential mutations on these positions and their effects to the protein.

The ConSurf Outputs

The ConSurf server was employed to identify conserved and unconserved regions of protein in the evolution of ANGPTL4.

The ConSurf server is a bioinformatics tool developed to predict the evolutionary conservation of amino/nucleic acid positions in a protein/DNA/RNA molecule by considering the phylogenetic relationships between homologous sequences (Ashkenazy et al., 2010; Ashkenazy et al., 2016; Berezin et al., 2004; Celniker et al., 2013; Sali, 1996; Meier and Söding, 2015). The results are given as in amino acid sequence format and as a three-dimensional model (Figure 7).

Figure 7 - The conservation output of The ConSurf server (3D model).



Analysis of the ConSurf results revealed that positions 40, 76, 80, and 223 from UniProtKB's mutagenesis data are conserved regions.

Furthermore, the server predicted the amino acids in the range of 161-164 to be un-conserved, yet prior studies have demonstrated that mutations occurring in this region can cause vital damage to the protein's structure.

Consequently, it was conjectured that other potential mutations at the 161-164 locations could also be damaging, and thus mutation analyses were carried out based on the probable mutations of the amino acids in this region.

PROVEAN Outputs

PROVEAN (Protein Variation Effect Analyzer) is a software tool utilized to predict the biological effects of possible mutations that may occur in certain positions of the amino acid sequence of ANGPTL4. PROVEAN is a software tool which estimates how given indel mutations effects the studied protein's biological function. The predictions are based on the score generated by PROVEAN's various calculations. A score of -2.5 or lower indicates that the mutation has a deleterious effect, while a score above -2.5 indicates that the mutation does not have a detrimental effect (Choi et al., 2012).

According to the PROVEAN results it is predicted that indel mutations at amino acids 161, 162 and 164 in ANGPTL4 will not have any effect on the biological function of the ANGPTL4. However, it predicts that the replacement of lysine amino acid at position 163 with cysteine (K163C), phenylalanine (K163F) or tryptophan (K163W) could cause damage to the biological function of the protein.

Obesity is a highly prevalent disorder triggered by multiple factors. The aim of this study is to use I-Mutant 2.0 and I-Mutant Suite tools to analyze the effect of any mutations that can occur in the structure of the ANGPTL4 protein on its stability and the potential of these effects to cause any disease. In addition, the HOPE tool will be used to predict the differences in sizes, hydrophobicity, and electrical charge between some possible variants of the ANGPTL4 protein

and its natural structure, and to examine the effect of the mutations and the damage they can do to the protein's protection. Since ANGPTL4 is a protein that circulates in the bloodstream, it is important to make docking modeling of the relationship between ANGPTL4 and different proteins that may be related to obesity in order to interpret the relationship and to comment on the impact of this protein on obesity.

Kaynakça

Abenavoli, L., Scarpellini, E., Colica, C., Boccuto, L., Salehi, B., Sharifi-Rad, J., Aiello, V., Romano, B., De Lorenzo, A., Izzo, A. A., & Capasso, R. (2019). Gut Microbiota and Obesity: A Role for Probiotics. *Nutrients*, *11*(11), 2690.

Aprile, M., Ambrosio, M. R., D'esposito, V., Beguinot, F., Formisano, P., Costa, V., & Ciccodicola, A. (2014). PPARG in human adipogenesis: differential contribution of canonical transcripts and dominant negative isoforms. *PPAR Research*, 2014.

Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic acids research*, 44(W1), W344- W350.

Ashkenazy, H., Erez, E., Martz, E., Pupko, T., & Ben-Tal, N. (2010). ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic acids research*, 38(suppl_2), W529-W533.

Bensadoun, A. (1991). Lipoprotein Lipase. *Annual Review of Nutrition*, 11(1), 217–237.

Berezin, C., Glaser, F., Rosenberg, J., Paz, I., Pupko, T., Fariselli, P., ... & Ben-Tal, N. (2004). ConSeq: the identification of functionally and structurally important residues in protein sequences. *Bioinformatics,* 20(8), 1322-1324.

Biterova, E., Esmaeeli, M., Alanen, H. I., Saaranen, M., & Ruddock, L. W. (2018). Structures of Angptl3 and Angptl4, modulators of triglyceride levels and coronary artery disease. *Scientific reports*, 8(1), 1-12.

Castaner, O., Goday, A., Park, Y. M., Lee, S. H., Magkos, F., Shiow, S., & Schröder, H. (2018). The Gut Microbiome Profile in Obesity: A Systematic Review. *International journal of endocrinology*, *2018*, 4095789.

Celniker, G., Nimrod, G., Ashkenazy, H., Glaser, F., Martz, E., Mayrose, I., ... & Ben-Tal, N. (2013). ConSurf: using evolutionary data to raise testable hypotheses about protein function. *Israel Journal of Chemistry,* 53(3-4), 199-206.

Choi, Y., Sims, G. E., Murphy, S., Miller, J. R., & Chan, A. P. (2012). Predicting the functional effect of amino acid substitutions and indels. *PloS one,* 7(10), e46688.

Choquet, H., & Meyre, D. (2011). Genetics of obesity: what have we learned?. *Current genomics,* 12(3), 169-179.

Dijk, W., & Kersten, S. (2014). Regulation of lipoprotein lipase by Angptl4. *Trends in Endocrinology & Metabolism*, *25*(3), 146-155.

González-Muniesa, P., de Oliveira, C., de Heredia, F. P., Thompson, M. P., & Trayhurn, P. (2011). Fatty acids and hypoxia stimulate the expression and secretion of the adipokine ANGPTL4 (angiopoietin-like protein 4/fasting-induced adipose factor) by human adipocytes. *Lifestyle Genomics*, *4*(3), 146-153

Gusarova, V., O'Dushlaine, C., Teslovich, T. M., Benotti, P. N., Mirshahi, T., & Gottesman, O. (2018). Genetic inactivation of ANGPTL4 improves glucose homeostasis and is associated with reduced risk of diabetes. Nat Commun.

Jin, W., Fuki, I. V., Seidah, N. G., Benjannet, S., Glick, J. M., & Rader, D. J. (2005). Proprotein covertases are responsible for proteolysis and inactivation of endothelial lipase. *Journal of Biological Chemistry*, *280*(44), 36551-36559.

Kaddatz, K., Adhikary, T., Finkernagel, F., Meissner, W., Müller-Brüsselbach, S., & Müller, R. (2010). Transcriptional Profiling Identifies Functional Interactions of TGFβ and PPARβ/δ Signaling: SYNERGISTIC INDUCTION OF ANGPTL4 TRANSCRIPTION [S]. *Journal of Biological Chemistry*, *285*(38), 29469-29479.

Koliaki, C., Liatis, S., & Kokkinos, A. (2019). Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism*, *92*, 98-107.

Kristensen, K. K., Midtgaard, S. R., Mysling, S., Kovrov, O., Hansen, L. B., Skar-Gislinge, N., Beigneux, A. P., Kragelund, B. B., Olivecrona, G., Young, S. G., Jørgensen, T., Fong, L. G., & Ploug, M. (2018). A disordered acidic domain in GPIHBP1 harboring a sulfated tyrosine regulates lipoprotein lipase. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(26), E6020–E6029.

La Paglia, L., Listì, A., Caruso, S., Amodeo, V., Passiglia, F., Bazan, V., & Fanale, D. (2017). Potential role of ANGPTL4 in the cross talk between metabolism and cancer through PPAR signaling pathway. *PPAR research*, *2017*.

Lei, X., Shi, F., Basu, D., Huq, A., Routhier, S., Day, R., & Jin, W. (2011). Proteolytic processing of angiopoietin-like protein 4 by proprotein convertases modulates its inhibitory effects on lipoprotein lipase activity. *The Journal of biological chemistry*, *286*(18), 15747–15756.

Malone, J. I., & Hansen, B. C. (2019). Does obesity cause type 2 diabetes mellitus(T2DM)? Or is it the opposite?. *Pediatric diabetes,* 20(1), 5-9.

Mandard, S., Zandbergen, F., Tan, N. S., Escher, P., Patsouris, D., Koenig, W., ... & Kersten, S. (2004). The direct peroxisome proliferator-activated receptor target fasting-induced adipose factor (FIAF/PGAR/ANGPTL4) is present in blood plasma as a truncated protein that is increased by fenofibrate treatment. *Journal of Biological Chemistry*, *279*(33), 34411-34420.

Marcelin, G., Silveira, A. L. M., Martins, L. B., Ferreira, A. V., & Clément, K. (2019). Deciphering the cellular interplays underlying obesity-induced adipose tissue fibrosis. *The Journal of Clinical Investigation*, *129*(10), 4032-4040

Meier, A., & Söding, J. (2015). Automatic prgeneediction of protein 3D structures by probabilistic multi-template homology modeling. *PLoS computational biology*, 11(10), e1004343

Michalik, L., Auwerx, J., Berger, J. P., Chatterjee, V. K., Glass, C. K., Gonzalez, F. J., ... & Wahli, W. (2006). International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacological reviews*, *58*(4), 726-741.

Morris, A. (2018). ANGPTL4—the link binding obesity and glucose intolerance. *Nature Reviews Endocrinology,* 14(5), 251-251.

Mysling, S., Kristensen, K. K., Larsson, M., Kovrov, O., Bensadouen, A., Jørgensen, T. J., Olivecrona, G., Young, S. G., & Ploug, M. (2016). The angiopoietin-like protein ANGPTL4 catalyzes unfolding of the hydrolase domain in lipoprotein lipase and the endothelial membrane protein GPIHBP1 counteracts this unfolding. *eLife*, *5*, e20958.

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., ... & Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic acids research*, 44(D1), D733-D745

Oughtred, R., Rust, J., Chang, C., Breitkreutz, B. J., Stark, C., Willems, A., Boucher, L., Leung, G., Kolas, N., Zhang, F., Dolma, S., Coulombe-Huntington, J., ChatrAryamontri, A., Dolinski, K., & Tyers, M. (2021). The BioGRID database: A comprehensive biomedical resource

of curated protein, genetic, and chemical interactions. *Protein science : a publication of the Protein Society*, 30(1), 187–200.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *Journal of computational chemistry,* 25(13), 1605– 1612.

Reyes-Farias, M., Fos-Domenech, J., Serra, D., Herrero, L., & Sánchez-Infantes, D. (2021). White adipose tissue dysfunction in obesity and aging. *Biochemical Pharmacology*, 192, 114723.

Sali, A. (1996). Comparative protein modeling by satisfaction of spatial restraints. *Immunotechnology,* 4(2), 279-280.

Santulli G. (2014). Angiopoietin-like proteins: a comprehensive look. *Frontiers in endocrinology*, *5*, 4.

Sayers, E. W., Bolton, E. E., Brister, J. R., Canese, K., Chan, J., Comeau, D. C., Connor, R., Funk, K., Kelly, C., Kim, S., Madej, T., Marchler-Bauer, A., Lanczycki, C., Lathrop, S., Lu, Z., Thibaud-Nissen, F., Murphy, T., Phan, L., Skripchenko, Y., Tse, T., … Sherry, S. T. (2022). Database resources of the national center for biotechnology information. *Nucleic acids research*, 50(D1), D20–D26.

Strub, C., Alies, C., Lougarre, A., Ladurantie, C., Czaplicki, J., & Fournier, D. (2004). Mutation of exposed hydrophobic amino acids to arginine to increase protein stability. *BMC biochemistry*, *5*, 9.

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic acids research*, 49(D1), D605–D612. 42

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38(7), 3022-3027.

The UniProt Consortium, UniProt: the universal protein knowledgebase in 2021, Nucleic Acids Research, Volume 49, Issue D1, 8 January 2021, Pages D480–D489

Walley AJ, Asher JE, Froguel P. The genetic contribution to non-syndromic human obesity. *Nat Rev Genet*. 2009 Jul;10(7):431-42.

Wang, H., & Eckel, R. H. (2009). Lipoprotein lipase: from gene to obesity. *American Journal of Physiology-Endocrinology and Metabolism,* 297(2), E271–E288.

Warde-Farley, D., Donaldson, S. L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C. T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G. D., & Morris, Q. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic acids research,* 38(Web Server issue), W214–W220.

World Health Organization, *Obesity,* 20 April 2022, https://www.who.int/health-topics/obesity,

Yin, W., Romeo, S., Chang, S., Grishin, N. V., Hobbs, H. H., & Cohen, J. C. (2009). Genetic variation in ANGPTL4 provides insights into protein processing and function. *The Journal of biological chemistry*, *284*(19), 13213–13222

Yin, W., Romeo, S., Chang, S., Grishin, N. V., Hobbs, H. H., & Cohen, J. C. (2009). Genetic variation in ANGPTL4 provides insights into protein processing and function. *Journal of Biological Chemistry*, 284(19), 13213-13222.

Effect of Cyclophosphamide on the Biosystem

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Cancer is an important health-threatening problem due to its number of cases, progression, and high mortality rates in the world and our country (Jemal et al., 2011). This disease, the leading cause of death in economically developed countries, is second only to cardiovascular disease in developing countries. This indicates that about 13% (7.6 million) of all deaths are due to cancer, and the global burden is increasing exponentially due to the aging and growth of the world population and the increase in cancer-causing behaviors, especially smoking (Schottenfeld et al., 2013; Zhang, 2012). Cancers of the lung, stomach, liver, colon, and breast account for most cases (Chen et al., 2014). Chemotherapy, one of the cancer treatments, is an antineoplastic drug, which is able to reverse or stop cancer development. Moreover, it is the generic name for its use. In the early forties, the growth of malignant cell agents and drugs has been developed (Gately and Howell, 1993).

The main goal of chemotherapy in cancer treatment is to prevent or destroy tumor growth and the division of cancer cells without harming the healthy cells of the cancer patient. Therefore, chemotherapy often uses agents that inhibit cell proliferation. However, these drugs inhibit the proliferation of rapidly dividing cells such as cancer cells, as well as intestinal epithelial cells, hematopoietic stem cells in bone marrow, and hair follicles. Chemotherapeutic agents not only inhibit cancer cells, but also cause many side effects in patients (Amjad et al., 2022; Wallace, 2006) including nausea, vomiting, weakness, fatigue, decreased desire for food, anemia, leukopenia, thrombocytopenia, hair loss, mucositis, skin problems, insomnia, psychological problems, and prolongation of reproductive time due to the negative effects on hematopoietic cells in the bone marrow (Miller and Kearney, 2001).

Drugs commonly used in chemotherapy are listed as follow (Bignold, 2006; Wormington et al., 2020):

1. Alkylating agents

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2. Antibiotics and cytotoxic drugs

3.Antimetabolites

4. Biological reaction modifiers

5. Histone deacetylase inhibitors

6.Hormonal agents

7. Peptide hormones

8. Monoclonal antibodies

9. Protein kinase inhibitors

10. Taxanes

11. topoisomerase inhibitors

12. vinca alkaloids

Alkylating agents

Alkylating agents are chemicals that bind to biological molecules such as DNA and proteins and degrade their structure and function. In particular, when these agents are covalently bound to DNA, they methylate DNA and cause errors in replication. They are also used in low doses to prevent cell division and cancer treatment. These agents have been reported to cause mitotic abnormalities, chromosome breaks, and mutations (Bignold, 2006).

The mechanisms of action of alkylating agents

Alkylating agents cause changes in their structure by covalent binding to nucleophilic groups such as amine, carboxyl, sulfhydryl, or imidazole structures in nucleic acids and proteins. Most alkylating drugs are monofunctional methylating agents such as temozolomide, N-methyl-N-nitro-N-nitrosoguanidine, and dacarbazine. In contrast, some agents such as nitrogen mustard (chlorambucil and cyclophosphamide (SC) and chloroethyl agents (like nimustine, carmustine, lomustine, and fotemustine) are different alkylated. Nitrogen mustard has a preference for guanine-N7 alkylation. chloroethyl agents are bifanctional DNA/RNA alkylating compounds. Single methylation factors are attached to the N and O bases of DNA. 80% of methylated bases are formed by nitrogen atom (N) methylation. The stability of the bases in methylated DNA differs from each other. In general, DNA damage caused by methylation of the oxygen atom is highly mutagenic and toxic. However, the damage caused by the alkylation of the N atom is more cytotoxic and less mutagenic (Kondo et al., 2010).

Cyclophosphamide

SC is a member of the oxazaphosphorine family of mustard alkylating agents. It has been used to treat malignant tumors since its discovery in 1958. SC, with a molecular weight of 261.08 g/mol, is currently used to treat many types of cancer, especially leukemia, lymphoma, and breast cancer (Swan et al., 2020). When SC is used in high doses, it has a cytotoxic effect due to its alkylating properties that cause DNA damage. However, at low doses, it has immunomodulatory effects. Although high and low doses have not been standardized in clinical studies, SC is administered as a single dose of 1-3 mg/kg in low doses, while in high doses it is administered 120 mg/kg (Swan et al., 2020).

Metabolism of cyclophosphamide

SC is a pharmacological precursor and is metabolized in the liver by P450 enzyme systems to 4-hydroxycyclophosphaide and its tomomer, aldophosphamide, which are actively taken into the cell by passive transport or by glycoproteins. This substance is then converted to the active metabolites acrolein and phosphoramide. Acrolein and phosphamide are covalently bound to DNA and cause breaks in DNA. Phosphoramide mustard also causes DNA cross-links that lead to apoptosis and necrosis in the cell. Aldehyde dehydrogenase converts aldophosphamide to the nontoxic carboxyphosphamide, while 4-hydroxycyclophosphaide is enzymatically converted to 4-ketocyclophosphamide. Carboxyphosphamide is stabilized by the antioxidant molecule glutathione (Bignold, 2006; Iqubal et al., 2019; Swan et al., 2020). It has been reported an increase in the amount of 4-hydroxycyclophosphamide and carboxyethyl phosphamide in the blood plasma of patients treated with SC (Campagne et al., 2020).

Mechanisms of action of cyclophosphamide

Phosphoramide and acrolein formed by the metabolism of SC are active metabolites and cause DNA alkylation. The alkylated DNA is immediately repaired. If this is impossible, the tumor suppressor protein p53 is activated, which arrests the cell cycle and buys time for DNA repair. When DNA repair is impossible, the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B cell leukemia-xL (Bcl-Xl) and nuclear factor kappa B (NF-kB) protein activation can be inhibited, leading to apoptosis. In addition, aldosphamide accelerates cell death by increasing the phosphorylation of mitogen-activated protein kinases (MAPK), c-Jun N-terminal kinase (JNK), and p38 (Voelcker, 2020). Apoptosis is base of the SC function (Figure 1). In vitro, acrolein is constructed as a toxic agent during phosphoramide mustard (PAM) formation, but in vivo, proapoptotic hydroxypropanal (HPA) is constructed. The SC metabolites created in sequence 4-hydroxycyclophosphamide (OHCP) are the important and powerful reason for toxicity, aldophosphamide (ALDO) is an active metabolite of pharmacology and HPA increases the cytotoxic apoptosis stimulated by alkylation of DNA via PAM. Bypassing OHCP, formation of ALDO enhanced antitumor activity, but greatly reduced toxicity. Moreover, the anti-tumor function against developed solid P388 tumors in CD2F1 mice is induced via DNA damage resulting from a modified PAM that is weakly repairable (Glufosfamide as a new oxazaphosphorine anticancer agent).

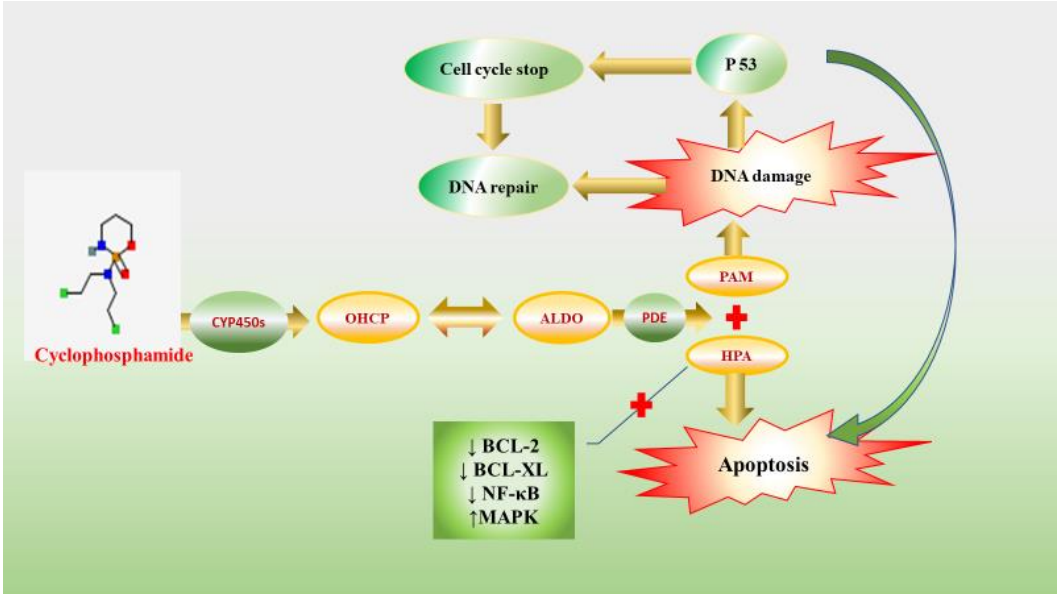

Figure 1*. Mechanism of action of cyclophosphamide (Voelcker, 2020)*

Oxidative stress

Free radicals contain unshared electrons in their outer shell. Therefore, they are unstable molecules and try to become stable by binding to other molecules. They cause changes and damage to the structure of DNA and the proteins, lipids and carbohydrates that bind with them, especially in cells. The most common radicals produced in cells are reactive oxygen species (ROS). Sources of ROS in cells include the mitochondrial electron transport chain, oxidation of unsaturated fatty acids and catecholamines, and NADPH-dependent oxidases. The production of ROS is increased in harmful gasses, radiation, drugs, alcohol, and pathogens (Birben et al., 2012; Pisoschi and Pop, 2015; Valko et al., 2006).

Reactive oxygen species

Superoxide radical: The superoxide radical ($O_2^{\bullet-}$) is formed when molecular oxygen gains an electron from the reducing agent. This radical, which has low reactivity, cannot pass through membranes and cannot be transported between cell layers because it is charged (Birben et al., 2012; Valko et al., 2006).

Hydrogen peroxide (H2O2): Hydrogen peroxide is formed by enzymatic two-electron reduction of oxygen or by enzymatic or non-enzymatic decomposition of superoxides. It is not radical but can easily penetrate cell membrane structures and diffuse readily into the cell nucleus, where DNA resides. The most significant damage caused by hydrogen peroxide is the formation of the hydroxyl ion, which is the most radical ROS, due to its interaction with metal ions (Valko et al., 2006).

Hydroxyl radicals ($OH^{\bullet}$): The most reactive type of oxygen in living systems is hydroxyl radicals ($OH^{\bullet}$). Because it has unpaired electrons, it interacts with all the major molecules it encounters in the cell (Halliwell, 2003, 2014; Valko et al., 2006).

Singlet oxygen ($^{-1}O_2$): Singlet oxygen is a highly excited form of molecular oxygen and is highly reactive. They carry two unpaired electrons in their molecular structure and interact with other molecules by forming covalent bonds. (Halliwell, 2003, 2014; Valko et al., 2006).

Peroxyl and ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals: Peroxy radical ($ROO^{\bullet}$) is formed as a result of the reaction of carbon-containing radicals with lipid, nucleic acid, carbohydrates and proteins with a hydroxyl radical followed by O2.The peroxy radical can also form the alkoxy radical ($RO^{\bullet}$) (Halliwell, 2003, 2014; Valko et al., 2006).

Cyclophosphamide-induced oxidative stress

SC increases MDA levels, a product of lipid peroxidation, in the brain (Dogan et al., 2015; Gaman et al., 2016; Oboh et al., 2011). This drug also increases the release of Tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) and, in parallel, the production of Cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), nuclear factor kappa light chain enhancer of activated B cells (NfkB), and p38-MAPK (Nafees et al., 2015) (Figure 2). Notably, COX2 and iNOS activity increases oxidative stress and oxidative damage to DNA. In addition, SC was found to decrease catalase and superoxide activity in the brain, heart, and lungs, as well as plasma antioxidant capacity. In addition, SC was found to reduce glutathione levels (Dogan et al., 2015; Ince et al., 2014).

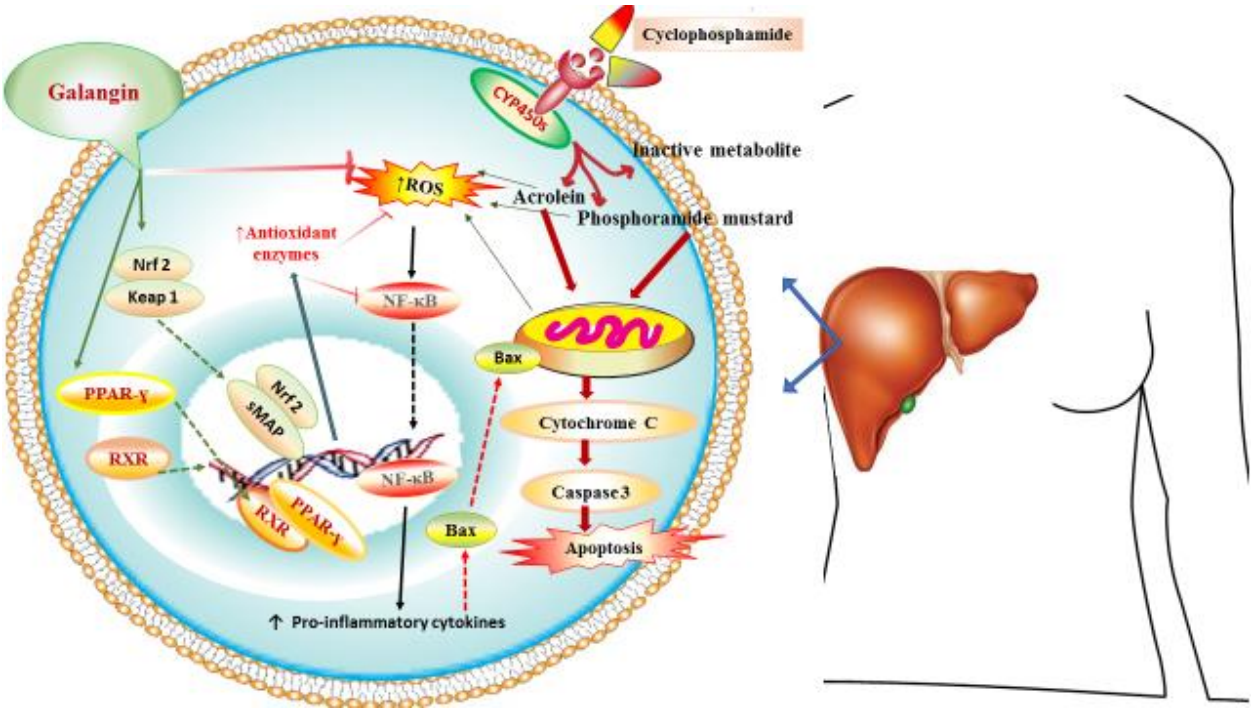

Figure 2*. Cyclophosphamide-induced ROS formation (Aladaileh et al., 2019)*

Effects of cyclophosphamide on the biosystem

Administration of SC can attack cancer cells and healthy cells during treatment, resulting in damage to many other organs (Swan et al., 2020).Several studies have investigated the effects of SC on the brain and cerebellum in animals. The study by Obhof et al. (2010) revealed that MDA levels, serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase expression were upregulated in the brain of rats following exposure to intraperitoneal administration of 75 mg/kg SC (Oboh et al., 2010). A study also demonstrated that SC caused an increase in glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase, and bilirubin in their serum (Oboh et al., 2011; Oboh and Ogunruku, 2010). Another investigation showed that different hypertrophies were observed in the neurons of the pathological cerebellar cortex of musculus rats that were treated with different doses of 75, 150, and 225 mg/kg for six weeks. In addition, vacuoles were found to increase neurons and fibers in the white matter of the brain tissue. Furthermore, the size and number of microglial cells increased in the cerebral cortexcortex (A. Al- Sabawy and J. Al Jumaily, 2006).

SC administration can damage the procine cells in the cerebellum and decrease the total volume of the cerebellum in 6-19 days old chicks (Singh et al., 1974). In another study, C57BL/6J mice were treated with 2 mg/kg doxorubicin (DOX), 50 mg/kg SC, and DOX + SC for four weeks. The density and morphology of spines in the hippocampus were measured ten weeks later in the dentate gyrus of the killed mice. A significant decrease in the intensity of short tracts was observed in the dentate gyrus rats (Flanigan et al., 2018).

SC using in cancer treatment causes damage to DNA through the formation of cross-links in DNA. Also, it has adverse effects on the functions of dendritic cells, effector T cells, and natural killer cells, which play essential roles in immune system (Swan et al., 2020). In addition, the administration of SC to patients has been shown to inhibit bone marrow cells and cause kidney inflammation and heart diseases such as pericardium and myocardium (Furst et al., 2011; Lotan et al., 2012; Wadia, 2015). Studies have reported that SC has teratogenic effects and that its long-term administration causes cancer in the bladder, esophagus, and lungs (Furst et al., 2011). There is also

a relationship between SC treatment and severe testicular damage such as histopathological changes, including extensive interstitial hemorrhage between testicular tubules, complete separation between different stages of spermatogenic cells, vacuoles in spermatogenic cells, and dispersion of spermatogenic cells(Mahmoud et al., 2016).

In an experimental study, spleen and bone tissues of rats was treated with 20 and 40 mg SC. There was found that SC induced a mutagenic effect on bone marrow (Moore et al., 1995). In a similar study with bones treated with high dose of SC, the number of nucleated cells in the bone marrow and the number of erythrocytes and leukocytes produced was decreased (Trasler et al., 1987). A previous study suggested that the cardiotoxic effects of SC were damage to cardiac tissue, morphologically characterized necrosis, hemorrhage, and subsequently fibrosis (Ludeman, 1999). In patients undergoing bone marrow transplantation, it has been reported that SC-induced endothelial damage may occur along with subsequent myocardial necrosis (Buja et al., 1976).

Many studies have reported that cardiotoxicity of SC may contribute to lipid peroxidation and oxidative stress (Fatani et al., 2010; Todorova et al., 2009). Myocardial tissue has aldehyde oxidase (AO) enzymes that protect it from oxidative damage. However, administration of SC causes inactivation of AO enzymes in rat myocytes (Selvakumar et al., 2004). SC (200 mg/kg) was found to cause the acute cardiotoxicity via reducing AO defense mechanisms in murine myocarium (Machida et al., 2003). Moreover, increased oxidative stress markers, decreased enzymatic and non-enzymatic AOs may promote carcinogenesis (Fisher-Wellman et al., 2009; Gupta et al., 2009). Indeed, a recent study demonstrated that cytotoxic effect of SC can induce ROS production and oxidative stress in rat (Tripathi and Jena, 2009). SC also cntributes to reduction in the number of platelet, leukocyte, and bone marrow cell by 54%, 92%, and 94%, respectively, respectively (Ayhanci et al., 2009; Taysi et al., 2008). Another study showed that 200 mg/kgof SC significantly reduced leukocyte (96%) and platelet (41%), as well as hemoglobin (21%) (Cengiz et al., 2018).

Other vital organs such as liver can also suffer serious complications following treatment with cyclophosphamide. In the liver tissue, intraperitoneal exposure to 200 mg/kg SC can lead to the reduction in the activity of endogenous antioxidant enzyme (Akamo et al., 2021). In addition, this chemotherapeutic drug contributes to inflamation via upregulation of expression of inflamatory cytocines, as well as oxidative sress via elavating production of oxidants, resulting in hepocyte dysfunction. In another report, SC caused DNA damage, apoptosis, and the architectural alteration derived from oxidative stress in the liver tissue (Patwa et al., 2020).

There are many reports surveyd a association between SC administration and alteration in urinary and reproductive systems. Toxic activity of SC can lead to disorders of testicular function and hemorrhagic cystitis via elavating cytocines such as IL 6 and IL 10, as well as reducing cyclic guanosine monophosphate (cGMP) as a regulator of cellular apoptosis (Ozatik et al., 2021). A study suggested that cystitis pain was attributed to marked increasein nitric oxide, malondialdehyde level, IL-1β, and TNF-α in bladder exposed to SC (Amanat et al., 2022). It has also been reported that 75 mg/kg SC can increase pro-inflammatory cytokines and lipid peroxidation, resulting in cellular apoptosis and nephrotoxicity (El-Shabrawy et al., 2020). In a research, measurment of total oxidant state and total antioxidant capacity also showed toxicity of SC on the kidney tissues (Gunes et al., 2018). Further investigation should be carried out on toxic side effect of SC on healthy organs.

References

A. Al- Sabawy, R., J. Al Jumaily, H., 2006. Histopathological Effects Induced by Cyclophosphamide in Brain Tissue of Albino Mice Mus musculus. Rafidain Journal of Science 17(13), 94-107.

Akamo, A.J., Rotimi, S.O., Akinloye, D.I., Ugbaja, R.N., Adeleye, O.O., Dosumu, O.A., Eteng, O.E., Amah, G., Obijeku, A., Cole, O.E., 2021. Naringin prevents cyclophosphamide-induced hepatotoxicity in rats by attenuating oxidative stress, fibrosis, and inflammation. Food Chem Toxicol 153, 112266.

Aladaileh, S.H., Abukhalil, M.H., Saghir, S.A.M., Hanieh, H., Alfwuaires, M.A., Almaiman, A.A., Bin-Jumah, M., Mahmoud, A.M., 2019. Galangin Activates Nrf2 Signaling and Attenuates Oxidative Damage, Inflammation, and Apoptosis in a Rat Model of Cyclophosphamide-Induced Hepatotoxicity. Biomolecules 9(8).

Amanat, S., Shal, B., Kyoung Seo, E., Ali, H., Khan, S., 2022. Icariin attenuates cyclophosphamide-induced cystitis via down-regulation of NF-small ka, CyrillicB and up-regulation of Nrf-2/HO-1 signaling pathways in mice model. Int Immunopharmacol 106, 108604.

Amjad, M.T., Chidharla, A., Kasi, A., 2022. Cancer Chemotherapy, StatPearls. Treasure Island (FL).

Ayhanci, A., Yaman, S., Appak, S., Gunes, S., 2009. Hematoprotective effect of seleno-L-methionine on cyclophosphamide toxicity in rats. Drug Chem Toxicol 32(4), 424-428.

Bignold, L.P., 2006. Alkylating agents and DNA polymerases. Anticancer Res 26(2B), 1327-1336.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. World Allergy Organ J 5(1), 9-19.

Buja, L.M., Ferrans, V.J., Graw, R.G.J., 1976. Cardiac pathologic findings in patiens treated with bone marrow transplantation. Human Pathol 7, 17.

Campagne, O., Zhong, B., Nair, S., Lin, T., Huang, J., Onar-Thomas, A., Robinson, G., Gajjar, A., Stewart, C.F., 2020. Exposure-Toxicity Association of Cyclophosphamide and Its Metabolites in Infants and Young Children with Primary Brain Tumors: Implications for Dosing. Clin Cancer Res 26(7), 1563-1573.

Cengiz, M., Yeşildağ, Ö., Ayhanci, A., 2018. Siklofosfamid Nedenli Hematoksisite Üzerine Karvakrolün Sitoprotektif Etkileri. Türkiye Tarımsal Araştırmalar Dergisi. 5, 125-130.

Chen, W., Zheng, R., Zhang, S., Zhao, P., Zeng, H., Zou, X., He, J., 2014. Annual report on status of cancer in China, 2010. Chin J Cancer Res 26(1), 48-58.

Dogan, Z., Kocahan, S., Erdemli, E., Kose, E., Yilmaz, I., Ekincioglu, Z., Ekinci, N., Turkoz, Y., 2015. Effect of chemotherapy exposure prior to pregnancy on fetal brain tissue and the potential protective role of quercetin. Cytotechnology 67(6), 1031-1038.

El-Shabrawy, M., Mishriki, A., Attia, H., Emad Aboulhoda, B., Emam, M., Wanas, H., 2020. Protective effect of tolvaptan against cyclophosphamide-induced nephrotoxicity in rat models. Pharmacol Res Perspect 8(5), e00659.

Fatani, A.G., Darweesh, A.Q., Rizwan, L., Aleisa, A.M., Al-Shabanah, O.A., Sayed-Ahmed, M.M., 2010. Carnitine deficiency aggravates cyclophosphamide-induced cardiotoxicity in rats. Chemotherapy 56(1), 71-81.

Fisher-Wellman, K., Bell, H.K., Bloomer, R.J., 2009. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid Med Cell Longev 2(1), 43-51.

Flanigan, T.J., Anderson, J.E., Elayan, I., Allen, A.R., Ferguson, S.A., 2018. Effects of Cyclophosphamide and/or Doxorubicin in a Murine Model of Postchemotherapy Cognitive Impairment. Toxicol Sci 162(2), 462-474.

Furst, D.E., Tseng, C.H., Clements, P.J., Strange, C., Tashkin, D.P., Roth, M.D., Khanna, D., Li, N., Elashoff, R., Schraufnagel, D.E., Scleroderma Lung, S., 2011. Adverse events during the Scleroderma Lung Study. Am J Med 124(5), 459-467.

Gaman, A.M., Uzoni, A., Popa-Wagner, A., Andrei, A., Petcu, E.B., 2016. The Role of Oxidative Stress in Etiopathogenesis of Chemotherapy Induced Cognitive Impairment (CICI)- "Chemobrain". Aging Dis 7(3), 307-317.

Gately, D.P., Howell, S.B., 1993. Cellular accumulation of the anticancer agent cisplatin: a review. Br J Cancer 67(6), 1171-1176.

Gunes, S., Sahinturk, V., Uslu, S., Ayhanci, A., Kacar, S., Uyar, R., 2018. Protective Effects of Selenium on Cyclophosphamide-Induced Oxidative Stress and Kidney Injury. Biol Trace Elem Res 185(1), 116-123.

Gupta, A., Bhatt, M.L., Misra, M.K., 2009. Lipid peroxidation and antioxidant status in head and neck squamous cell carcinoma patients. Oxid Med Cell Longev 2(2), 68-72.

Halliwell, B., 2003. Methods in Biological Oxidative Stress: K. Hensley and R.A. Floyd (Eds), 2003. Humana Press, New Jersey. Free Radic Res 37(10), 1145.

Halliwell, B., 2014. Cell culture, oxidative stress, and antioxidants: avoiding pitfalls. Biomed J 37(3), 99-105.

Ince, S., Kucukkurt, I., Demirel, H.H., Acaroz, D.A., Akbel, E., Cigerci, I.H., 2014. Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere 108, 197-204.

Iqubal, A., Iqubal, M.K., Sharma, S., Ansari, M.A., Najmi, A.K., Ali, S.M., Ali, J., Haque, S.E., 2019. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: Old drug with a new vision. Life Sci 218, 112-131.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA Cancer J Clin 61(2), 69-90.

Kondo, N., Takahashi, A., Ono, K., Ohnishi, T., 2010. DNA damage induced by alkylating agents and repair pathways. J Nucleic Acids 2010, 543531.

Lotan, E., Leader, A., Lishner, M., Gottfried, M., Pereg, D., 2012. Unrecognized renal insufficiency and chemotherapy-associated adverse effects among breast cancer patients. Anticancer Drugs 23(9), 991-995.

Ludeman, S.M., 1999. The chemistry of the metabolites of cyclophosphamide. Curr Pharm Des 5(8), 627-643.

Machida, Y., Kubota, T., Kawamura, N., Funakoshi, H., Ide, T., Utsumi, H., Li, Y.Y., Feldman, A.M., Tsutsui, H., Shimokawa, H., Takeshita, A., 2003. Overexpression of tumor necrosis factor-alpha increases production of hydroxyl radical in murine myocardium. Am J Physiol Heart Circ Physiol 284(2), H449-455.

Mahmoud, A.M., Soilman, H.A., Abd El-Hameed, A.M., Abdel-Reheim, E.S., 2016. Wheat germ oil attenuates cyclophosphamide-induced testicular injury in rats. World Journal of Pharmacy and Pharmaceutical Sciences 5(5), 40-52.

Miller, M., Kearney, N., 2001. Oral care for patients with cancer: a review of the literature. Cancer Nurs 24(4), 241-254.

Moore, F.R., Urda, G.A., Krishna, G., Theiss, J.C., 1995. An in vivo/in vitro method for assessing micronucleus and chromosome aberration induction in rat bone marrow and spleen. 2. Studies with chlorambucil and mitomycin C. Mutat Res 335(2), 201-206.

Nafees, S., Rashid, S., Ali, N., Hasan, S.K., Sultana, S., 2015. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFkappaB/MAPK pathway. Chem Biol Interact 231, 98-107.

Oboh, G., Akomolafe, T.L., Adefegha, S.A., Adetuyi, A.O., 2011. Inhibition of cyclophosphamide-induced oxidative stress in rat brain by polar and non-polar extracts of Annatto (Bixa orellana) seeds. Exp Toxicol Pathol 63(3), 257-262.

Oboh, G., Akomolafe, T.L., Adetuyi, A.O., 2010. Inhibition of cyclophosphamide-induced oxidative stress in brain by dietary inclusion of red dye extracts from sorghum (Sorghum bicolor) stem. J Med Food 13(5), 1075-1080.

Oboh, G., Ogunruku, O.O., 2010. Cyclophosphamide-induced oxidative stress in brain: protective effect of hot short pepper (Capsicum frutescens L. var. abbreviatum). Exp Toxicol Pathol 62(3), 227-233.

Ozatik, F.Y., Ozatik, O., Teksen, Y., Yigitaslan, S., Ari, N.S., 2021. Protective and therapeutic effect of hydrogen sulfide on hemorrhagic cystitis and testis dysfunction induced with cyclophosphamide. Turk J Med Sci 51(3), 1531-1543.

Patwa, J., Khan, S., Jena, G., 2020. Nicotinamide attenuates cyclophosphamide-induced hepatotoxicity in SD rats by reducing oxidative stress and apoptosis. J Biochem Mol Toxicol 34(10), e22558.

Pisoschi, A.M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 97, 55-74.

Schottenfeld, D., Beebe-Dimmer, J.L., Buffler, P.A., Omenn, G.S., 2013. Current perspective on the global and United States cancer burden attributable to lifestyle and environmental risk factors. Annu Rev Public Health 34, 97-117.

Selvakumar, E., Prahalathan, C., Mythili, Y., Varalakshmi, P., 2004. Protective effect of DL-alpha-lipoic acid in cyclophosphamide induced oxidative injury in rat testis. Reprod Toxicol 19(2), 163-167.

Singh, S., Sanyal, A.K., Kar, A.K., 1974. The effect of cyclophosphamide on the morphogenesis of the cerebellum in chick embryos. Anat Rec 178(1), 127-137.

Swan, D., Gurney, M., Krawczyk, J., Ryan, A.E., O'Dwyer, M., 2020. Beyond DNA Damage: Exploring the Immunomodulatory Effects of Cyclophosphamide in Multiple Myeloma. Hemasphere 4(2), e350.

Taysi, S., Memisogullari, R., Koc, M., Yazici, A.T., Aslankurt, M., Gumustekin, K., Al, B., Ozabacigil, F., Yilmaz, A., Tahsin Ozder, H., 2008. Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. Int J Radiat Biol 84(10), 803-808.

Todorova, V., Vanderpool, D., Blossom, S., Nwokedi, E., Hennings, L., Mrak, R., Klimberg, V.S., 2009. Oral glutamine protects against cyclophosphamide-induced cardiotoxicity in experimental rats through increase of cardiac glutathione. Nutrition 25(7-8), 812-817.

Trasler, J.M., Hales, B.F., Robaire, B., 1987. A time-course study of chronic paternal cyclophosphamide treatment in rats: effects on pregnancy outcome and the male reproductive and hematologic systems. Biol Reprod 37(2), 317-326.

Tripathi, D.N., Jena, G.B., 2009. Intervention of astaxanthin against cyclophosphamide-induced oxidative stress and DNA damage: a study in mice. Chem Biol Interact 180(3), 398-406.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1), 1-40.

Voelcker, G., 2020. The Mechanism of Action of Cyclophosphamide and Its Consequences for the Development of a New Generation of Oxazaphosphorine Cytostatics. Scientia Pharmaceutica 88(4).

Wadia, S., 2015. Acute Cyclophosphamide Hemorrhagic Myopericarditis: Dilemma Case Report, Literature Review and Proposed Diagnostic Criteria. J Clin Diagn Res 9(11), OE01-OE03.

Wallace, V.A., 2006. Cancer biology: second step to retinal tumours. Nature 444(7115), 45-46.

Wormington, A.M., De Maria, M., Kurita, H.G., Bisesi, J.H., Jr., Denslow, N.D., Martyniuk, C.J., 2020. Antineoplastic Agents: Environmental Prevalence and Adverse Outcomes in Aquatic Organisms. Environ Toxicol Chem 39(5), 967-985.

Zhang, J., 2012. Can We Discover "Really Safe and Effective" Anticancer Drugs? Advances in Pharmacoepidemiology & Drug Safety 01(05).

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