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EDITORS
Prof. Dr. Engin ŞAHNA
Prof. Dr. Hasan Hüseyin DOĞAN

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CHAPTER 1

THE EFFECT OF DIFFERENT LAYERS OF OĞUZLAR 77 WALNUT, A LOCAL VARIETY, ON DEVELOPMENT, LONG LASTING AND OXIDATIVE STRESS

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1. Introduction

Scientifically demonstrating the effectiveness of certain substances/ foods used in nutrition or herbal products considered medically important in the prevention and treatment of diseases increases the importance of nutritional support in the protection of our health. Many of the alternative medicine products used without fully elucidating the mechanisms of action cause irreversible damage. For this reason, trial studies of functional foods in creatures with short life forms have become increasingly common. In today's conditions, where the importance of using different parts of herbal products (seeds, fruits, flowers, roots, etc.) in various alternative treatments (food supplements) is increasing, not only the effect of the product on living things; The seeds used, soil properties, irrigation, environmental factors, growing conditions and environment (around the road or factory, etc.), storage conditions affect the product and living things, in short, people indirectly (Biçer, 2020).

In this study, the effect of different parts of the walnut, which is one of the most consumed foods in our country (green shells, hard shells, walnut skin, walnut curtain and walnut kernel), on D. melanogaster model organism was investigated. Walnut fruits consist of three structures on the tree. These; walnut kernel, hard shell and green shell. Although the fruit weight of the walnut varies according to the varieties and ecological conditions, it varies between 2-25 g. The shell thickness of the walnut can vary greatly between varieties. In addition to thin-shelled walnuts, which are also called paper-shelled walnuts, there are also very hard and thick walnut varieties called hard walnuts (Koçtürk and Gürhan, 2007). Walnuts are mostly consumed in the form of dried internal fruit. The green fruit bark, tree bark, leaf parts and fruit bark of the walnut plant are widely valued in the cosmetic and pharmaceutical industry. It is also used as a dyestuff in the textile and carpet industries (Oliveira et al., 2008).

Walnuts are consumed alone in every period, and continue to be consumed together with some other different foods. In addition, there are different opinions on the consumption and effects of different parts of the walnut plant. For this purpose, the effects of different parts of the walnut were evaluated separately in our study.

Walnuts are also a good source of protein (12-15%), fat (50-80%), carbohydrates, all amino acids, mineral compounds (3%), vitamins and low sugar content (2.5-4%). diet fruit (Mitrovic et al., 1997). It can also be used as a concentrated food in terms of high amount of protein and oil it contains. Walnut contains vitamin C and B group vitamins (B1, B2 and B6). In addition to vitamins; It is also rich in zinc, iron, copper, magnesium, potassium and phosphorus. It is poor in terms of cellulose and sodium content

(Anonymous, 1986). In the structure of 100 g walnut, approximately 14.10 g protein, 630.00 kcal energy, 68.00 g total fat, 3.20 g total carbohydrate, 9.70 g cellulose, 1.80 g ash, 3.20 g moisture and 391.00 mg potassium, 348.00 mg phosphorus, 89 It contains .00 mg of calcium, 113.00 mg of magnesium, 10.00 mg of sodium and 2.40 mg of iron (Akça, 2009). In addition to the rich unsaturated fatty acids it contains, walnut plays an important role in human nutrition in terms of micro and macro nutrients (Drehar et al., 1996). In a study, they stated that frequent consumption of minerals such as magnesium and potassium in the structure of walnuts provides protection against coronary cardiovascular diseases and regulates blood pressure (Prineas et al., 1993).

Walnut (Juglans regia L). Within the genus Juglans, there are 18 walnut species whose characteristics have been determined. Among these species, Juglans regia L., which is also known as Anatolian walnut, Persian walnut and English walnut, is the first thing that comes to mind when walnut is mentioned with superior fruit quality (Şen, 1986).

In this study, Oğuzlar 77 walnut, which is one of the walnut species grown in our country and produced in our province and has become very popular in recent years, was used (Picture 1.1). It is known that the walnut, which is estimated to have a lifespan of more than three hundred years, is the native walnut of the Oghuzes. Oğuzlar walnut has been known by the synonym of Aliağa for years. The fact that the Oğuzlar walnut variety belongs to the Oğuzlar district has been proven by the presence of trees 200 years and older in the district.

Picture 1.1. *Oğuzlar walnut and walnut tree*

When walnuts are to be used, they are consumed by discarding the green shell, hard shell and curtain and often using the part we call the walnut kernel. In the same way, only walnuts or hard-shelled versions are sold in markets and nuts. This situation causes about 50% of the walnut to go to waste. However, today, under the name of alternative medicine, it is used in different ways in these different parts. In this study, the effects of these parts, which were thrown away without being used or evaluated in different ways, on D. melanogaster individuals were evaluated separately on surviv-

al-development in larvae and life-length in adults. In addition, biochemical parameters such as Total antioxidant capacity (TAS), total oxidant stress (TOS), oxidative stress index (OSI) and Malondialdehyde (MDA) levels were evaluated in adults fed with different walnut concentrations.

The loss of electrons of an atom or molecule is called oxidation, and gaining electrons is called reduction (Çaylak, 2011). Free radicals are chemical products that carry at least one unpaired electron in their outer orbital (superoxide radical, hydrogen peroxide radical, hydroxyl radical, hydroperoxyl radical, alkoxyl radical). It is also formed during the conversion of nutrients into energy using oxygen (Vijayakumar et al., 2004). It is also produced as a by-product of metabolism in the body, as a result of exposure to radiation, environmental pollutants and sun rays, due to smoking and excessive alcohol consumption, as a result of feverish diseases, iron overload, excessive physical exercise and a diet rich in polyunsaturated fatty acids (Oksante, 2012). . The body's susceptibility to free radical-mediated damage is related to the balance between oxidant load and the adequacy of antioxidant defense (Vijayakumar et al., 2004). It is stated that reducing the damage can be possible by increasing the antioxidant concentration in the tissues (Vijayakumar et al., 2004). Oxidative stress is defined as the deterioration of oxidative balance as a result of the increase of free radicals formed during cellular metabolism, which detoxifies them, and the insufficiency of antioxidants. Having a balance between oxidants and antioxidants is essential for life (Yılmaz, 2010). Increased oxidative stress promotes aging by damaging many biological materials such as lipid, deoxyribo nucleic acid (DNA), coenzymes, cardiovascular, hypertension, various types of cancer, diabetes, obesity, lung diseases, allergies, skin aging, sunburns, dermatitis, There are studies showing that it causes many diseases such as Alzheimer's, Parkinson's, migraine, autism, cataract, retinal degeneration, weakening of the immune system, and degenerative diseases of the nervous system (Oksante, 2012).

Food antioxidants are substances that can reduce the negative effects of free radicals (Fang, et al., 2002). The antioxidant content of foods and the rate of benefiting from antioxidants vary according to the type of foodstuff, harvest time and harvesting methods, climate, temperature, humidity, light of the storage and preservation environment, preparation of the food and even the consumption habits of the person and society (Yılmaz, 2010).

Antioxidants can be divided into two groups as primary and secondary antioxidants according to their working mechanisms. primary antioxidants; They react with existing radicals and prevent them from turning into free radicals, which are more harmful forms, that is, free radical formation. Enzymes such as superoxide dismutase (SOD), Glutathioneperoxidase (GSHPx) and Catalase (CAT) are capable of destroying free radicals. Sec-

ondary antioxidants capture the oxygen radical and break the radical chain reactions. These are compounds such as vitamin C, vitamin E, uric acid, polyphenols (Yılmaz, 2010).

In inferential models for vertebrates such as humans, living organisms such as Drosophila are used to understand whole body oxidation and antioxidant mechanism with very little adipose tissue or total body size corresponding to liver tissue. It is preferred more because the number of replications does not require ethical and easy supply and easy cultivation in laboratory conditions. In our study, D. melonagaster model organism was used to observe the change in antioxidant activity against oxidation and to understand detoxification.

Drosophila larvae are also called fruit flies because they develop on sour fruits (Picture 1.2). D. melanogaster is one of the species in the family Drosophiidae (Adams et al., 2000). D. melanogaster, which has a short developmental biology, is known for its nutritional needs (Sang, 1956) and is used in obesity studies; The effects of some chemicals (such as organic insecticides) on lifespan, oxidant-antioxidant balance, hunger and excessive food intake are studied (Tettweiler et al., 2005). Drosophila is also used to understand the effects of nutrients on non-target organisms and target agricultural pests.

Picture 1.2. *Adults and larvae of Drosophila melanogaster*

As in mammals, in flies, food is made available to the organism by a digestive system, which is similar to the stomach and intestines and is divided into highly specialized sections (Padmanabha and Baker, 2014). In addition to its digestive function, the intestinal epithelium is a barrier between the internal and external environment (Buchon et al., 2009). The Drosophila digestive system creates a selective barrier that allows for the absorption of nutrients, ions, and water, and limits contact with potentially harmful substances such as toxins and pathogens. This selectivity is supported by a strong mucosal immune system. Fruit flies also have an antioxidant system that works similar to that in mammals. Enzymes in this antioxidant system; SOD is CAT, GPx, GST and GR, and in addition,

Drosophila has thioredoxin peroxidase (TRXP) (Güneş, 2013). Eun - Mi Ha et al. (2005) demonstrated in their study that immune-regulated catalase (IRC) provides a unique antioxidant defense system in Drosophila. Drosophila has many enzymes that detoxify toxins. The increase in SOD, CAT, GST activities against the increase in MDA is the body's natural defense mechanism (Ataş et al., 2017).

Researchers examined the antioxidant capacities of flavonoids, genotoxic and antioxidant capacities of chlorogenic acid, kaempferol, quercetin and quercetin 3a-d_glycoside against oxidative damage produced in vivo by hydrogen peroxide. It is stated that oxidative stress has occurred by the tested oxidate and reactions due to the destruction of peroxides by CAT, which is thought to occur in the case of CAT (Sotibrán et al., 2011).

In our study, the effect of Oğuzlar 77 walnuts on life expectancy in Drosophila melanogaster individuals was investigated. Aging, which started with the history of humanity and whose causes are wondered, maintains its interesting feature today and constitutes the subject of various researches. The most important reason for these studies is the increase in the elderly population, especially since the 19th century, and more and more people are getting older. With the attainment of growth, development and reproductive competence from the moment of birth, aging begins and the inevitable end is approached. The mechanism of aging is a multifactorial phenomenon that still contains unanswered questions. Different theories have been developed to explain this process. According to various researchers, internal factors such as hereditary characteristics, nutrition, living habits, environmental and psychological external factors and the biochemical and immunological reactions in the metabolism that they affect determine the lifespan (Bishop and Guarente, 2007). Nutrition is one of the important environmental stress factors affecting the longevity of insects as in all living things. The presence or absence of protein at low rates in the medium content may cause a decrease or complete cessation of egg production in adult individuals of Drosophila (Ashburner 1989).

2. Materials and Methods

2.1. Materials

2.1.1. Oğuzlar 77 walnuts

In the study, Oğuzlar 77 walnuts were obtained from a local producer serving in Oğuzlar district in July and August 2021. Due to the fact that the walnuts supplied are in the fruiting period of the plant, care has been taken to ensure that they are green-shelled and not damaged. The obtained walnuts were kept under room conditions after being sterilized.

2.1.2. Insect Culture

Wild-type adults (W1118) of D. melanogaster (Meigen) have been cultured since 2017 in the incubator in Amasya University Biological Research Laboratory (25 ± 2°C and 60-70% relative humidity in 200 ml culture bottles, 12 hour light 12 hour dark photoperiod).

2.2. Method

2.2.1. Preparation of Drosophila melanogaster individuals for chemical analysis and studies

Oregon (wild type) strain of Drosophila melanogaster individuals was used in the study. In order to eliminate the age and gender difference between the individuals to be used, pre-crossing was performed. For this purpose, 25♂x25♀ cross was made between flies kept alive with standard Drosophila ready-made medium in 200ml bottles. The 3rd stage larvae that emerged from the eggs obtained as a result of crossing were added to the medium and used in the control and experimental groups. Adult individuals obtained from larvae were used for enzyme measurements. Of the adult individuals, only male individuals were used. Due to the effect of factors such as egg laying behavior in females, they were not included in the enzyme measurements.

2.2.2. Collection of adults and application of different walnut layers for lifespan experiment

Male and female individuals of the Oregon-R strain of D.melanogaster were placed in bottles containing fresh medium and crossed (♀10 X ♂10). From the pupa formation, as the adults started to emerge, male individuals were collected every 4-5 hours and stocks were prepared for the control and experimental groups. Thus, 100 individuals of the same age (1-3 days/72±4 hours) were collected for 3 days for each walnut layer and each dose. Five different walnut layers were added to the standard Dro-

sophila medium (SDB) medium at 2 different doses, 0.25 and 0.5g. For the control groups, bottles containing SDB prepared with only distilled water were prepared. All applications were started simultaneously and environmental conditions were kept stable by ensuring that the ambient conditions were 60% relative humidity, 25±1°C and a permanent dark environment. The media were renewed twice a week, and during this change, all individuals were checked and counted dead and removed from the environment. The experimental procedure was continued until the last individual died in both the control and treatment groups.

2.2.2. Getting walnuts ready for work

The walnuts supplied were subjected to smashing and grinding processes after undergoing sterilization processes in order to use different parts. Different parts of the walnut, which were processed through grinding, were infused in order to add them to the fly medium. The infusion process is done by pouring boiling water on the plant parts, in a closed container or in a water bath, stirring frequently, for 5 minutes. It is the process of filtration after keeping it in a closed container and cooling it. The green shell of the walnut, the hard shell, the walnut veil, the walnut kernel and the walnut shell were subjected to infusion process and added to the fly medium.

2.2.3. Trial Pattern

Different parts of Oğuzlar 77 walnuts to be tried in this study were dissolved in pure water by infusion method and added to Drosophila medium (culture food) at rates of 0.25mg/ml and 0.5mg/ml. After determining the amount that the insect can live and develop by making preliminary experiments, biochemical analyzes were made according to the lower and upper limits to be added. Oxidation was induced by feeding insects with hydrogen peroxide as a positive control. Except for the preparation of the food and the transfer of adults to the food for laying eggs, all of the feeding experiments were carried out under the conditions in which the stock culture of insects was grown. Male and female individuals were taken from the culture for the trial design (25♂x25♀) and they were mated and their eggs were collected after six hours. Third instar larvae (72 h, 50 pcs) from collected eggs were inoculated into each flask. Newly hatched larvae (3rd stage larvae) of D. melanogaster were grown up to adult stage with foods containing different amounts of different parts of the walnut specified in the experimental design. The insect's survival, development and adult lifespan from egg to adult were determined. The amount of MDA, which is a product of lipid peroxidation, oxidative stress index (OSI), total antiox-

idant level and total oxidation level (TAS/TOS) analysis were determined in adult males and all experiments were repeated three times.

2.2.4. In vivo Biochemical analyzes

Concentrations were determined as a result of preliminary experiments, taking into account the studies conducted with Drosophila and different organisms (Sortibrán et al., 2015). The larvae were fed with these concentrations until they reached adulthood. In order to see more clearly the effects of the determined concentrations that occur very close to each other; Biochemical analyzes of control, lowest and highest concentrations were made. Since larval and adult nutritional levels are important in holometabol insects, biochemical analyzes were performed on adult individuals. In experiments on adult individuals, at least 20 individuals are studied (Taşkın et al., 2007). In our study, 25 adults were collected, paying attention to the collection of male individuals from each concentration. Samples were used by homogenizing three times with 1ml cold buffer (0.5M Potassium Phosphate buffer pH 7.2) (Homogenizer, Branson). The supernatants of the samples were collected and stored at -18°C until biochemical analysis (30 min at 4°C and 20.000g).

In the TAS measurement based on the reduction of the dark blue-green colored ABST radical to the colorless form by the antioxidants in the sample; The absorbances of the samples were measured at 660 nm in a spectrophotometer (Biochrom Libra S22) as specified in the kit procedure using commercial kits (Rel Assay Diagnostics) (Erel, 2004). TAS levels (mmol Trolox Eq L) of the samples were calculated according to the standard formula given in the studies (Erel, 2004). In the TOS measurement, which is based on the principle that the oxidants in the sample oxidize the ferrous ion-chelator complex to ferric ions, the ferric ions form color with the chromogen substance in the acidic environment; The absorbances of the samples (at 530 nm) were measured in a spectrophotometer (Biochrom Libra S22) using the commercial kit (Rel Assay Diagnostics) and the kit procedure. The TOS levels (μmol H_2O_2Eq L) of the samples were calculated according to the standard formula given in the studies (Erel, 2005). Experiments were repeated 3 times; TOS/TAS levels and OSI values were determined (Erel, 2005). MINDRAY-BS400 device was used for TAS and TOS measurement. Kits with catalog number RL0017 were used for TAS measurement and kits with catalog number RL0024 for TOS measurement. Based on the method used by Jain and Levine for the Measurement of Malondialdehyde (MDA) Amount, the amount of MDA, the end product of lipid peroxidation reacting with dethiobarbituric acid (TBA) at 532 nm, was measured (Jain and Levine, 1995). The amount of MDA was given as nmol/mg protein using a constant coefficient of 1.56 x 105 M-1 cm-1. In

the study, brand REL BIOCHEM-REL ASSAY device was used to measure the amount of MDA. Otto scientific kit with catalog number Otto1001 was used to measure the amount of MDA.

3. Results

In this study, primarily the effect of Oğuzlar 77 walnut on larval mortality rates in Drosophila melanogaster larvae was evaluated (Table 4.1). Then, the effect of walnut layers added to the medium after egg production in fruit flies on the pupalization and maturation rate and duration was evaluated (Table 4.2). The life span of the adults obtained from the larvae fed with walnut layers was investigated. Only male individuals were evaluated in the lifespan study (Table 4.3). The effects of different layers of walnut fruit on total antioxidant capacity, total oxidative stress, oxidative stress index (Table 4.4) and malondialdehyde enzyme levels were evaluated (Table 4.5).

Table 4.1. Effect of different parts of Oğuzlar 77 walnut on larval mortality rate of *Drosophila melanogaster*

W.G.S.: Walnut Green Shell, W.S.: Walnut Skin, W.H.S.: Walnut Hard Shell, W.K.: Walnut Kernel, W.C.: Walnut Curtain N.C.G: Negative Control Group, P. C.G: Positive Control Group

In this study, the change in the development time of *Drosophila melanogaster* larvae fed with different layers of Oğuzlar 77 walnuts was investigated. The effect of walnut layers added to Drosophila medium on pupation time, maturity rate and duration is as shown in Table 4.2.

Experimental groups	Larva (n)	Pupa (n)	Adult (n)	Percentage of Life (%)
W.G.S. (0,25 g)+H_2O_2	100	85	80	%80
W.G.S. (0,5 g)+H_2O_2	100	85	80	%80
W.K. (0,25 g)+H_2O_2	100	84	79	%79
W.K. (0,5 g)+H_2O_2	100	80	76	%76
W.S. (0,25 g)+H_2O_2	100	87	80	%88
W.S. (0,5 g)+H_2O_2	100	98	93	%93
W.H.S. (0,25 g)+H_2O_2	100	75	73	%73
W.H.S. (0,5 g)+H_2O_2	100	79	76	%76
W.C (0,25 g)+H_2O_2	100	87	86	%86
W.C. (0,5 g)+H_2O_2	100	92	91	%91
N.C.G. (Distiled water)	100	85	83	%83
P.C.G. (H_2O_2)	100	60	55	%55

Table 4.2. *The effect of different parts of Oğuzlar 77 walnut on the developmental period of Drosophila melanogaster.*

Experimental groups	3rd stage larva	3. Stage access time	1. Pupa being rate	Pupa being time	Adult being rate	Adult being time
W.G.S. (0,25)+H_2O_2	88±1,2	3,49±2,1	82±1,2	5,17±1,3	77±2,6	11,01±2,1
W.G.S. (0,5 g)+H_2O_2	85±1,1	3,99±1,3	78±1,6	5,99±1,6	72±2	11,87±3,7
W.K. (0,25 g)+H_2O_2	89±2,1	3,21±2,9	82±2,3	5,11±2,3	81±2,1	10,01±1,6
W.K. (0,5 g)+H_2O_2	89±0,8	2,99±3,1	83±2,6	4,77±1	79±1,1	10,21±2.5
W.S. (0,25 g)+H_2O_2	94±1,1	2,77±2,4	89±3,1	4,12±2	87±2,4	9,89±1,9
W.S. (0,5 g)+H_2O_2	93±1,5	2,87±1,6	91±3,8	4,86±1,4	89±3,1	9,56±2,2
W.H.S. (0,25g)+H_2O_2	88±2,7	3,77±3.2	83±2,5	5,21±2,1	78±3,6	10,22±1,3
W.H.S. (0,5 g)+H_2O_2	85±0,8	4.01±4.6	78±3,3	4,99±2,4	73±2,7	10,01±1,5
W.C (0,25 g)+H_2O_2	95±1,6	2,77±3.5	92±2,5	4,22±3,2	91±1,9	9,21±1
W.C. (0,5 g)+H_2O_2	92±2,3	2,56±1.7	90±1,8	4,55±2,2	89±3,2	9,88±1,7
N.C.G. (Distiled water)	91±1,7	3.31±2.4	88±2,3	4,75±2,5	83±2,4	10,01±1,5
P.C.G. (H_2O_2)	65±2,1	4,12±3,2	56±2,8	5,88±1,7	49±2,2	11,88±1,3

W.G.S.: Walnut Green Shell, W.S.: Walnut Skin, W.H.S.: Walnut Hard Shell, W.K.: Walnut Kernel, W.C.: Walnut Curtain N.C.G: Negative Control Group, P. C.G: Positive Control Group

Aging, which is almost universal and inevitable, progresses at varying rates and rates among populations, species and individuals, depending on internal and external factors. The life span, which is affected by many factors, continues to attract the attention of the scientific world. In our study, a longevity study was conducted to evaluate the nutritional factor, which is one of the factors affecting lifespan. Experiments were conducted on male individuals only. Average lifespan and maximum lifespans of flies fed with different layers of Oğuzlar 77 walnuts were determined. It has been determined that the walnut skin and walnut veil, which can be seen in Table 4.3, cause an increase in the life span in both ratios (0.25, 0.5g) compared to both the control groups and the other layers.

Table 4.3. *The effect of different parts of Oğuzlar 77 walnut on lifespan in male individuals of Drosophila melanogaster.*

Experimental groups	Number of Individuals	Max. life span	Average lifespan
W.G.S. (0,25 g)+H_2O_2	100	73	65+1.,3
W.G.S. (0,5 g)+H_2O_2	100	71	63+0,79
W.K. (0,25 g)+H_2O_2	100	77	70+1,79
W.K. (0,5 g)+H_2O_2	100	79	69+0,99
W.S. (0,25 g)+H_2O_2	100	86	81+1,18
W.S. (0,5 g)+H_2O_2	100	92	84+1.23
W.H.S. (0,25 g)+H_2O_2	100	78	65+2,11
W.H.S. (0,5 g)+H_2O_2	100	76	67+1,97
W.C (0,25 g)+H_2O_2	100	92	85+0,78
W.C. (0,5 g)+H_2O_2	100	91	80+0,89
N.C.G. (Distiled water)	100	75	69+1,34
P.C.G. (H_2O_2)	100	54	41+1,38

W.G.S.: Walnut Green Shell, W.S.: Walnut Skin, W.H.S.: Walnut Hard Shell, W.K.: Walnut Kernel, W.C.: Walnut Curtain N.C.G: Negative Control Group, P.C.G: Positive Control Group

It is accepted that phytochemicals such as phenolic compounds in walnut are beneficial for human health, reducing the risk of degenerative diseases by reducing oxidative stress and preventing macromolecular oxidation. In addition to their anticarcinogenic properties, these compounds have been shown to have free radical scavenging and metal chelating activities (Middleton, 1998). In our study, unlike other studies, the effects of all layers of Oğuzlar 77 walnuts on total antioxidant capacity (TAS), total oxidative stress (TOS), oxidative stress index (OSI) and malonaldehyde (MDA) enzyme levels were evaluated (Table 4.4, 4.5). In our study, biochemical analyzes of adults fed with different layers of walnut for 15 days were made. All of the adult individuals used in the study were selected from male individuals, female individuals were not included in the study. All studies were performed in 3 replicates and results are the average of these 3 studies.

Table 4.4. *TAS, TOS and OSI values in Drosophila melanogaster adults fed with different walnut layers*

Experimental groups	TAS (mmol/L)	TOS (µmol/L)	OSI
N.C.G. (Distiled water)	1,84	1,8	0,098
P.C.G. (H_2O_2)	0,96	3,92	0,408
W.G.S. (0,25 g)+H_2O_2	1,36	2,4	0,176
W.G.S. (0,5 g)+H_2O_2	1,44	2,48	0,172
W.K. (0,25 g)+H_2O_2	1,08	3,6	0,333
W.K. (0,5 g)+H_2O_2	1,12	3,52	0,314
W.C (0,25 g)+H_2O_2	1,99	1,48	0,074
W.C. (0,5 g)+H_2O_2	2,89	2,72	0,094
W.S. (0,25 g)+H_2O_2	1,48	1,64	0,111
W.S. (0,5 g)+H_2O_2	2,44	1,6	0,066
W.H.S. (0,25 g)+H_2O_2	2,12	4	0,189
W.H.S. (0,5 g)+H_2O_2	2,4	3,44	0,143

W.G.S.: Walnut Green Shell, W.S.: Walnut Skin, W.H.S.: Walnut Hard Shell, W.K.: Walnut Kernel, W.C.: Walnut Curtain N.C.G: Negative Control Group, P.C.G: Positive Control Group

Table 4.5. *MDA concentrations in adults of Drosophila melanogaster fed with different walnut layers.*

Experimental groups	absorbans	blank	abs-blank	result(n-mol/L)
N.C.G. (Distiled water)	0,06	0,03	0,03	1,88
P.C.G. (H_2O_2)	0,19	0,03	0,16	3,71
W.G.S. (0,25 g)+H_2O_2	0,93	0,03	0,9	21,21
W.G.S. (0,5 g)+H_2O_2	1,01	0,03	0,98	23,83
W.K. (0,25 g)+H_2O_2	0,34	0,03	0,31	6,28
W.K. (0,5 g)+H_2O_2	0,34	0,03	0,31	6,28
W.C (0,25 g)+H_2O_2	0,11	0,03	0,08	2,54
W.C. (0,5 g)+H_2O_2	0,04	0,03	0,01	1,63
W.S. (0,25 g)+H_2O_2	0,11	0,03	0,08	2,54
W.S. (0,5 g)+H_2O_2	0,07	0,03	0,04	2,01
W.H.S. (0,25 g)+H_2O_2	0,11	0,03	0,08	2,54
W.H.S. (0,5 g)+H_2O_2	0,26	0,03	0,23	4,85

W.G.S.: Walnut Green Shell, W.S.: Walnut Skin, W.H.S.: Walnut Hard Shell, W.K.: Walnut Kernel, W.C.: Walnut Curtain N.C.G: Negative Control Group, P.C.G: Positive Control Group

5. Conclusion and Discussion

When the results obtained in the study are examined, the survival rate in the control group individuals is 95% in the larvae, while it can go down to 80% during the maturation process. When Table 4.1 is examined, it is seen that walnut veil and walnut shell obtained higher data than the control group at both concentrations. While the survival rate was 83% in the control group, these rates increased to 88% and 93% in walnut shells. Again, when looking at the walnut screen, it was determined that the survival rates increased to 86% and 91% (Table 4.1).

The development period is 7-8 days in total from larva to adult. In the study, it was determined that hydrogen peroxide in the positive control group prevented the survival of the insect, and that the walnut layers were added to the medium, increasing the survival rate and shortening the development period (Table 4.2). Considering the rates of reaching the 3rd stage, it was determined that the highest rate was in the walnut curtain and walnut shell. The mating period of larvae (0.25g, 0.5g) fed with walnut curtain is 9.21 and 9.88 days, respectively. In larvae fed with walnut membrane, the maturation period is 9.89 and 9.56 days, respectively. In the larvae fed with walnut green shell, the rate of reaching the 3rd stage and 85% in both ratios, while the maturation period was extended to 11 days. When the effect of different walnut layers on the lifespan of Drosophila melanogaster male individuals is examined, it is seen that the longest average lifespan is 85 days in individuals fed with walnut curtains. While the average life span was 69 days in individuals in the negative control group, this period decreased to 41 days in the individuals in the positive control group. Lower results were obtained in the walnut green shell and walnut hard shell from the walnut layers at both concentrations compared to the negative control group. When the data on the maximum life span is examined, it is seen that the highest yield is observed in the flies fed with walnut skin and walnut screen (Table 4.3).

Studies have been carried out on larval mortality, maturation time and lifespan of feeding with different foods in relation to Drosophila. In the study conducted with Drosophila species, it is stated that Cucurbita pepo L. provides 79% of live emergence.

Nutrients and their contents can both decrease and increase the survival rate. It is known that palmitic acid, which is used as a high-fat diet, adversely affects survival (Güneş et al., 2019). In insects, both environmental factors such as stress, temperature, climate, light, nutrition, humidity and genetic factors such as mutation or interspecies diversity affect lifespan and aging (Uysal et al., 2015). While population density affects body size and maturity, maturity affects lifespan in individuals (Benli & Türkoğlu,

2017).

Although walnuts are known to have antioxidant properties in various studies, no study has been found on total antioxidant capacity (TAS), total oxidative stress (TOS), oxidative stress index (OSI), and Malomaldehyde (MDA) levels of Drosophila melanogaster individuals of Oğuzlar 77 walnuts. Reactive oxygen species (ROS) cause oxidative stress by transporting molecular oxygen due to attack of unshared electron pairs; Thus, macromolecules such as fats and proteins peroxide increase total oxidation and toxic effects are observed in Drosophila (Çolak and Uysal, 2018). As a result of oxidative damage with TOS, cellular or dietary antioxidants are activated and ROS are removed, but in cases where TAS mechanism is insufficient, adequate or insufficient response to stress is created according to OSI (Güneş, 2016). In most of the methods used, stable peroxidation products are generally preferred (Dotan et al., 2000). Malondialdehyde (MDA), a thiobarbutyric acid reagent, is the end product of lipid peroxidation and is the most common and reliable compound in the determination of oxidative stress (Akşit et al., 2014). Peroxidation of fatty acids with three or more double bonds results in MDA production (Deveci, 2007).

When Table 4.4 is examined, TAS (1.84 mmol/L) in the flies in the negative control group was determined as TOS (1.8 (μmol/L) while the oxidative stress index (OSI) was found to be 0.098. The hydrogen peroxide used as the positive control group was determined as the TAS and TAS in the flies. When the TOS values were examined, TAS (0.96 mmol/L), TOS (3.92 μmol/L) were found, and the OSI value was 0.408 (Table 4.4). TAS in the walnut membrane was detected at two different concentrations of 1.48 mmol/L and 2.44 mmol/L. In the walnut screen-fed flies, the TAS levels were 1.99 mmol/L and 2.89 mmol/L, respectively. The flies with the highest TOS level were the ones fed with walnut kernel and walnut shell (Table 4.4). . When the effect of walnut hard shell on OSI values was examined, it was seen that it did not increase the stress, and values close to the control group were obtained.

When the concentrations of malondialdehyde (MDA), which is the end product of lipid peroxidation and the most common and reliable area of use in the determination of oxidative stress, are examined, it is seen that the highest values are in the flies fed with walnut kernel and hard shell. It was 1.88 nmol/L in the negative control group and 3.71 nmol/L in the positive control group. In flies fed with walnut green shell, this rate is lower than the negative control group at both concentrations. MDA levels are 2.54 nmol/L and 2.01 nmol/L in flies fed with walnut membrane (0.5g) and walnut screen (0.5g) (Table 4.5).

It has been stated that walnut polyphenols have antioxidant and im-

mune-enhancing properties. It has been reported that polyphenols in walnuts are mostly located in the thin brown shell surrounding the outside of the fruit (Anderson et al., 2001). Walnut green shell and leaf parts are used in traditional medicine.

It is known and used among the people for its vaso-strengthening, astringent, anthelmintic, antidiarrheal, antifungal, hypoglycemic, hypotensive and sedative properties. Green bark and leaf parts are very rich in phenolic substances and flavonoids. These phytochemicals provide a protective effect against degenerative diseases by reducing oxidative stress and preventing macromolecular oxidation, and their free radical scavenging effects also show anti-carcinogenic properties (Pereira et al., 2008). Phytochemicals found in the leaf and green shell parts of the walnut (Juglans regia L.) plant can be used as natural preservative additives in food processing due to their good antioxidant and antimicrobial properties (Amaral et al., 2014). Studies have found significant differences in oil content, fatty acids and tocopherols between different varieties of walnut depending on environmental conditions (Amaral et al., 2005). In addition, it has been reported that walnut has a high content of α-tocopherol, a vitamin E family compound with a high antioxidant effect in inhibiting the lipid oxidation process (Koksak et al., 2006).

Walnuts have a high nutritional value as they contain high fat and protein. The most important feature of walnut oil is that it is very rich in unsaturated fatty acids. The high amount of linoleic acid in walnut oil makes walnuts a unique food. In addition, high biological quality protein, vitamins and minerals in its composition increase its nutritional value. (Yiğit and Ertürk, 2005). In traditional medicine, the leaf parts and green shell of the walnut have been found and used among the people to have hemorrhagic, vascular strengthening, anthelmintic, sedative, antidiarrheal, hypotensive, hypoglycemic and antifungal properties. In Asian and European countries, dried walnut leaves are widely used in the form of tea in rural areas. Leaf parts and green bark are very rich in flavonoids and phenolic substances. These phytochemicals show anticarcinogenic effects by preventing the oxidation of macromolecules and reducing oxidative stress, providing free radical scavenging effects and protective effects against degenerative diseases (Pereira et al., 2007).

6. Suggestions

Today, prevention or treatment of some diseases is very important along with natural, high quality and balanced nutrition. Foods contain different proportions of protein, fat, carbohydrates, minerals and vitamins, as well as one or more of the antioxidant substances. Walnuts are consumed as an important part of the nutritional diet in our country in the form of fruit. The fruit characteristics of the plant are extremely valuable. According to the results we obtained in this study, we think that the hard shell parts, walnut skin and walnut curtain can be evaluated as much as the fruit part of the walnut plant.

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CHAPTER 2

A BRIEF OVERVIEW OF DEVELOPMENTS IN NANOTECHNOLOGY AND NANOMEDICINE

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INTRODUCTION

Today's achievements in science and technology provide obvious benefits to people, but they also bring with them some problems. These problems can negatively affect the environment as well as people. Considering the benefits it provides, nanotechnology, which is considered as today's industrial revolution, pioneers many innovations and developments in our lives.

Nanotechnology is the creation of new structures by changing the properties of matter through manipulations at the atomic and molecular levels (Hong et al., 2009). It can also be defined as the science of creating useful materials suitable for a purpose by manipulating and rearranging atoms and molecules one by one. Nanotechnology is used in a wide variety of fields (computer, agriculture, energy, medicine, health, chemistry, aviation, etc.) (Mamalis, 2007). Nanotechnology is very meaningful and unique because it integrates different fields, branches of science and disciplines (health, personal care, defense, etc.) for a certain purpose (Park, 2007). It is possible to see nanotechnological products in every aspect of our lives (Haverkamp & Marshall 2009). Nanotechnological products have been produced and offered for human use in many fields, from industry to textiles, from medicine to electronics (Demirkıran, 2019). Stain-resistant fabrics, scratch-proof surfaces, color-changing paints, anti-aging cosmetic products and many more can be given as examples of nanotechnological products.

Nanoparticles, which are products of nanotechnology, differ from other materials in both their size and physical, chemical and biological properties. Development of nanotechnological products; They can be evaluated as first generation products, second generation products, third generation products and fourth generation products. Passive nanostructures, introduced in 2000, constitute the first generation products. The products introduced in 2005 are active nanostructures and constitute second generation products. The products introduced in 2010 constitute the third generation products. The molecular and atomic sized devices in our lives between 2015 and 2020 constitute the fourth generation products (Demirkıran, 2019).

Two methods are used to create nanostructures. One of these is the Bottom-up method and the other is the Top-down method. In the first method, the Bottom-up method, there is an application carried out from atom to molecule and from molecule to product. In this method, nanoparticles; They are formed by reducing macromolecules through some applications such as crushing and grinding. The second method, the Top-down method, is a method that works from material to molecule and from molecule to

atom. In this method, the material is separated into atoms and rearranged by some mechanical or chemical applications (Sanguansri et al. 2006; Zhao et al. 2010).

1- HISTORICAL DEVELOPMENT OF NANOTECHNOLOGY and THOSE WHO PIONEERED THE DEVELOPMENT OF NANOTECHNOLOGY

1-1-Historical development of nanotechnology

In the historical evaluation of nanotechnological products, it is seen that the use of these products dates back to ancient times. As a matter of fact, the Lycurgus Cup is a good example of this. This cup is known as the first nanotechnological product. The cup exhibited in the British Museum can change color due to the soda-lime glass it contains, gold (1%), silver and manganese (0.5%). When light is given from the front, it turns green, and when light is given from the back, it turns red (Tolochko, 2009). Stained Glass Windows are also a nanotechnological product and have historical value. Stained glass windows were used in European cathedrals and contain gold chloride and chloride nanoparticles (Tolochko, 2009).

1-2- Those who pioneered the development of nanotechnology

Michael Faraday, who had the biggest role in the development of nanotechnology, prepared aqueous colloidal mixtures and investigated the optical and electrical properties of these mixtures in 1857. The researcher used gold nanoparticles when preparing these mixtures. The concept of nanometer was first introduced to the literature by Richard Zsigmondy in 1925. Richard Feynman said in 1959 that it was possible to produce products at the size of atoms and molecules. However, Richard Feynman is considered the father of nanotechnology. Because the term Nanotechnology emerged when the physicist named Richard Feynman claimed that nano-sized structures could be measured and used for certain purposes (Sahoo and Labhasetwar, 2003; Zhang and Webster, 2009). However, the first person to use the term nanotechnology was Norio Taniguchi in 1974.

2-NANOMEDICINE and NANOTECHNOLOGICAL SYSTEMS

2-1-NANOMEDICINE

Nanotechnological studies carried out in the field of medicine are collected under the title of nanomedicine. Nanomedicine is the use of chemical, physical, biological, etc., in accordance with the purpose of application of natural or synthetic nanoscale materials for the early diagnosis of diseases, application of necessary treatments and taking precautions. It emerged as a result of taking advantage of its features (Tüylek, 2019). It is a field used to monitor and treat human biological systems with some nanostructured devices (Zhang and Webster, 2009). Nanomedicine deals with the production of molecular tools for early diagnosis, treatment and protection by keeping cellular functions under control and using molecular information in the body (Syed et al. 2013). The primary aim of nanomedicine is to examine human biological systems at the molecular level and provide the necessary improvement (Zhang et al. 2014). The US National Institute of Health maintains its global leadership in the field of nanomedicine by using nanotechnological applications necessary for the prevention and diagnosis and treatment of diseases (Park, 2007).

Materials used today such as smart drug carriers, orthopedic prostheses, cardiovascular implants, neural and dental implants, insulin pumps, medical imaging devices, biosensors, nanorobots, implants and artificial tissue can be counted among the products developed with nanotechnology (Sahoo et al. 2007; Zhang et al. .2014). Abciximab, which is also produced using nanotechnology, can be counted among these. Abciximab reduces the chance of heart attack because it is used in percutaneous coronary treatment to open the blocked heart vessel (Sahoo et al. 2007). In the healthcare sector, nanotechnology is used in many areas such as drug development and drug delivery systems, gene transfer, patient monitoring and treatment (Singh et al. 2009; Sahoo et al. 2007; Staggers et al. 2008). Significant developments have been achieved in many areas such as gene therapy, drug delivery systems, and new drug discovery through nanomedicine (Zhang and Webster, 2009).

Nanosensors produced through nanotechnology are used in the early diagnosis of some diseases. Parkinson's disease, cancer and diabetes mellitus can be given as examples. In studies, the affinity of PLGA nanoparticles to tumor cells was investigated and it was determined that the retention of this nanoparticle to tumor cells was high. In another study, the binding rate of the same PLGA nanoparticle on lung cancer cells was increased (Chung et al. 2010; Nafee et al. 2007).

Nanostents used in heart patients are designed using nanotechnology and are used in blood clotting and treatment processes (Naschie, 2006). Nanotechnology has been used in the field of oncology, especially for the diagnosis and delivery of chemotherapeutic drugs (Laiva et al. 2015).

2-2- NANOTECHNOLOGICAL SYSTEMS

Biochip, biosensor, nanosphere and nanorobot applications are nanotechnological systems used in the field of medicine.

2-2-1-Nanospheres

The surface and release properties of nanospheres used to trap, attach or adsorb drugs may vary depending on the preparation method (Behera et al., 2010).

2-2-2-Nanorobots

Respirocytes created in the laboratory are created by mimicking erythrocytes in living tissues. Respirocytes are known as nanorobots. Running for 15 minutes without breathing or staying under water for 4 hours without breathing is possible with these respirocytes.

2-2-3-Biosensors

In recent years, more advanced biosensor designs have been made based on performance criteria such as sensitivity, selectivity, ease of construction and cheapness. Nanomaterials are used to increase the performance criteria of biosensors (Gooding, 2006).

2-2-4-Biochips

Biochips, which are electronic devices made to perform certain biological functions, have the ability to perform many tests simultaneously and within seconds and result in thousands of biological reactions. It has the ability to be programmed to perform different functions. When the majority of today's analyzes begin to be performed thanks to biochip technology, applications such as electrophoresis and gel preparation kits will be used less (Cheng et al., 1999).

3- HEALTH BENEFITS OF NANOTECHNOLOGY, RISKS AND CONCERNS ABOUT NANOTECHNOLOGY

3-1-HEALTH BENEFITS OF NANOTECHNOLOGY

With specific materials and devices prepared using nanomaterials, treatments with very low side effects and high therapeutic effectiveness can be performed (Sahoo et al. 2007). Nanotechnology can provide a unique benefit, especially when it comes to drug delivery in the body. Nanomedicines have distinct advantages over conventional drugs. While controlled releases stand out among these advantages, there are also some features that cannot be ignored, such as reaching the target region and showing its effect only on this region. In addition, the therapeutic effectiveness of nanomedicines prepared using nanoparticles is longer, as their dissolution, retention time in the blood (bioavailability) and retention time in the body are increased. Since they are prepared by protecting them from interaction with the biological environment, they have many advantages, such as reducing premature deterioration and improving their retention within the cell (Park, 2007). Since the drugs reach the target directly, healthy tissues are not damaged (Atlı-Şekeroğlu 2013).

Nanotechnology not only provides significant benefits in many diseases such as neurodegenerative diseases, diabetes and cancer, but also makes positive contributions to humanity in terms of improving the quality of life (Maheshwari and Gupta, 2012). Regarding cancer, benefits have been obtained from nanomedicine in terms of imaging and drug delivery systems (Kawasaki and Player, 2005). Tumor structure can be detected by using magnetic iron oxide nanoparticles. Special antibodies developed against the tumor sought in the body and marked with iron oxide nanoparticles are given to the body. If the relevant tumor is present in the body, the labeled antibodies attach to antigens on the tumor surface. Iron oxide particles found in antibodies collected in the tumor tissue send some signals, and thus the tumor structure can be detected with MRI.

In the nano-ear used in those with hearing loss, nano devices, nanoparticles and drugs are carried to the damaged area in the inner ear by means of chips and transmitted to the brain from there. Thus, the person with hearing problems is enabled to hear better (Abeer 2012). In addition, nanofibers obtained by nanotechnology can be used in wound care and burn treatment. Nanofibers have a healing role on the cell due to their highly porous structure and large surface area (Hromadka et al., 2008).

3-2- RISKS AND CONCERNS ABOUT NANOTECHNOLOGY

Nanotechnology has provided convenience in many areas with the opportunities it offers, and has started a new era for humanity with the tools and equipment produced in various fields. So much so that people's lifestyles have changed thanks to these nanotechnological products (Sia 2017). Although nanotechnology has so many benefits, it also has some risks and uncertainties (Moore 2006; Medina et al. 2007; Yazıcı 2009). For example, nanoparticles that can enter the body and cells may accumulate excessively in cells and tissues and damage the defense mechanism (Moore 2006). Some nanoparticles that can pass into the blood may negatively affect some organs (Chau et al. 2007). Particularly metal nanoparticles enter the cell and the nucleus, causing DNA damage and creating free radicals above physiological limits. When nano titanium dioxide was used in pregnant mice, it was determined that the offspring had brain damage and reproductive problems in the male offspring. Brain damage was detected in fish exposed to nanoparticles for 48 hours (Syed et al. 2013; Donaldson et al. 2010).

In addition, it is not clear what by-products may occur in the production of nanoparticles and what consequences they will have in the environment. It is also not known what the consequences of long-term use on living tissue will be (Çelik, 2011). Problems experienced in the use of nanodevices may not be understood by immediate observation. Or even if it is understood, the problem may not be eliminated or solving the relevant problem may require separate expertise. The issue of safety is of great importance in nanomaterials used for therapeutic purposes. Nanotechnology studies in all fields require both a good budget and expertise in their safe use. It should also be noted that defensive policies may also be necessary (Staggers et al., 2008).

CONCLUSION

Thanks to nanotechnology, changes can be made in materials and substances can be obtained at the atomic and molecular level. All of these add value to our standard of living. Nanotechnology, which has an important role in improving the quality of life, seems to offer humanity a healthier and safer living space with the gains it creates in the field of medicine. Thanks to nanotechnology, efficiency is increased and labor and time are saved. It seems that in the near future, thanks to nanotechnology and nanomedicine, there will be developments in the field of health that we cannot even imagine. In fact, developments in the field of nanomedicine will develop depending on our imagination. However, when incorporating this technology into our lives, a solid infrastructure must be created. Therefore,

it may be necessary to train expert personnel in this field and provide training on developping technology.

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CHAPTER 3

EMPOWERING HEALTH ORGANIZATIONS FOR PATIENT SAFETY: STRATEGIES FOR CAPACITY BUILDING IN THE EUROPEAN UNION CONTEXT

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Patient safety is an active and systematic approach implemented during the delivery of health services to minimize harm that patients may encounter. It encompasses the prevention, reduction, and mitigation of errors, adverse events, and other preventable harms, with the ultimate goal of improving the patient's well-being. Patient safety is defined as "the reduction of risk of unnecessary harm associated with healthcare to an acceptable minimum" (WHO, 2019). This definition emphasizes the need to identify and address potential risks and hazards in healthcare services, acknowledging that complete elimination of all risks is not always possible. It underscores the importance of a risk-based approach prioritizing the patient's welfare and aiming to achieve an acceptable level of safety (WHO, 2019). Another definition of patient safety describes it as the "prevention of harm due to errors or negligence in the delivery of healthcare." This definition highlights the role of healthcare providers in ensuring safe care, addressing both procedural issues (e.g., medication errors) and negligence (e.g., diagnostic errors or treatment delays) that could harm the patient (AHRQ, 2021).

In Turkiye, the Ministry of Health evaluates patient safety indicators in healthcare facilities through biannual internal audits. Independent teams appointed by the Ministry conduct these assessments (Saglık Bakanlıgı, 2023). The regulations outlined in the 'Regulation on Ensuring Patient and Employee Safety', published in the official journal in 2011, define patient safety practices and plan their implementation in healthcare institutions. These practices include identifying and verifying patient identity, obtaining patient consent for interventional procedures, ensuring communication safety in healthcare service delivery, ensuring medication safety, ensuring transfusion safety for blood and blood products, ensuring surgical safety, preventing patient falls, ensuring radiation safety, and planning for disabled patients.The Turkish Ministry of Health updated the National Patient Safety Goals in December 2022. New quality criteria were established by the Ministry in March 2023, aligning with the 23 goals under the National Patient Safety Goals (Saglık Bakanlıgı, 2023).

Ensuring patient safety in healthcare services is crucial, especially in surgical clinics and departments, given the significant risks associated with surgical interventions (WHO, 2011; OECD, 2015; Kohn et al., 2000). Surgical clinics and departments, characterized by complex procedures, intense work traffic, and potential risks, prioritize patient safety in all processes (WHO 2011). Surgical interventions, with their immediate and long-term impact, emphasize the importance of prioritizing patient safety to prevent any harm (Kohn et al., 2000; Güner et al., 2018).

Key Dimensions of Patient Safety:

Patient-centered care, emphasizing the active involvement of patients in their own care, is a fundamental dimension of patient safety. It underscores the importance of addressing patient values, preferences, and concerns and providing personalized health experiences (Epstein and Street, 2011). Patient engagement and empowerment are crucial for promoting patient safety in health information management. Engaging patients in their care, providing access to their health information, and involving them in decision-making contribute to a safer and more patient-centered healthcare system. Safety culture expresses the shared values, beliefs, attitudes, and practices related to safety within a healthcare organization. It plays a significant role in shaping the overall approach to patient safety, encouraging open communication, and accountability (Epstein and Street, 2011; Ginsburg et al., 2012).

Effective communication and collaboration are critical dimensions for patient safety. Communication breakdowns can lead to errors and undesired events, making it essential to ensure accurate and timely information exchange (Manser, 2009). Continuous learning and improvement are vital dimensions for patient safety. Healthcare organizations should actively identify improvement areas, analyse adverse events, and adopt an initiative-taking approach to prevent future errors (Leistikow et al., 2010). The integration of technology and innovation is an increasingly important dimension of patient safety. Advances in health technologies, such as electronic health records and clinical decision support systems, can reduce errors while coordinating care and treatment (Bates et al., 2014).

Ethics is a critical dimension of patient safety, emphasizing respect for patient autonomy, privacy, and confidentiality. Ethical rules and principles guide healthcare professionals in making decisions that prioritize patient welfare and safety (Beauchamp and Childress, 2001).

The integration of evidence-based practice into healthcare delivery is a fundamental dimension of patient safety. Using the best available evidence, clinical expertise, and patient values guides health decisions and interventions (Melnyk and Fineout-Overholt, 2018).

Environmental and physical safety is another important dimension for ensuring patient safety. Healthcare organizations must ensure that the physical environment is safe and suitable for providing care. Verifying patient identity is one of the most critical aspects of patient safety. Standardized protocols and technologies for patient identification are recommended to reduce the risk of misidentification and medical errors (AHRQ, 2017). Obtaining informed consent is a crucial ethical principle and a legal requirement in healthcare services. It ensures that patients understand their

diagnoses, treatments, associated risks, benefits, and alternatives, supporting autonomous decision-making and strengthening legal procedures for patient safety (Beauchamp and Childress, 2001).

Effective and safe communication is vital for ensuring patient safety during health service delivery. Maintaining communication safety involves protecting patient information, preserving privacy, and facilitating accurate and timely communication among healthcare professionals

- Electronic Health Records (EHR): The use of Electronic Health Records (EHRs) has become widespread in healthcare service delivery. EHRs facilitate the storage, access, and sharing of patient information among healthcare providers (Adler-Milstein et al., 2017).
- Health Information Exchange (HIE): Health Information Exchange (HIE) enables secure sharing of patient information among different healthcare organizations and systems (Adler-Milstein et al., 2017).
- Secure Messaging Systems: Secure messaging systems provide a secure and effective method for healthcare professionals to communicate and share patient information (Adler-Milstein et al., 2017).
- Privacy Policies and Procedures: Healthcare organizations should establish clear privacy policies and procedures for managing patient information, and addressing the collection, storage, and sharing of patient data (Adler-Milstein et al., 2017).
- Access Control Measures: Implementing strong access control measures is crucial to protect against unauthorized access. Assigning unique user identities and passwords, limiting access privileges based on job roles, and implementing multi-factor authentication are necessary (Adler-Milstein et al., 2017).
- Secure Communication Systems: Communication systems play a crucial role in facilitating communication among healthcare professionals, especially in emergency situations. Implementing secure communication systems is essential to safeguard patient information and maintain confidentiality (Adler-Milstein et al., 2017).
- Encryption and Decryption Protocols: Encryption and decryption protocols help secure the transmission of patient information. These protocols ensure that data is transmitted and stored in a secure format, protecting it from unauthorized access (Adler-Milstein et al., 2017).

Training and education are essential components of ensuring patient safety in health information management. Healthcare professionals should receive adequate training on privacy laws, security measures, and the proper handling of patient information (Adler-Milstein et al., 2017). Compliance with privacy regulations possess a fundamental aspect of patient safety in health information management. Healthcare organizations must adhere to local and international privacy laws to protect patient information and avoid legal consequences (Adler-Milstein et al., 2017). Usability and user experience are essential considerations in the design and implementation of health information management systems. Systems that are user-friendly and intuitive contribute to better patient safety outcomes by reducing the risk of user errors and improving overall efficiency. Interoperability is a critical factor in ensuring patient safety in health information management. Seamless exchange of information among different healthcare systems and providers improves care coordination, reduces errors, and enhances overall patient safety (Williams et al.; 2019).

Continuous monitoring and auditing of health information management systems are essential for identifying and addressing potential security breaches and vulnerabilities. Regular audits help ensure compliance with privacy and security policies, safeguarding patient information (Adler-Milstein et al., 2017). Establishing an incident reporting and response system is crucial for addressing and mitigating potential breaches of patient information. Healthcare organizations should have clear protocols for reporting and responding to incidents, including investigating and remedying security breaches. Confidentiality agreements play a role in ensuring patient safety in health information management. These agreements establish legal obligations for individuals with access to patient information, emphasizing the importance of maintaining confidentiality and preventing unauthorized disclosure (Adler-Milstein et al., 2017). Data encryption is a fundamental security measure to protect patient information in health information management. Encrypting sensitive data helps prevent unauthorized access and ensures that patient information remains confidential and secure. Error prevention and reporting are crucial dimensions of patient safety. Identifying and addressing potential errors and system failures can significantly reduce the likelihood of adverse events and harm to patients. A culture based on error reporting and learning is critical in promoting an initiative-taking approach to patient safety (Evans et al., 2015; Adler-Milstein et al., 2017).

Disaster recovery and business continuity planning are essential components of patient safety in health information management. Establishing robust plans ensures that patient information remains secure and accessible, even in the event of disasters or system failures (Adler-Milstein et

al., 2017). Conducting regular risk assessments and implementing risk management strategies are essential for patient safety in health information management. Identifying and addressing potential risks helps prevent security breaches and protects patient information (Adler-Milstein et al., 2017). Implementing patient identification protocols is crucial for ensuring patient safety in health information management. These protocols help prevent misidentification and ensure that patient information is accurately matched to the correct individual (AHRQ, 2021).

Robust authentication measures are essential for patient safety in health information management. Implementing strong authentication processes, such as multi-factor authentication, helps ensure that only authorized individuals have access to patient information (Adler-Milstein et al., 2017).

Collaboration and information sharing among healthcare organizations are essential for patient safety in health information management. Establishing effective communication channels and sharing relevant information contribute to better care coordination and improved patient outcomes. Ensuring the accuracy and integrity of patient data is fundamental for patient safety in health information management. Implementing measures to verify and maintain the accuracy of patient information helps prevent errors and supports informed decision-making. Developing and implementing clear health information privacy and security policies is crucial for patient safety in health information management. These policies guide the responsible and secure handling of patient information, protecting it from unauthorized access and disclosure (Adler-Milstein et al., 2017).

Providing ongoing training and ensuring the competency of staff involved in health information management is essential for patient safety. Well-trained and competent staff are better equipped to manage patient information securely and contribute to overall data accuracy and integrity. Educating patients about health information privacy is an important aspect of patient safety in health information management. Informing patients about how their information is used and protected fosters trust and supports their active engagement in their healthcare (Bates et al., 2014).).

Adhering to legal and ethical considerations is paramount for patient safety in health information management. Healthcare organizations must comply with relevant laws and ethical standards to protect patient information and maintain the trust of patients and the public (Adler-Milstein et al., 2017).

These key dimensions collectively contribute to creating a comprehensive framework for ensuring patient safety in health information management. By addressing these dimensions, healthcare organizations can

establish robust practices and systems that safeguard patient information, promote privacy and security, and support high-quality care delivery. Patient safety in health information management is an ongoing process that requires continuous monitoring, assessment, and improvement to adapt to evolving technologies, threats, and regulatory requirements. It is a shared responsibility among healthcare organizations, healthcare professionals, technology vendors, policymakers, and patients to collaboratively work towards achieving and maintaining patient safety in health information management.

Examination of Patient Safety in the Context of European Union Health Policies

The European Union (EU) is an organization formed by a group of European countries to promote economic cooperation, political stability, and social harmony (Dinan 2017; Nugent 2017; Peterson and Shackleton 2016). The EU was established in 1993 with the Maastricht Treaty and has since grown in size, currently being one of the world's largest economies with a population exceeding 447 million. The roots of the EU trace back to the post-World War II period when several European countries sought closer economic ties to promote growth and stability (Jones 2019; Kelemen 2018).

1. Maastricht and Copenhagen Criteria: The Maastricht and Copenhagen Criteria are essential requirements that countries must meet to join and become members of the European Union (EU) (European Commission, 2021).

- Maastricht Criteria: Also known as convergence criteria, these were established by the Maastricht Treaty in 1992. They are economic in nature and designed to ensure that countries aspiring to join the EU have stable and sustainable economies compatible with the EU's single market (European Council, 1992; Harris, 2015).

- Copenhagen Criteria: Also known as political criteria, these were defined at the Copenhagen European Council in 1993. These criteria are political and intended to ensure that countries aspiring to join the EU have stable democratic systems, respect human rights, and uphold the rule of law (European Council, 1993; Jensen, 2006).

2. Turkey's EU Membership Process: Turkey first applied to join the European Economic Community, the precursor to the EU, in 1959. However, it did not officially apply for EU membership until 1987. Since then, Turkey has embarked on a lengthy and com-

plex negotiation and reform process aimed at meeting the EU's accession criteria.

Stages of the EU Membership Process for Turkey:

- Application Process: The first step is Turkey's formal application for EU membership, which involves submitting an official application to the European Commission, subsequently evaluated by EU member states (Ülgen, 2017).
- Screening Process: After receiving the application, the European Commission conducts a screening process to assess whether Turkey is ready to adopt and implement EU laws and regulations. This involves a comprehensive review of Turkey's legal and institutional frameworks (Ülgen, 2017; White, 2011).
- Negotiation Process: If the screening process is successful, formal accession negotiations begin. These negotiations cover various policy areas, known as chapters, and require Turkey to align its legislation and practices with EU standards (Ülgen, 2017).
- Accession and Ratification: Once negotiations are concluded, the EU and Turkey sign an accession treaty. Subsequently, the treaty must be ratified by the existing EU member states and the Turkish government (Ülgen, 2017).

European Union Acquis: The European Union Acquis refers to the entirety of laws and regulations that have been adopted by the member states of the European Union (EU) and are binding for all EU institutions and member states (European Commission; 2019). The term "Acquis" denotes the accumulated body of laws and regulations developed by the EU since its inception. The EU Acquis encompasses a variety of instruments, including EU treaties, regulations, directives, decisions, and other legal tools that cover diverse areas of EU policy such as competition law, environmental law, consumer protection, and human rights (Peers and Ward, 2017).

These legal instruments play a crucial role in shaping the functioning of the EU as a single market and legal community. Having a common legal framework ensures that all member states share the same legal foundation and provides the necessary legal tools for EU institutions to implement policies (Maurer and Potì, 2016).

Importance of the EU Acquis: The EU Acquis serves as a fundamental element of the EU's legal system and plays a critical role in ensuring the functioning of the EU as a political and economic union. It establishes a shared legal framework that regulates the activities of EU institutions and member states. Additionally, it ensures a level playing field for businesses

operating in the EU and protects consumers from unfair practices (Papafilippou and Vlachos, 2018). Furthermore, the EU Acquis is instrumental in the expansion process of the EU. As new countries seek to join the EU, they are required to adopt the EU Acquis and align their legal systems with EU standards. This alignment helps new member states fully integrate into the EU and contribute to the common goals of the union (Vandeginste, 2019).

The health-related section of the EU Acquis is a key area that influences EU laws and regulations affecting patient safety. It covers various aspects, including the regulation of medicines, medical devices, and the rights of healthcare workers, as well as measures related to disease prevention and the promotion of health (European Commission, 2017).

One significant benefit of the health-related section is its provision of a common framework for ensuring high standards of patient safety across the EU (European Commission, 2013). By establishing common rules and regulations for health products and services, the EU Acquis helps ensure that patients receive consistent and high-quality care regardless of their location within the EU. The EU has created a unified framework for patient safety standards throughout the union. However, the EU remains committed to improving patient safety by enhancing regulatory frameworks to meet changing needs (Permanand, Mossialos, and Baeten, 2017; Sánchez-Recio, Márquez-Calderón, and Valdés-Ortiz, 2018).

European Union Competence Framework: The European Union has identified a set of competencies that its citizens need to fully participate in today's rapidly changing society. The European Commission has proposed competencies, including digital skills, language skills, social and civic competencies, health literacy, and cultural competencies. These competencies are relevant to patient safety and represent important areas for development for both healthcare professionals and patients (Kushniruk et al., 2016).

- Digital Skills: The European Commission recommends that all citizens of member states possess basic digital literacy. This includes the ability to use digital tools and technologies for communication, information management, and problem-solving. Digital technologies have been shown to enhance patient safety by reducing medication errors, improving the clinical decision-making process, and enhancing communication between healthcare providers and patients (Kushniruk et al., 2016). Additionally, digital technologies empower patients to manage their health more effectively and access information about their treatments (Berkowitz et al., 2017).

- Language Skills: Considering the diversity of languages spoken in the EU, the European Commission suggests that all citizens should have at least basic proficiency in at least two EU languages. This approach promotes intercultural communication and understanding and is particularly crucial for healthcare professionals to communicate effectively with patients who speak different languages (Karliner et al., 2010).
- Social and Civic Competencies: The European Commission recommends that all citizens have basic social and civic competencies, including the ability to collaborate with others, participate in democratic decision-making processes, and respect diversity and human rights. These competencies can contribute to creating a culture of safety and respect in healthcare settings and are vital for promoting patient safety. Other social and civic competencies, such as collaboration with other professional groups and participation in democratic decision-making processes, are essential for promoting patient safety. Teamwork and communication in healthcare settings are critical for ensuring that patients receive safe and effective care. By promoting social and civic competencies, the European Commission helps healthcare professionals work effectively in teams, communicate with patients and families, and advocate for patient safety (European Commission, 2019).
- Health Literacy: The European Commission emphasizes the importance of health literacy for all citizens of member states. Health literacy includes the ability to understand and use health information, make informed decisions about health services, and effectively navigate the healthcare system (European Commission, 2019).
- Cultural Competency: Cultural competency is another crucial competency for healthcare professionals. Patients from different cultural backgrounds may have diverse health beliefs, practices, and expectations, and healthcare professionals need to be aware of these differences. The European Commission also highlights the importance of cultural competency for healthcare professionals, especially as healthcare services become more globalized and diverse (Lambert et al., 2021). By promoting cultural competency, the European Commission helps healthcare professionals effectively collaborate with patients from different cultural backgrounds and ensures that they are equipped to provide safe and effective care (European Commission, 2019). The competencies proposed by the European Commission are based on research and best practices in the health sector and, when implemented, have

significant positive effects on patient safety and health outcomes (European Commission, 2019).

European Skills/Competences, Qualifications and Occupations (ESCO) Framework: The ESCO classification system, initiated by the European Commission, aims to improve the transparency, recognition, and comparability of skills and qualifications across different countries and sectors. The ESCO system is based on a standard language that defines skills, competences, qualifications, and occupations, making it easier for employers, job seekers, and educational institutions to understand and compare different aspects of the labour market in the EU (Papafilippou and Vlachos, 2018).

Enhancing Institutional Capacity in the Scope of Patient Safety: The ongoing global changes affect all sectors, with patient safety being a critical concern for healthcare organizations. Patient safety is on the agenda of all international organizations, and various approaches have been developed to enhance patient safety (WHO, 2011b). Achieving sustainable practices in patient safety and ensuring the adoption of these practices by all employees require the development of institutional capacity. Capacity development is defined as the process of increasing knowledge and skills through training (WHO, 2011b; UNDP, 2009). Capacity development involves enhancing the abilities of individuals, groups, institutions, and communities. This approach supports the development of health policies during the adaptation process of our country to the European Union. Enhancing the capacities of healthcare institutions is a topic emphasized by other international organizations as well (WHO, 2018; OECD, 2018; UNDP, 2009; EU, 2022).

The accession process to the European Union involves various dimensions related to institutional capacity, partnership structures, and the development of patient safety (Veronesi and Martinelli, 2020). Developing institutional capacity for EU candidate countries is characterized by the improvement of the workforce, institutions, policies, and knowledge, leading to the strengthening of the international partnership network of the institution, positively impacting patient safety (Stegeman et al., 2009; Aroni, 2012; Greer et al., 2019; WHO and EU, 2020). The support of healthcare infrastructure, human resources, and equipment investments associated with improving the quality of health services strengthens with the accession process (Atun et al., 2015). The accession process to the EU requires candidate countries to strengthen their institutional capacities in various areas, contributing to the effective management of health systems, resource allocation, and the effective delivery of health services (Kutzin et al., 2019). The accession process leads to the adoption of EU health policies and regulations, standardizing health practices and ensuring high-quality care for

patients (Mladovsky et al., 2012). By promoting evidence-based practices and the adoption of quality standards, the accession process supports the improvement of patient safety (Cameron et al., 2018). The EU membership process requires candidate countries to meet requirements such as reducing medical errors, improving care quality, and strengthening patient safety (Tarnovska and Liaropoulos, 2019). This process supports the development of new partnerships for both the candidate country and its health institutions, encouraging knowledge exchange and sharing best practices (OECD, 2015). It also encourages partnerships between health sector and other organizations outside the health sector.

In summary, the steps to be taken for the regulation of health services within the scope of patient safety in the EU membership process are outlined as follows (Brown et al., 2001; Labonte and Laverack, 2001a; UNDP, 2008; Stegeman et al., 2009; WHO, 2010; Aroni, 2012; ECDPC, 2015; EC, 2016; WHO, 2019; Zhu and Lee, 2021; EU4Health, 2021):

Institutional Capacity Development:

- Strengthening regulatory frameworks for health service delivery,
- Improving health systems and processes,
- Organizing capacity development training for healthcare workers, Development of Partnership Structures:
- Developing partnerships between public institutions, civil society organizations, and institutions to improve health services,
- Collaboration with international organizations, including the World Health Organization, the European Commission, and the European Centre for Disease Prevention and Control,
- Collaboration with patient organizations and other relevant stakeholders to promote patient-centred care, Patient Safety:
- Developing and implementing policies and procedures to improve patient safety,
- Developing systems for reporting and analysing adverse events,
- Establishing patient safety committees and teams to make improvements in patient safety,
- Implementing measures to empower patients and encourage their participation in their care.

Conclusion: During the European Union (EU) accession process, it has been emphasized that establishing partnerships and networks in the development of capacity-building programs related to patient safety is a

fundamental element. The enhancement of institutional structures is another crucial aspect in the capacity-building process. Studies suggest that evaluating practices in this process should be incorporated into the scope of quality management (Brown, LaFond, and Macintyre, 2001; Labonte and Laverack, 2001a; UNDP, 2008; Stegeman et al., 2009; Aroni, 2012). Various studies propose including the assessment of these practices within quality management (Crisp, Swerissen, and Duckett, 2000; Potter and Brough, 2004) or evaluating them under a separate title (Labonte and Laverack, 2001b; WHO, 2001; UNDP, 2008). European Union policies place significant importance on the evaluation processes in healthcare services and the monitoring of incidents related to patient safety (Stegeman et al., 2009; EC, 2016; EU4Health, 2021).

Another crucial aspect in capacity-building practices is the development of employees' skills and competencies through training. Research highlights this as a fundamental element in capacity-building (Crisp et al., 2000; Potter and Brough, 2004; UNDP, 2008; Rowe et al., 2010; WHO and EU, 2020). Access to information is also a key topic in capacity-building efforts within the scope of patient safety based on EU health policies (Crisp et al., 2000; Stegeman et al., 2009; Aroni, 2012; Greer et al., 2019; WHO and EU, 2020).

The EU collaborates closely with international organizations such as the World Health Organization (WHO) and the United Nations Development Programme (UNDP) to support capacity-building in patient safety in healthcare organizations. Consequently, EU health policies have a global impact on healthcare services and support their development. The EU places significant importance on capacity-building in healthcare organizations, as evident in joint reports with the WHO and the 2021-2027 health strategy reports. Specifically, the EU prioritizes strengthening "effective, accessible, and innovative health systems" to enhance the quality of healthcare, develop the workforce, and provide high-quality and universal health services.

The European Commission emphasizes patient safety as a central theme in all its reports and research related to the health system. Patient safety is consistently placed at the forefront of all health-related policies of the EU (EC, 2016; Greer et al., 2019; WHO and EU, 2020; EU4Health, 2021).

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CHAPTER 4

ARTIFICIAL INTELLIGENCE IN DRUG CANDIDATE DISCOVERY & PRODUCTION

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1. Drug and Artificial Intelligence Relationship

Despite progress in the understanding of disease etiology and extreme advances in innovation, bringing novel drugs to market remains a time-consuming and sumptuous process, primarily due to the significant costs associated with failures in clinical trials (Schneider, 2020).

If the pharmaceutical form transport processes of the molecule developed after drug candidate development and design should be exemplified for institutions such as FDA, EMEA, TITCK: It consists of pre-clinical, Phase I, Phase II, Phase III, and ongoing phase IV in the clinic. In all of these stages, economic burden and time are essential criteria. But today, after the development of computer science and the emergence of the concept called artificial intelligence, the concepts of time and economy have turned into an advantage.

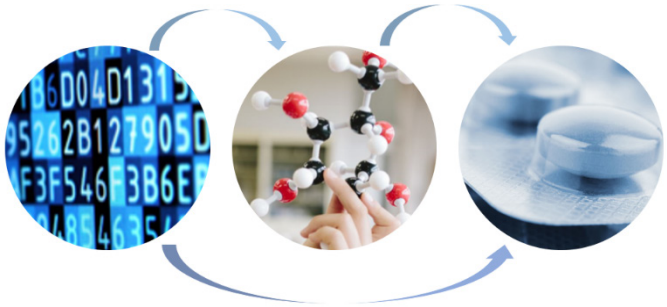

Figure 1. *Relationship-Diagram*

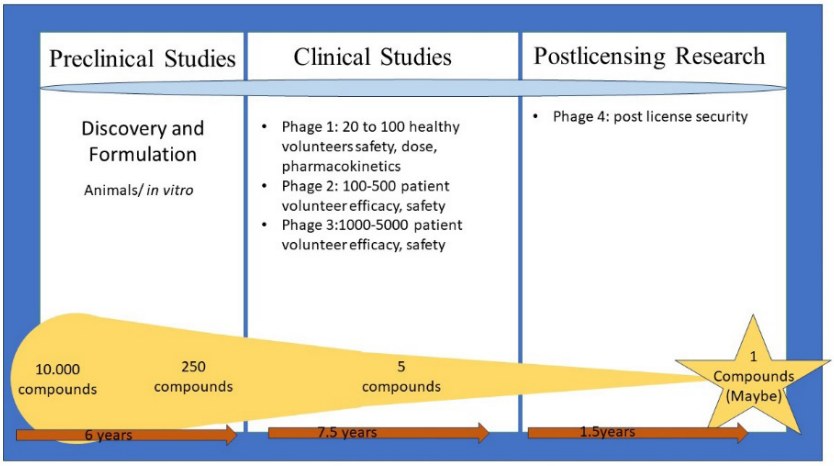

Figure 2. *The adventure of the drug*

As time progresses, the value given to information science is increasing day by day, and to eliminate the time taken for drug design in Figure 1, the concept of artificial intelligence takes its place in drug development.

Artificial intelligence, in short, can be called the ability to process data. It is known as machine learning and deep learning as its sub-steps.

It involves drug research and development processes and can sometimes result in frustration. It is an undeniable fact that preliminary results reduce the possibility of this disappointment. In these preliminary predictions, benefiting from auxiliary elements such as machine learning, deep learning, and artificial intelligence provides many advantages.

For example, if *in silico* methods were used to survey the cytotoxic effects of *Inula viscosa* extract, both less time and less material would have been used to determine the activity values (Hepokur, 2019).

On the contrary, in this study by Çöl et al., time and economic efficiency were provided by using in silico studies in some of the studies (Çöl, 2022).

2. AI for Drug Discovery

2.1. Drug Discovery with Artificial Intelligence

The concept of artificial intelligence emerged in the mid-20th century with the claim that computers could think. It is a general field that encompasses artificial intelligence, machine learning, and deep learning, but also does not involve any learning (Chollet, 2021),(Figure 3).

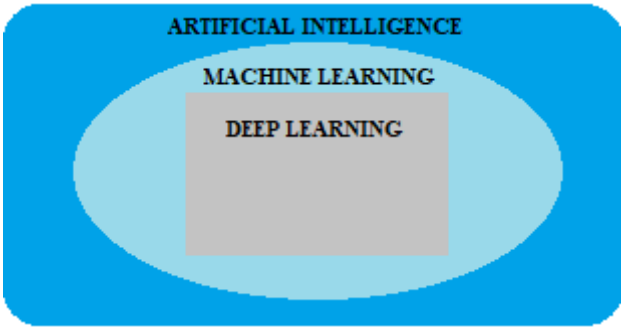

Figure 3. *Cluster representation of artificial intelligence and its sub-branches*

Artificial intelligence is becoming a widespread application in many fields. Especially the developments in the range of natural language processing have shed light on the use of artificial intelligence in drug discovery stages. Sub-fields of artificial intelligence, called machine learning and deep learning, have developed over time. The use of machine learning and

deep learning in drug research and development studies is increasing and becoming widespread day by day. These developments lead to a swift increase in studies in this field (Raschka, 2017) (Figure 4).

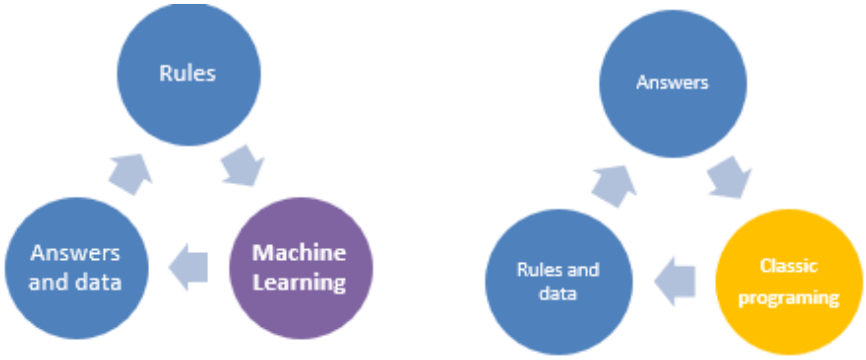

Figure 4. *Artificial intelligence principles*

2.3. Artificial Intelligence in *Silico* Studies

When artificial intelligence and in silico studies are the common keywords, the first concept to emerge in the name of drug development will be Computer-aided drug design.

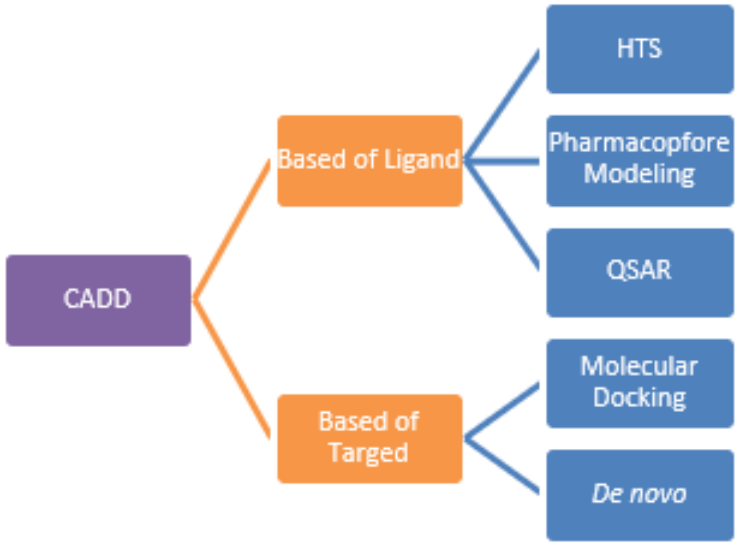

Figure 5. *CADD Diagrams*

Indispensable in chemoinformatics is the chemical discipline and the effort to provide maximum chemical data that these disciplines will provide to wet experiments (Gasteiger, 2003).

As seen in Figure 5, the distinction between ligand and target-based design is mentioned, and when evaluated in terms of artificial intelligence, the main distinction is the way they use databases. While compound libraries are used in ligand-based design, illuminated bioinformatics data of the target is used in target-based design (Bacilieri and Stefano Moro, 2006),(Russel, 2023).

High-efficiency virtual scanning studies are a set of studies that include the use of databases and the principles of elimination or elimination according to some rules. These studies have been brought together in the connection of artificial intelligence in drug development works, both manually and automatically (Mayr, 2009).

Pharmacophores capture the nature and three-dimensional arrangement of chemical functionalities in ligands that are relevant for molecular interactions with the macromolecular target. Thus, chemical functionalities are classified according to more general pharmacophore properties such as hydrophobic domains, aromatic ring systems, hydrogen bond acceptors, hydrogen bond donors, negatively ionizable groups, and positively ionizable groups. Less common types of interactions that contribute to ligand binding, such as metal coordination and halogen bonds, are either already implemented in most software packages or require user definition (Schaller, 2020).

At the first stage of rational drug design, parts of existing compound molecules with new structures can be created by combining them or using optimization algorithms such as genetic algorithms. With the rapid development of computer science and high-performance computing, artificial intelligence (AI) approaches have been successful in areas such as picture processing, pattern recognition, and natural language processing. In recent years, machine learning, particularly deep learning, has also been applied to drug discovery, such as predicting compound properties and activities and their interactions with protein targets. In the last few years, deep generative models that attempt to learn the probability distribution of training data, extract representative features, produce a low-dimensional continuous representation, and eventually generate new data by sampling the learned data distribution have attracted increasing attention (Tong, 2021).

Different applications of generative models have displayed outstanding results in rendering images. When applied to construct molecules, the essence of the generative model is to learn the distribution of molecules in the training set so as to obtain molecules similar to but different from the molecules in the training set. By combining the evolutionary algorithm or reinforcement learning, the specified properties of the produced molecules can be further optiimized. In this Perspective, we focus on the applica-

tion of generative models in de novo drug design. Primarily, the models of recurrent neural networks, autoencoders, generative competitor networks, converters, and deep generative (Tong, 2021).

We briefly introduce frequently used generative models, such as hybrid models that combine with reinforcement learning. Second, we extensively review benchmarks and benchmarks to evaluate the latest developments in the application of various generative models in drug design and their performance. Finally, we discuss the possibility of generative models for drug design (Tong, 2021).

2.4. Machine Learning in Drug Design

The goal of QSAR studies in drug development is to find the mathematical relationship between the activity of the molecule and its chemical properties. In particular, on a related data set in QSAR studies with the Random forest algorithm; easily interpreted, robust models with good predictive performance have been established, for example, Profile-QSAR (pQSAR). This RF algorithm allows for an appropriate chemical and biological interpretation of the molecular identifiers and structural motifs considered important by the model. SVM is another popular technique used in QSAR studies. in QSAR studies (Ramsundar, 2015).

There are also studies focusing on the effects of the size of the data set and parameter values on the performance of SVM. Various deep learning algorithms have been used in multi-target studies. 37.8 million experimental data of over 200 target 1.6 million compounds and extended fingerprints of these molecules were created. The multi-target neural network outperformed logistic regression, RF, and single-target neural networks. Although performance improvements resulting from multi-target deep neural networks have been reported by many different groups, few studies have focused based on this effect (Xu, 2017)

In one of their studies, Xu et al found that a task embedded in a multitasking deep neural network can “borrow” information from other QSAR tasks during the training process. It has been shown by many studies that deep neural network models show better results in QSAR studies than traditional machine learning models. There is not yet an optimal way to adjust the hyperparameters of the deep neural network model. In a study, it was shown that tuning certain hyperparameters, including the activation function, dropout, number of hidden layers, and the number of neurons it has, significantly affects the performance of the deep neural network model (Wu,2018).

Inappropriate ADMET properties can lead to late-stage failure of drug candidate molecules and withdrawal of approved drugs. It is important to

determine the ADMET properties of a compound at the beginning of drug development studies. Therefore, the development of in silico models that can reduce these problems has become one of the main points of industry and academic studies. In recent years, models that predict ADMET properties based on molecular properties have been developed with artificial intelligence techniques (Morgan, 2012).

Models that affect the pharmacokinetic properties of molecules and rapidly predict physicochemical parameters (such as LogP, KÇç) have been developed. The physicochemical properties of a drug candidate compound affect the absorption, distribution, metabolism, elimination, toxicity, and ADMET properties, potency, and selectivity, and thus significantly affect the treatment process (Duran-Iturbide, 2015).

Minor molecule drug candidates must exhibit sufficient solubility and permeability to reach their sites of action and bind to their targets. In this context, it is very focal better understand and accurately predict the physicochemical properties for the design of compounds with desired pharmacokinetic and pharmacodynamic profiles(Waring, 2010).

3. Artificial İntelligence for Drug Production

While focusing on the pharmaceutical industry, the role of artificial intelligence cannot be ignored due to its wide applications at various stages. Artificial intelligence has a huge impact on all stages of pharmaceutical products, from drug discovery to product development. Artificial intelligence technologies are used in both drug discovery and drug design. In pharmaceutical product development, artificial intelligence is used to select appropriate excipients, select the development process and ensure that specifications are achieved according to compatibility during development. Methods such as model expert system (MES) and artificial neural networks (ANN) are used in pharmaceutical product development. In production, artificial intelligence is used in automatic and personalized production by matching production errors according to limits. Artificial intelligence technologies such as meta classifier and tablet classifier are used to achieve the desired quality in the final product. Incorporating AI into clinical trials helps select subjects and monitor the trial, and machine learning (ML) is being used in clinical trials. Additionally, artificial intelligence technologies such as ML and natural language processing (NLP) tools are used in market analysis, product positioning and product costing (Bhattamisra, 2023).

Producing a commercial product from a simple formulation or more complex formulations with properties such as controlled release is time-consuming and complex. An initial formulation is usually prepared

with a variety of excipients and one or more active ingredients, and as development progresses, their selection and quantities and the manufacturing process are optimized through intensive, time-consuming experimentation. This process results in large amounts of data that are difficult to process and understand. In recent years, it has been shown that artificial neural networks will provide an alternative approach to manage this process more easily. Neural networks are mathematical structures that can "learn" relationships within data without requiring any prior knowledge from the user. The neural network makes no conventions about the functional form of the relationships; it creates and evaluates a series of simulations to determine the one that best fits the experimental data provided to it. Therefore, artificial neural networks are increasingly used to model complex behavior in problems such as pharmaceutical formulation and processing (Ibrıc, 2009).

Leading pharmaceutical companies are collaborating with AI suppliers and leveraging AI technology in R&D, drug discovery and manufacturing processes. Reports show that approximately 62 percent of healthcare organizations are considering investing in AI in the near future, and 72 percent of companies believe AI will be very critical in the future. In order to better understand the future of artificial intelligence in the industry, Pharma News Intelligence examines in depth the current artificial intelligence use cases, the best uses of the technology, and the future of artificial intelligence and machine learning. According to researchers, the use of these technologies improves decision-making, optimizes innovation and increases the efficiency of research/clinical trials (Aziz, 2022).

The use of AI and ML in drug discovery and development in FDA applications has been increasing since the 2010s and therefore the FDA has had to investigate how to regulate this nascent form of therapeutic development, application and selection. In 2019, the FDA published a discussion document titled " Proposed Regulatory Framework for Changes to Artificial Intelligence/Machine Learning (AI/ML) Based Software (SaMD) as a Medical Device - Discussion Paper and Request for Feedback ", which primarily aims to raise users' awareness of self-learning and machine learning-based changes and developments and to confirm that the risk assessment of these changes is carried out during and after the FDA approval process (Parvathaneni, 2023).

Artificial intelligence can be used in a variety of ways, such as predicting the behavior of drug carrier systems in the body, predicting drug interactions and optimizing drug formulations. Machine learning algorithms can be used to analyze large datasets of drug behavior in the body and predict drug response. This may be useful in designing optimized drug delivery systems for specific patient populations. Machine learning algorithms can be trained on large datasets of drug behavior in the body to predict the

optimal formulation for a given drug, reducing the time and cost associated with formulation development and leading to better drug efficacy. Another example of an AI-supported drug delivery system is the use of neural networks to predict drug interactions. Neural networks can be useful in designing drug carrier systems that minimize drug interactions and reduce side effects by training on large datasets of drug interactions to predict potential interactions between different drugs. Artificial intelligence can play an important role in the optimization of drug delivery systems by designing new drug carriers, predicting drug release profiles, and optimizing drug dosages (Gupta, 2023).

3.1. Using Artificial Intelligence in Drug Formulation

With advances in new drug discovery methods, the use of advanced drug delivery systems is rapidly expanding, providing benefits in areas such as safety, efficiency and patient compliance. Compared to traditional drug carriers, advanced drug carrier systems can increase the effectiveness and stability of drugs while reducing their adverse effects, and targeting is also possible with these systems. However, the preparation of a suitable carrier system is quite complex as it depends on the active ingredients, excipients and conditions (such as temperature, time and mixing speed). Experiments alone cannot screen all of these parameters. In addition to determining the molecular target and biological activity of a drug, artificial intelligence can also accurately predict the conditions for creating the drug's optimal carrier system (Lu, 2023).

Shamay at al. designed quantitative structure-nanoparticle assembly prediction (QSNAP) models to identify and legalise electrotopological molecular descriptors as highly predictive indicators of nanoparticle size. This method also revealed important molecular structural features that allow self-assembly and formation of nanoparticles. With the help of sulfated indocyanine, these drugs were formed into nanoparticles with 90% loading efficiency. Moreover, the created nanoparticles selectively targeted kinase inhibitors in caveolin-1-expressing human colon cancer and autochthonous liver cancer models to achieve high therapeutic effects while preventing pERK inhibition in healthy skin. These findings enable the computational design of nanopharmaceuticals based on quantitative models for the selection of drug load (Shamay, 2018).

Leonardi at al. have prepared chitosan microparticles containing benznidazole for use in the treatment of Chagas disease. In this study, process parameters such as encapsulation efficiency, size, yield and dissolution rate were optimized using artificial neural networks (ANN). Validation experiments were performed to show that the optimized properties were in good agreement between the predicted and experimental values.

This study demonstrated that ANNs are a valuable tool for the development of optimized benznidazole chitosan microparticles (Leonardi, 2009).

Takagaki et al. created a tablet database containing various active ingredients for a standard tablet formulation. Tablet hardness and disintegration time were measured before and after storage for 30 days at 40°C and 75% relative humidity. An ensemble artificial neural network (EANN) was used to predict responses to differences in the amounts of excipients in the tablets and the physical-chemical properties of the active ingredients. The prediction abilities of EANNs using linear, radial basis function, general regression and multilayer perceptron algorithms are compared. EANNs using the general regression algorithm accurately predicted pharmaceutical responses in tablet hardness and disintegration time. This study has shown that EANNs with a general regression algorithm, when used in conjunction with a tablet database, may have the capacity to identify acceptable candidate tablet formulations (Tagaki, 2010).

3D printing (3DP) is a recent technology in pharmaceutical formulation development. Despite its promising advantages, its transition to clinical settings remains slow. In order to make the transition to clinical applications and improve patient care, 3DP needs to make use of modern technologies. Machine learning (ML), which is an effective branch of artificial intelligence, may be important for 3DP. Together, 3DP and ML can make use of human learning-based intelligence to accelerate drug product development, confirm stringent quality control, and design innovative dosage forms. Thanks to the capabilities of ML, 3DP drug delivery can enable personalized treatment. Advanced forms of machine learning, including ANNs, along with pharmaceutical 3DP, have also been extensively trialled by traditional pharmaceutical technologies, providing numerous successful examples (Elbadawi, 2021).

3.2. Using Artificial Intelligence in Drug Release Studies

The release of a drug is very important for the treatment of the disease. Developing drugs that release depending on the differences in various organ and tissue physiological signals can increase the effectiveness of the drug and achieve safe and definitive treatment by preventing toxic and side effects caused by release in non-targeted areas. Drug release in the designed carrier systems can occur depending on pH, temperature, osmotic pressure and many endogenous substances such as enzymes, various ions. At this point, AI can facilitate the evaluation of drug release and provide feedback for the formulation of drug carriers through machine learning (ML) (Lu,2023).

Boztepe et al. synthesized doxorubusin hydrogels. Release studies were carried out on the prepared hydrogels and then these data were successfully modeled using artificial neural network (ANN), least squares support vector machine (LS-SVM) and support vector regression (SVR) methodologies. To evaluate the performance of these models, four statistical parameters were calculated: correlation coefficient (R), root mean square error (RMSE), mean square error (MSE), and mean absolute percentage error (MAPE). Results obtained from ANN, LS-SVM and SVR methods used to model doxorubicin release behavior from hydrogels showed good agreement between predictions and observations. It was thought that the ANN model, which showed the best performance among these, could be a reliable method for modeling drug release behavior from highly swellable pH and temperature sensitive hydrogels (Boztepe, 2020).

3.3. Artificial Intelligence in Quality Control and Quality Assurance

Through the great integration potential within the quality by design framework, starting from formulation development, these data science tools enable a better understanding of pharmaceutical formulations and related processes. The current approach to pharmaceutical development should be based on quality by design (QbD) principles. The first step in the QbD approach is to define the quality target product profile (QTPP) and then define the critical quality attributes (CQA) of the product. The most important aspect of QbD is the establishment of the relationship(s) between critical material properties (CMA) and/or critical process parameters (CPP) that influence CQAs. Once these relationships are defined and measured, design space can be assigned to provide the opportunity to optimize and continuously control the quality of the product. Assignment of quantitative analysis and design area; It is based on experimental design, regression methods, and traditional statistical analysis. However, there are no limitations on the methods that can be used for quantitative assessments in the context of QbD. In fact, there is a wide range of techniques available under data science that can be used efficiently for various QbD elements. ML algorithms, especially ANN, have been used in numerous examples of QbD-based pharmaceutical development, particularly due to their nonlinear nature and ability to capture complex relationships between CMAs and/or CPPs and CQAs for various pharmaceutical dosage forms (Djuris, 2021).

Maintaining consistency between batches produced and performing quality control tests on products require human intervention. This shows the need for artificial intelligence application right now. Artificial intelligence can also be used to regulate in-line production processes in order to

achieve the target product standard. The freeze-drying process is monitored using an ANN-based method that uses a combination of self-adaptive evolution, local search, and backpropagation algorithms. Additionally, an automated data entry platform such as the Electronic Laboratory Notebook combined with advanced, intelligent algorithms can ensure product quality. Data mining in total quality management expert system and knowledge discovery techniques can be used as valuable approaches in making difficult decisions, this can lead to the development of new technologies for intelligent quality control (Wanare, 2022).

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CHAPTER 5

EXERCISE-ADAPTIVE RESPONSES: THE POTENTIAL ROLE OF EPIGENETIC INTERACTIONS AND SKELETAL MUSCLE METABOLISM

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ABSTRACT

Frequent exercise has many health advantages and lowers the risk of metabolic and cardiovascular disorders. Additionally, studies have demonstrated that in high-risk populations, a regular exercise and diet regimen can halve the risk of major health issues like type 2 diabetes. Even though scientists have long explained the advantages of regular exercise for health, the number of people who lead sedentary lifestyles is rising daily. But research into the molecular mechanisms of exercise in the body has shown that it alters gene expression across a wide range of bodily tissues. Similarly, it was only recently discovered that regular exercise could alter the human genome's overall DNA methylation pattern. Frequent exercise, particularly in skeletal muscle tissue, is also beneficial for mitochondrial biogenesis and adapting to increased oxidative capacity. Regular exercise is directly linked to living a long and healthy life. It has been suggested that mitochondria may play a role in the improvement of metabolic health in skeletal muscle brought about by partial training. Conversely, low aerobic capacity brought on by a sedentary lifestyle plays a role in the emergence of health issues like metabolic disease and low muscle mitochondrial content. Exercise training causes many tissues, particularly skeletal muscle, to adapt. Cellular adaptations in skeletal muscle are driven by both extra- and intramuscular factors. Exercise-induced intracellular signaling enhances mitochondrial function and leads to changes in gene and protein structure, which improves skeletal muscle. Cell perturbations include variations in oxygen tension, energy levels, pH, and temperature. The purpose of this review is to provide an explanation of the advancements in the fields of epigenetics and regular exercise.

Key words: Exercise Adaptation, Skeletal Muscle Metabolism, DNA methylation, exercise epigenetics.

ÖZET

Düzenli yapılan egzersiz metabolik ve kardiyovasküler hastalıkların riskini azalttığı gibi sağlığa da birçok sayısız faydası vardır. Ayrıca, düzenli egzersiz ve diyetle oluşturulan yaşam tarzının yüksek risk gruplarında tip 2 diyabet gibi ciddi sağlık sorunları oluşturan hastalık riskinin yarıya indirilebileceği de gösterilmiştir. Araştırmacılar uzun yıllardır düzenli egzersizin sağlığa faydalarını açıklamalarına rağmen hareketsiz yaşam süren insanların sayısı her geçen gün sürekli artmaktadır. Bununla birlikte, egzersizin vücutta oluşturduğu moleküler mekanizmalar araştırılmış ve egzersizin birçok dokuda gen ekspresyonu değiştirdiği belirlenmiştir. Keza, yakın zamana kadar düzenli egzersizin insanlarda genom çapında DNA metilasyon modelini değiştirebileceği bilinmiyordu. Aynı zamanda düzenli egzersiz mitokondriyal biyogenez ve özellikle de iskelet kası dokusunda gelişmiş oksidatif kapasiteye adaptasyonda da etkilidir. Düzenli egzersiz ile uzun ve sağlıklı yaşam arasında doğru orantılı bir ilişki vardır. Bu da kısmi antrenmanına bağlı oluşan iskelet kasındaki metabolik sağlığın iyileşmesinin mitokondrierle ilişkili olabileceği bildirilmiştir. Aksine, hareketsiz yaşam tarzına bağlı oluşan düşük aerobik kapasite, kas mitokondriyal içeriği ve metabolik hastalık gibi sağlık problemlerinin gelişimine katkıda bulunur. Birçok doku özellikle de iskelet kası egzersiz eğitimine adapte olur. Kas dışı ve kas içi faktörleri iskelet kasındaki hücresel adaptasyonları yönlendirilir. Egzersizin uyardığı hücre içi sinyaller, oksijen gerilimi, enerji seviyeleri, pH ve sıcaklıktaki değişiklikler gibi hücresel pertürbasyonlar aracılığıyla gelişen mitokondriyal fonksiyonlar iskelet kasını iyileştirerek gen ve protein yapısında değişikliklere yol açar. Bu derleme, düzenli egzersiz ve epigenetik alanındaki gelişmeleri açıklamak adına düzenlenmiştir.

Anahtar Kelimeler: Egzersiz Adaptasyonu, İskelet Kası Metabolizması, DNA Metilasyonu, Egzersiz Epigenetiği.

INTRODUCTION

Chronic illnesses linked to obesity and/or inactivity are growing more prevalent in today's society. The health benefits of exercise are numerous (Khan MAB et. al., 2020). Additionally, it is well established that exercise helps prevent a wide range of chronic illnesses, such as type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular disease, and certain types of cancer. Numerous adaptive responses in other tissues, mainly in skeletal muscle, are also brought on by regular exercise. Numerous adaptations are beneficial consequences of exercise that enhance overall health (Khan MAB et. al., 2020-3; Lee CG, et. al., 2002; Zhang X, et. al., 2017).

It is noteworthy how little research has been done on humans to examine how consistent physical activity affects epigenetic adaptation. Not everyone can achieve the levels of exercise necessary to reap the many health benefits of exercise for various reasons. Nevertheless, depending on the type, intensity, and duration of exercise, it is not possible to predict with our current data the intensity and duration of exercise that results in such intricate disruptions to homeostasis. Still, it would be naive to expect that medications could replicate all of the health benefits of exercise that result in such intricate disruptions of homeostasis. There is growing evidence regarding the influence of environmental modifications on epigenetic adaptation, leading to changes in health and illness across multiple generations. There has been a rise in interest lately in using drugs to mimic the overall health benefits of exercise (Corinne C, et. al., 2015; van Tienen FHJ, et. al., 2012; Poppelreuther M, et. al., 2023; Kostek M, 2019; Keller P, et. al., 2011; Dillon EL, et. al., 2019; Fritzen AM, et. al., 2020; Lotte L, et. al., 2010; Islam H, et. al., 2018; McGee SL, et. al., 2006). Studies on the molecular mechanisms underlying this phenomenon imply that the structure of skeletal muscle's exercise-responsive genes undergoes structural changes over time (Muhammad S, et. al., 2022). Novel therapeutic modalities that mediate this response might be discovered by comprehending the underlying molecular mechanisms. It has been discovered that certain environmental contaminants, like benzo[a]pyrene and dioxin, are linked to epigenetic modifications in DNA methylation. Exercise has been shown to cause genome-wide changes in DNA methylation regardless of an individual's presence or absence of metabolic diseases like Type II diabetes (Dolinoy DC, et. al. 2007) . An increasing number of people are interested in improving their lifestyles through exercise training and dietary selection and restriction. These practices can lead to positive, heritable epigenetic changes that enhance disease-protective transcriptional programs, such as those that prevent cancer, heart disease, and metabolic dysfunction (Sato F, et. al., 2011). The objective of this review is to present a conceptual summary of exercise adaptations, their multitude of health benefits, and

the hierarchy of molecular mechanisms underlying them. It has also been investigated to highlight how knowledge of exercise biology identifies molecular targets that can be treated therapeutically to yield major health advantages.

METHYLATION OF DNA

Up until recently, little was known about whether exercise affects a person's epigenetic makeup. Numerous biological processes are influenced by epigenetics, with DNA methylation being the most well-studied marker. The cytosines in CpG dinucleotides undergo DNA methylation in differentiated mammalian cells. Methyl groups are added to the mammalian genome by DNA methyl transferases (DNMTs), which include DNMT1, DNMT3a, and DNMT3b. DNMTs are involved in methyl group transfers and have S-adenosyl methionine as an auxiliary substrate. Since increased DNA methylation can draw transcriptional co-repressors and histone deacetyltransferases (HDACs), it has been linked to decreased transcriptional activity and suppression of transcription factors' binding to promoter regions (McNamee MJ, et. al., 2009; Gray B, Semsarian C., 2020). Inactive genes and a dense chromatin structure are further effects of DNA methylation. Additionally, recent techniques have demonstrated that DNA methylation may affect alternative splicing events and/or has a positive correlation with active gene transcription in gene bodies (Ling C. and Groop L., 2009; Pesta D, et. al., 2021). It's likely that the function of DNA methylation varies depending on the external stimuli and genomic context, resulting in a complex relationship with transcription of genes. In one study (Gensous N, et. al., 2019), it was discovered that the DNA methylation of 2817 and 7663 genes (or near) in skeletal muscle and adipose tissue, respectively, had been altered after multiple testing was corrected for using false discovery rate analysis. The majority of the identified genes exhibited increased DNA methylation in adipose tissue in response to exercise (Brøns C, et. al., 2010), whereas approximately 75% of the genes showed decreased DNA methylation in skeletal muscle (Jacobsen SC, et. al., 2012). This suggests that epigenetics may have different effects in the two tissues. This shows that muscles are sensitive to the hormone adiponectin, which is released by adipocytes. On the other hand, we can state that skeletal muscle and adipose tissue exhibit altered DNA methylation of type 2 diabetes candidate genes as a result of exercise. People who are genetically predisposed to diabetes may exercise more frequently as a result of this information. The muscle and fat papers (Malmgren S, et. al., 2013; Liu J, et. al., 2021) demonstrated that high DNA methylation directly impairs transcriptional activity and also found a correlation between differential DNA methylation and gene structure. MEF2A, a transcription factor that controls the expression of glucose transporter member 4 (GLUT4) in human muscle in

response to exercise (Sandovici I, et. al., 2021), NDUFC2, a gene that encodes a protein involved in the first complex of the respiratory chain, RUNX1, a transcription factor that is known to be regulated by exercise (Taillandier D, Polge C., 2019), and THADA, a type 2 diabetes candidate gene (Zhang Y, et. al., 2023) are among the genes investigated using the luciferase assay. In addition, it was discovered in a study (Huntera DJ, et. al., 2019) that physical activity lowers the DNA methylation of PPARGC1A, the transcriptional co-activator PGC1a (peroxisome proliferator-activated receptor gamma coactivator 1 alpha), which is known to control the expression of genes influencing the skeletal muscle's mitochondrial oxidative phosphorylation. The luciferase assay is utilized to exhibit how adipocytes' elevated methylation of the RALBP1 promoter results in a reduction in transcriptional activity (Swiatowy WJ, et. al., 2021). This gene has a history of being linked to the pathophysiology of metabolic syndrome (Liu J, et. al., 2021; Sandovici I, et. al., 2021), and it has been demonstrated to affect Glut4 trafficking in response to insulin. By raising RALBP1 DNA methylation and lowering mRNA expression, the luciferase assay is a test used to replicate the conditions in adipose tissue following exercise. Reducing the expression of two genes in cultured adipocytes (HDAC4 and NCOR2, which demonstrated increased DNA methylation and decreased expression in fat in response to exercise) causes an increase in lipogenesis in adipocytes, simulating the conditions in human adipose tissue (Chelsea MP, et. al., 2023; Dayeh TA, et. al., 2013).Exercise increases nutrient storage in adipocytes and decreases glucose and/or lipid uptake in other organs, such as the liver, muscle, and pancreas, if it regulates genes that induce lipogenesis in adipose tissue. Together, these studies found that middle-aged sedentary men who engaged in regular, moderate-intensity exercise twice a week for six months showed improvements in their clinical and metabolic traits. These improvements may have been caused by epigenetic modifications that were followed by altered expression in tissues that play a key role in metabolism. In an intriguing contrast to what was observed during exercise, young men were placed on bed rest for nine days in a different study by Alibegovic et al. In response to bed rest, PPARGC1A DNA methylation rose and gene expression dropped in skeletal muscle (Brøns C,, et. al., 2020). In a similar vein, young men exposed to a high-calorie, high-fat diet for five days saw an increase in PPARGC1A DNA methylation in their skeletal muscle (Martinia M, et. al., 2020; Calen PR, Christopher WK, 2020). Moreover, patients with type 2 diabetes exhibit decreased PPARGC1A DNA methylation and increased DNA methylation (Ahmed SAH, et. al., 2020). Conversely, both short-term and long-term exercise reduced PPARGC1A DNA methylation [Brøns C,, et. al., 2020]. When considered collectively, these findings imply that while exercise has the opposite effect on a person's "poor epigenetic phenotype," a

sedentary lifestyle, which includes neither physical activity nor a high-calorie diet, may cause one. According to a different study, variations in the enzymes that control the quantity of methyl donors in top athletes may have an impact on myoblast differentiation and proliferation, which in turn may have an impact on muscle mass (Hunter DJ, et. al., 2021). In addition to assessing the degree of DNA methylation at individual CpG sites, other assays measure multiple CpG sites in repetitive elements dispersed across the genome to ascertain the overall level of DNA methylation. Using this method, it has been shown that exercise raises the global DNA methylation levels in peripheral blood cells (Nitert MD, et. al., 2012). Additionally, the study by White et al. (2019) revealed that increased exercise raises the level of DNA methylation. Because cancer patients have lower global DNA methylation levels, the results are consistent with the previously reported link between exercise and a lower risk of developing the disease. Restrictions enzymes are an additional technique for measuring global DNA methylation, and their application has been shown to result in a global reduction in leukocyte DNA methylation in response to physical activity. It is obvious that more investigation is required to comprehend the differences in DNA methylome between various genomic regions and cellular subfractions within a tissue. The precise function of medications that target epigenetic factors—such as HDAC inhibitors—is still unknown, despite the fact that they are currently used to treat a number of illnesses, including cancer and epilepsy. According to recent research, regular exercise alters the genome-wide epigenome, which may pave the way for the creation of medications that replicate this effect. Additionally, since physical activity lowers the risk of cancer, some research has looked at the effects of exercise on DNA (Helios P-G, et. al., 2014). It's interesting to note that in peripheral blood leukocytes of breast cancer patients taking part in a randomised clinical exercise study, six months of moderate-intensity aerobic exercise changed the DNA methylation of 43 genes (Zeng Y, et. al., 2021). Physical activity may impact the epigenetic regulation of tumour suppressor genes following a breast cancer diagnosis, according to Zeng et al. (2021). Additionally, two studies on rodents have demonstrated that exercise can alter the brain's epigenetic makeup (Horsburgh S, et. al., 2015; McEwen BS, Bulloch K, 2019). Moreover, Abel and colleagues proposed that the beneficial impacts of exercise on behavior in teenagers can be explained by epigenetic modifications in their brains (McEwen BS, Bulloch K, 2019). Lastly, it can be challenging to investigate how genetics and epigenetics influence a phenotype because of their potential interactions (Jacobsen SC, et. al., 2012; Huntera DJ, et. al., 2019; Sandovici I, et. al., 2011). Exercise's impact on the epigenome may therefore also be influenced by an individual's genotype. Thus, it is important to think about how

genetic, epigenetic, and non-genetic factors—like exercise—interact with disease phenotypes in future research.

ENZYMES REGULATING DNA METHYLATION IN SKELETAL MUSCLE DURING EXERCSIE

There are currently few studies looking at how exercise affects DNA methylation in skeletal muscle. According to the information that is currently available, acute exercise lowers skeletal muscle gene methylation. Barres et al. (2012), for instance, demonstrated that in human skeletal muscle, high-intensity exercise (80% VO2 max) resulted in an abrupt decrease in methylation levels at the promoter regions of PGC1a, PDK4, and PPARd, which was linked to increased transcription of these genes (Barre` s R, et. al., 2012) But the same exercise modality at 40% VO2 max, which is a lower intensity, did not change DNA methylation. This implies that the level of exercise may have a significant impact on DNA methylation. Some of these results were confirmed in a mouse study, which revealed that exercise reduced PGC1a promoter methylation, which was linked to higher basal transcription of this gene (Lochmann TL, et al. 2015). In a different study, it was shown that cycling for 120 minutes at 60% of one's maximum oxygen consumption (VO2 max) increased methylation in the promoters of the genes FABP3 and COX4L1, which was linked to lower expression of these genes in healthy people (Lane SC, et al. 2015). These studies clearly show that DNA methylation may be an important part of the immediate physiological response to an acute exercise, and they also suggest that changes in DNA methylation may occur more quickly than previously believed. The precise control of DNMTs and enzymes involved in DNA demethylation is probably crucial in this process, even though the precise mechanism underlying the changes in DNA methylation patterns in skeletal muscle after exercise is unknown. TET1 has been linked to hypoxia-induced and upregulation of glycolytic gene expression in several cell lines (Tsai SQ, et al., 2014), and in vitro knockdown of DNMT3B has been demonstrated to prevent saturated fatty acid-induced methylation of the PGC1a promoter in a muscle cell line (Barres R, et al. 2009). However, little is known in this field. To fully comprehend the role of DNA methylation and demethylation in the adaptive response to acute exercise, more research is required. Finding out whether these changes persist over time and how soon they disappear before another exercise is needed to have the same impact would be especially interesting.

CONCLUTION

Gene structure responses in all organisms are regulated by epigenetic mechanisms that control gene structure in response to environmental cues. As of now, posttranslational modification of histones and methylation of DNA have been identified as the main epigenetic control mechanisms that have been studied in experimental settings. We still don't fully understand the complex spatially and temporally dependent interactions between DNA methylation and various histone modifications at particular gene loci, but these interactions should become clearer as the technology to study them becomes more widely available. The role of epigenetics in phenotypic adaptations to exercise and transcriptional responses is currently poorly understood. According to preliminary research, this response is influenced by acute reductions in DNA methylation and increases in histone acetylation. Further research indicates that class IIa HDAC regulation plays a significant role in exercise adaptations. Given that several metabolites are known to be exercise-rate limiting, another developing field of study will be how variations in muscle metabolism impact epigenetics and exercise adaptations. Reactions or the activity of epigenetic modifiers are directly regulated by epigenetic modification. Lastly, whether our knowledge of the epigenetics of exercise can be used to identify therapeutic targets and eventually cure diseases like obesity, type 2 diabetes, and cardiovascular diseases—diseases that are exacerbated by inactivity—will be an exciting field for future research. In order to provide an exercise-epigenetic roadmap, future research should methodically investigate the effects of exercise modality, intensity, and duration while examining the interactions between epigenetic mechanisms. This will become feasible with the integration of new omics technologies, such as unbiased proteomics that includes post-translational modifications into current epigenetic pipelines. This could have significant implications for comprehending the health benefits of exercise that may be applied to medicine.

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CHAPTER 6

THE ROLE OF FOLIC ACID (VITAMIN B9) IN IMMUNITY

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Introduction

The immune system is likened to a team effort with many different players interacting with each other to provide a strong defense against invading pathogens such as foreign macromolecules, bacteria, viruses, parasites, neoplastic cells or harmful attacks. (Alpert, 2019; Elmadfa & Meyer, 2019; Sompayrac, 2022). While recognizing and eliminating pathogens, situations such as recognizing, tolerating, and storing non-threatening antigen sources in immunological memory must also be corrected and kept under control (Calder, 2013; Chaplin, 2010; Elmadfa & Meyer, 2019). The organism distinguishes between self and non-self through innate and adaptive immune systems (Alpert, 2019; Gartner & Hiatt, 2007; McComb et al., 2019). Both systems include blood-borne factors and many different immune cells (Alpert, 2017; Calder, 2013).

It is reported that micronutrients increase body resistance to infections and inflammation by modulating immunity and that they are necessary to maintain immune sufficiency (Alpert, 2017; Chandra, 2002; Wintergrest et al., 2007). These nutrients protect immune cells from oxidative stress by maintaining antioxidant/oxidant balance (Calder & Kew, 2002). It is stated that micronutrients such as iron, folic acid, and zinc are involved in the synthesis of nucleotides and nucleic acids (Calder, 2013).

Folate, also known as vitamin B9, is the general term for the family consisting of folic acid and its derivatives such as 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, 10-formyl-methyltetrahydrofolate, 5,10-methylene-methyltetrahydrofolate, unmodified methyltetrahydrofolate (Scaglione & Panzavolta, 2014; Wright et al., 2005). Folate, a major micronutrient; it serves as a cofactor in one-carbon metabolism that gives methyl groups to creatine, adrenaline, choline phospholipids and DNA (Scaglione & Panzavolta, 2014; Shulpekova et al., 2021). Since mammals cannot synthesize folate, folate must be obtained externally through supplements in the form of folic acid, folinic acid or 5-methyltetrahydrofolate (Mitchell et al., 2014; Shulpekova et al., 2021). Folic acid, which is a member of the vitamin B family, is water-soluble, oxidized, and synthetic. In order for folic acid, which is inactive as a coenzyme, to be transformed into the active tetrahydrofolate form in nucleic acid and protein synthesis, it must go through several metabolic stages within the cell (Forssen et al., 2000; Mikkelsen & Apostolopoulos, 2019).

Folic acid was first discovered by Lucy Wills as a result of her work on poor pregnant textile workers. It is derived from the Latin word "folium" meaning leaf and was isolated from spinach in 1941 (Shulpekova et al., 2021). This acid, an important component for cellular functions, plays a role in DNA synthesis, repair, cell division, energy production, meth-

ylation, maintenance of cellular/humoral immune functions and maintenance of this balance. Deficiency or excess may lead to negative changes in immune functions. It is said that folic acid, in particular, has significant effects on Treg cells (Alpert, 2017; Henry et al., 2017; Kunisawa et al., 2012; Mansouri et al., 2016; Meadows et al., 2015; Mikkelsen & Apostolopoulos, 2019). It works together with vitamin B12 and vitamin B6 to convert homocysteine into methionine in methylation reactions (Mikkelsen & Apostolopoulos, 2019). Folate and vitamin B12 metabolism are closely related, as methylcobalamin is required in the regeneration of tetrahydrofolate from methyltetrahydrofolate. Therefore, the balance between vitamin B12 and folate is important (Elmadfa and Meyer, 2019; Paul & Selhub, 2017). It is also stated that folic acid has a protective effect against ischemic events and cancer (Shulpekova et al., 2021). Studies have reported that insufficiency or excess folate intake causes many health problems. Especially during pregnancy, folic acid deficiency can cause neural tube defects in the fetus (Barry et al., 2023; Morris & Wald, 2023). It is stated that excessive folate load during the embryogenesis period can cause disorders in brain development and may have a growth advantage for precancerous altered cells (Shulpekova et al., 2021).

This study aims to provide information about the effects of folic acid, an effective micronutrient in important functions of the body, on immune cells.

Folic Acid and Immune Cells

A diet containing plenty of fresh fruits and vegetables is essential to strengthen the immune system. In recent years, studies have been reported that vitamins and their metabolites have specific effects on the immune system (Alpert, 2017; Kunisawa & Kiyono, 2013; Mansouri et al., 2016). Continuous renewal and maintenance of immune cells depends on adequate energy and nutrient supply. B group vitamins, which are necessary for cytotoxic cellular immunity, have a critical role in the modulation of T cell responses (Ibrahim et al., 2017; Katona & Aktona-Apte, 2008). Vitamin B9, one of the B group vitamins, is known as folate or folic acid. Folic acid serves as a cofactor in many physiological events, especially amino acid metabolism, energy metabolism and nucleic acid synthesis. It also has regulatory effects on immune responses. (Elmadfa & Meyer, 2019; Mansouri et al., 2016; Paul &Selhub, 2017). Responding quickly and effectively of the immune system to antigens and neoplastic formations depends on the distribution of lymphocyte and granulocyte subsets (Abe et al., 2013; Shirato et al., 2007).

Monocytes

Monocytes, one of the mononuclear cells of the immune system, differentiate into macrophages and dendritic cells against cytokines and microbial agents (Mikkelsen & Apostolopoulos, 2019). Macrophages in connective tissue spaces produce cytokines necessary for inflammatory and immune responses and present epitopes to T lymphocytes (Hiatt & Gartner, 2007). The effect of folic acid on DNA methylation is important in regulating monocyte subgroups and functions (Xiang et al., 2022). Additionally, folic acid inhibits homocysteine-induced nuclear factor kappa B, which has a critical role in the regulation of proinflammatory cytokines in macrophages (Au-Yeung et al., 2006). In vascular injuries, mononuclear cells bind to damaged endothelial cells, leading to the formation of vascular diseases. Folic acid prevents the formation of atherosclerotic plaques by reversing these processes (Kolb & Petrie, 2013; Xiang et al., 2022).

Lymphocytes

Lymphocytes, one of the most important members of immunity, are divided into 3 functional classes as T lymphocytes (cells), B lymphocytes (cells) and null cells. These cells, which are not functional in the circulation, show their immune functions when they pass into the connective tissue. While B lymphocytes are responsible for humoral immunity, T lymphocytes play an important role in cell-mediated and partly humoral immunity (Hiatt & Gartner, 2007; Sauls et al., 2023). The best-known T cells are CD4+T cells, CD8+T cells, and T regulatory cells. These cells can recognize through their T cell receptors (TCR). T cell function varies depending on the type of T cell (Lyu et al., 2019; Sauls et al., 2023). CD4+T cells are responsible for humoral immunity as they activate B cells to produce immunoglobulin as well as cell-mediated immunity (Bourne et al., 2019; Mikkelsen et al., 2017; Shen et al., 2019). There are three different subtypes of CD4+T cells as TH1 CD4+T cells (in autoimmune reactions and diseases), TH2 CD4+T cells (in helminthic infections), and TH17 CD4+T cells/MAIT cells (in mucosal immunity and fight against extracellular bacteria/fungi). In activation of macrophages and intracellular infections, TH1 CD4+T cells secrete the cytokines IFN-gamma and TNF-alfa, while TH2 CD4+T cells produce IL-4, IL-5 and IL-13 in helminthic infections. Additionally, TH17 CD4+T cells secrete IL17A, IL17F, and IL-22 for pro-inflammatory function (Sauls et al., 2023). CD4+T cells have an important role in recruiting polymorphonuclear leukocytes to inflammation regions and in regulating immune responses with the secretion of cytokines and chemokines (Mikkelsen et al., 2017; Zhu & Paul, 2008). CD8+T cells perform the killing function when they find their antigenic target through the production of TNF-α and interferon gamma (cytokines), the release of per-

forin and granzymes (cytotoxic granules), and caspase-triggering Fas/FasL interactions (Dhur et al., 1991; Lyu et al., 2019). T regulatory cells are involved in the modulation of immune responses and inhibition of autoimmune processes (Batista-Duharte et al., 2018; Jeon & Oh, 2017). Diets low or high in folate lead to permanent DNA damage, faulty nucleotide synthesis, disorders in lymphocyte production, and defective cell cycles (Elmadfa & Meyer, 2019; Henry et al., 2017; Kunisawa et al., 2012; Mikkelsen & Apostolopoulos, 2019). In addition, the high expression of folate receptor 4 (FR4), as a Treg cell marker, on the surfaces of these cells indicates that folic acid plays a critical role immunologically in the survival and differentiation of Treg cells (Kunisawa et al., 2012; Mansouri et al., 2016).

Elmadfa & Meyer (2019) report that B group vitamins modulate T cell responses and are necessary for cytotoxic cellular immunity. Studies have shown that folic acid affects the immune system by making changes in the rate, number and differentiation of T cells (Henry et al., 2017; Kunisawa et al., 2012; Mikkelsen & Apostolopoulos, 2019; Wu et al., 2017). Folic acid deficiency inhibits CD8+T cell activity, reducing lymphocyte proliferation and natural killer cell activity, and as a result, immune resistance against infections decreases (Elmadfa & Meyer, 2019; Mansouri et al., 2016). One of the important effector lymphocytes of the innate immunity are natural killer cells (NK cells). These cells, which are constantly located in the bloodstream, have the ability to directly kill pathogens through cytotoxic pathways (Mikkelsen et al., 2017). Studies have reported that high or low folate status also causes impaired NK cell functions because it causes disorders related to blood production (Bayer & Fraker, 2017; Paniz et al., 2017). Moreover, it has been reported that there is a decrease in Treg cells in case of deficiency of folate, which acts as a Treg cell marker (Yamaguchi et al., 2007). It has been reported that the number of B lymphocytes is more sensitive than T lymphocytes and NK cells in rats fed a diet deficient in folic acid. In summary, it is stated that folate deficiency causes lymphocytopenia and granulocytopenia in a cell-specific manner (Abe et al., 2013).

Granulocytes

Neutrophils, which constitute the majority of white blood cells and are granulocytes, are phagocytes that eliminate bacteria occupying connective tissue spaces by using the contents in their granules. It is stated that eosinophils, a member of leukocytes, eliminate antigen-antibody complexes and parasite invasions while basophils, which are functionally similar to mast cells, initiate the inflammatory process. (Gartner & Hiatt, 2007). It is known that folic acid plays an active role in the production of these blood cells originating from pluripotent hemopoietic stem cells (McNulty, 2022). Abe et al. (2013) reports that neutrophil and eosinophil numbers decrease

in folic acid deficiency. The same researchers found that in the cell ratio analysis, the decrease in the number of eosinophils was greater than the number of neutrophils.

McNulty (2022) says that the earliest morphological change in the hematopoietic system caused by folate deficiency is hypersegmented neutrophils in the peripheral blood. It has been reported that the number of segments in neutrophils, which normally have three or four nuclear segments, increases as a result of abnormal cell replication (McNulty, 2022; Metz, 2008). Following this, anemia characterized by the formation of macroovalocytic erythrocytes also occurs (Chan et al., 2013). It has been reported that there is an inverse relationship between the number of eosinophils (Denlinger et al., 2017), which play an significant role in the emergence, development and treatment of asthma, a chronic airway disease, and serum folate level. The formation of this situation also affects the immune system (Wen et al., 2023). Studies have reported that serum folate levels are an effective factor in the pathophysiology of asthma, which has a multifactorial etiology (Han et al., 2020; Kim et al., 2022; Wen et al., 2023). Affecting DNA methylation and increasing or decreasing asthma-prone gene expressions are important in the pathogenesis of asthma (Wen et al., 2023). As is known, folate, which has an important place in cell growth and proliferation by regulating DNA and RNA synthesis and stabilization (Abe et al., 2013), serves as a methyl donor source for DNA methylation and is also necessary for purine and pyrimidine synthesis (Naderi & House, 2018). Abe et al. (2013) report a temporary increase in the number of circulating basophils despite a decrease in the number of neutrophils and eosinophils in rats fed a diet deficient in folic acid. This increase is thought to reflect decreasing plasma folate levels. As a result, it is argued that the basophil production mechanism follows a different path than other granulocytes (Shirato et al., 2006), and that the number and cell ratio of basophils in the bone marrow should also be taken into account, as well as changes in plasma folate levels (Abe et al., 2013).

Effects on The Offspring's Health of Maternal Folic Acid

Iron and folic acid supplementation is important in reducing maternal deaths that occur as a result of anemia, which is frequently seen in pregnant women (Sanghvi et al., 2010; WHO, 2007). Folic acid, which mediates DNA methylation in one-carbon metabolism, is one of the maternal nutrients (Liu et al., 2020; Wolff et al., 2009). Folic acid is considered an essential nutrient for the development of a healthy fetus and placenta (Bulloch et al., 2018; Kalhan & Marczewski, 2012). S-adenosylmethionine, the methyl donor for 5-methylcytosine, is derived from folate one-carbon metabolism. Epigenetic information encoded by 5-methylcytosines has an import-

ant place for fetal development, brain development and offspring health (Liu et al., 2020; Roza et al., 2010). It is very important to have adequate amounts of folate during events such as cell division, angiogenesis, trophoblast invasion and endothelium-dependent vascular relaxation (Williams et al., 2011). Folate, which is effective in the development of the central nervous system, plays an important role in the synthesis of many neurotransmitters by affecting neuronal/glial growth and proliferation (Craciunescu et al., 2004). During pregnancy, the need for folate also increases due to the increase in the total number of cells dividing for fetal growth. A decrease in erythrocyte folate level, an increase in homocysteine concentration, and megaloblastic changes in the bone marrow and rapidly dividing cells occur in the offspring when this increased need cannot be met (Lassi et al., 2013). It is reported that maternal and even paternal folate status are effective in childhood diseases and can lead to congenital malformations (Liu et al., 2020; Sahara & Matsuzawa, 2019). Maternal folic acid intake is characterized by DNA methylation changes in the offspring (Richmond et al., 2018). Maintaining DNA methylation balance, especially during pregnancy, is very important to prevent congenital malformations, neurodevelopmental disorders such as hyperactivity and schizophrenia, and behavioral and emotional problems in later life in the offspring (Gonseth et al., 2019; Kofink et al., 2013; Roza et al., 2010; Zaganjor et al., 2016; Zammit et al., 2007). For this reason, the United States Food and Drug Administration has mandated folic acid supplementation since 1998 (Liu et al., 2020).

Many studies have been conducted investigating the relationship between the risk of congenital heart defects, one of the birth defects of newborns, and folic acid supplementation before and/or during pregnancy. While some of them find that folic acid supplementation can reduce the risk of congenital heart defects, others suggest that it increases this risk or has no relationship (Leirgul et al., 2015; Mao et al., 2017; Obermann-Borst et al., 2011). Lassi et al. (2013) declared that folic acid supplementation throughout pregnancy showed improvement in average birth weight and reductions in the risk of megaloblastic anemia. It is stated that folic acid supplementation may help prevent the development of preeclampsia, which is important for maternal and newborn health. Continuing folic acid supplementation throughout pregnancy may protect against the high plasma homocysteine concentration associated with preeclampsia (Bulloch et al., 2018). Caffrey et al. (2021) stated that folic acid supplementation during pregnancy contributes to the child's neurocognitive development by affecting cognitive performance and brain function.

Folate concentrations in different trimesters of pregnancy are said to be of critical importance for the occurrence of certain childhood diseases. While it is stated that folate deficiency causes especially neonatal neural

tube defects such as spina bifida and anencephaly (Roza et al., 2010; Zaganjor et al., 2016), orofacial clefts (Gonseth et al., 2019; Jahanbin et al., 2018) and neurodevelopmental disorders (Craciunescu et al., 2004; Kofink et al., 2013) in the first trimester of pregnancy, it is suggested that excessive folate in the perinatal period can also cause immune diseases, autism and lipid disorders in the offspring (Liu et al., 2020). Kiefte-de Jong et al. (2012) found periconceptional and first trimester folic acid supplementation to the mother throughout pregnancy to be a risk factor for atopic dermatitis, an allergic disease, while Fortes et al. (2019) argued that this supplement had a protective effect. It is also reported that folic acid taken after the 12th week of pregnancy is associated with wheezing, asthma and eczema (Liu et al., 2020). Sreenivas et al. (2015) associated the timing of folic acid intake during pregnancy with childhood asthma. Catrina et al. (2017) declared that folic acid promotes the allergic phenotype through changes in DNA methylation, leading to the development of allergic disease in childhood. Another study showed that the mother's high folate level poses a risk of autism spectrum disorders in the offspring (Raghavan et al., 2018). It is also reported that high folate levels in the mother lead to lipid metabolism disorders in the offspring, such as high insulin resistance, colitis, increased body weight, and increases in serum and liver triglyceride levels (Liu et al., 2020).

Conclusion

Folic acid is an important micronutrient that plays a role in many functions of immune responses by participating in cellular and molecular mechanisms. Deficiency or excess of this acid, which is a B group vitamin, can lead to many negative effects. While its deficiency causes disruption of many processes, from neural tube defects to weakened immune responses, its excess contributes to embryonal brain development disorders and the development of pre-cancerous cells. Suppression or impairment of immune functions can lead to decreased resistance to all kinds of factors and susceptibility to infections/diseases. For these reasons, taking folic acid in sufficient amounts is very important for both organ development during embryogenesis and the proliferation and functioning of immune cells. Furthermore, many studies have reported that maternal folate concentrations in different trimesters of pregnancy are important in terms of childhood diseases. Researchers recommend further research on this subject to determine the correct dosage and timing of folic acid, which has a very important role in preventing neurodevelopmental disorders, for the health of the offspring.

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CHAPTER 7

ROOT CANAL TREATMENT IN PRIMARY TEETH

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INTRODUCTION

Health and protection of primary teeth in the mouth; skeletal and muscle development, growth and development of the jaws, occlusion, aesthetics, chewing, speech functions and protection from abnormal habits are critical in child development (Bolette *et al.,* 2016). Early loss of primary teeth is considered an oral health problem due to its functional and psychological damage. The most common etiological causes are trauma, deep dentin caries, newborn tooth extraction and early root resorption (Nadelman *et al.,* 2021).

Dental infection, which can develop with the irritation of the pulp and periapical tissues in primary teeth, can cause local micro-abscess formation in the region, digestion of the pulp tissue by proteolytic enzymes, and finally necrosis of the entire dental pulp. (Rechenberg, 2017; Bjørndal & Ricucci, 2014).

Root canal treatment is the treatment applied when clinically irreversible pulp inflammation findings are observed in the radicular pulp tissue of primary teeth or when signs of pulp necrosis are observed and minimal or no resorption is observed in the roots of the relevant primary teeth. The procedure of the root canal treatment consists of excising of the inflamed or necrotic pulp tissue in the root canals, instrumenting of the intracanal area at the specified working length, disinfecting the area with appropriate irrigation agents, and then hermetically filling the canals. The purpose of this procedure; To eliminate the infection in the primary teeth, to eliminate and control the inflammation, to alleviate the pain, to prevent the pathological effects of the inflammation on the successive permanent teeth, and to prolong the usage period of the primary teeth in the oral cavity (Yu, 2020; Daloğu & Güzel, 2017; Allen, 1979; Winters *et al.,* 2013).

MORPHOLOGICAL CHARACTERISTICS OF PRIMARY TEETH

For successful pulp therapy to be performed on the primary dentition, it is necessary to fully understand root formation, the differences between primary teeth from permanent teeth, their pulpal morphology and specific characteristics related to physiological primary tooth roots resorption (Ralph *et al.,* 2010). Permanent teeth are larger in all dimensions than their corresponding successive primary teeth (Ralph *et al.,* 2010; Nelson & Ash, 2010). The primary molars' roots are thinner, narrower, longer in comparison with the permanent molars' roots, and expand outward from the cervical region, according to the crown-to-root ratio. In addition, the pulpal cervical region is closer to the apex than permanent molar roots in primary teeth. This causes the pulp size to be larger than the crown sizes (Ralph *et*

al., 2010; Nelson & Ash, 2010; Cameron & Widmer, 2021; Camp, 2006). Dentin thickness between the enamel and pulp chamber is more in permanent dentition than in primary dentition. The pulp chambers are relatively smaller in permanent teeth compared to the pulp chambers of primary teeth, and the pulp horns are lower in permanent molars than in primary molars, especially the mesial horns in permanent molars. Due to the mesial pulp horns, which are closer to the occlusal surface than permanent teeth, the risk of exposure to the pulp tissue of primary teeth increases in caries or trauma (Ralph *et al.,* 2010; Nelson & Ash, 2010; Camp, 2006; Camp, 2008; Dean & Sanders, 2021). The roots of primary anterior teeth are long and narrow relative to crown width and length. The roots of permanent molars are less divergent than the roots of primary teeth; this characteristic provides more space for the development of successive premolars between the roots of primary molars (Ralph *et al.,* 2010; Camp, 2006; Dean & Sanders, 2021; Miller, 2017).

In primary dentition, the morphology of the root canals in the anterior teeth shows a structuring in accordance with the shape and form of the teeth roots. The permanent incisor and canine germs are located and develop in a position that coincides with the lingual and apical of the primary and canine teeth. Therefore, physiological root resorption of primary anterior incisors and canine teeth begins on the lingual side in the apical third of the roots. In anterior deciduous teeth, which usually have a single root canal, apical branching, lateral canals and accessory are uncommon. However; In maxillary primary canines, variations such as double canals and the presence of accessory roots and root canals can be encountered (Waterhouse *et al.*, 2011; Cleghorn *et al.*, 2010; Mochizuki *et al.*, 2001).

Root number and position of primary molars and permanent molars are the same. Maxillary primary molars have one palatal, two facial and three roots. There are two located roots as mesial and distal for mandibular primary molars. The inner surface of the primary teeth close to the inter-radicular septum is usually the area where physiological root resorption begins. (Camp, 2006; Ahmet *et al.*, 2017; Reddy *et al.,* 2018; Meryem *et al.,* 2019). When root formation is complete in primary molars, each root has a single root canal. However, with continued dentin deposition, isthmus or lateral canals may form, which can split the root canals into two or more, connecting the canals. In primary teeth, it has been reported that secondary dentin deposition occurs after root length formation is complete. This may produce variations in the size and number of root canals, resulting in some changes in the initial basic root canal morphology. Secondary dentin deposition begins simultaneously with the onset of physiological root resorption. Changes in canal form are observed more prominently in teeth with root resorption (Camp, 2006; Reddy *et al.,* 2018; Hibbaed, 1957). The

biggest change observed in root canal morphology is dentin agglomeration, which occurs in the mesial roots of maxillary and mandibular primary molar teeth, which partially or completely separates the root canal into two or more separate canals. These variations, which are found especially in the mesial roots of primary molars, can also be found in the distal and lingual roots. However; these are fewer common variations compared to mesial roots. Apical branching, lateral canals and accessory canals in the pulp are observed in 10% to 20% of primary molars (Camp, 2006; Waterhouse *et al.*, 2011; Ahmet *et al.*, 2017; Reddy *et al.,* 2018; Meryem *et al.,* 2019; Hibbaed, 1957). In primary teeth series, some canal anomalies such as taurodontism, radix entomolaris, root fusions, enamel pearls, dens evaginates, C-shaped canals, the fusion that can occur between supernumerary teeth and primary dentition teeth, accessory canals, apical deltas, para molar canals in the furcation region, dilacerations in the roots can be encountered. Due to the accessory canals seen in primary molars, destruction and lesion in the bone can be found in any area along the root structure, especially in the interradicular area in the furcation region (Camp, 2006; Ahmed & Dummer, 2018; Moskovitz & Tickotsky, 2016). The unusual internal geometry of the pulp cavity occurs when dentin accumulates within the root canal system after beginning root resorption of primary teeth, which can remarkably change the size, number, and/or form of root canals. For these reasons, endodontic treatment of primary teeth is considered quite complex (Dean & Sanders, 2021; Reddy *et al.,* 2018; Katge & Dixit, 2022; Ozcan *et al.,* 2016).

Since the root lengths of primary teeth are shorter compared to permanent teeth, root development is completed in a shorter time (Camp, 2006; Li *et al.,* 2017). After about 16 to 20 months with eruption into the oral cavity, the roots of the primary teeth are completely occurred. At roughly three years, the primary tooth roots begin to resorption from the apex or a lateral surface close to the apex (Salama *et al.*, 1992).

INDICATIONS AND CONTRAINDICATIONS FOR PRIMARY ROOT CANAL TREATMENT

Root canal treatment is applied to primary teeth in the following cases; radiographically and clinically, the existence of acute or chronic infection in the root pulp or pulp necrosis, minimal or no root resorption, interroot bone loss not exceeding 1/3 of the root, spontaneous pain due to the related tooth, or presence of long-term pain complaint, treatment of the existing abscess and fistula tract caused by pulpal infection, presence of infected primary teeth in congenital deficiency of the consecutive permanent tooth, unstoppable, dark red colored bleeding or no bleeding, bleeding that lasts longer than 5 minutes during pulpotomy treatment, minimal mobility in

the relevant tooth. However, for applying root canal treatment to primary teeth, the root length of the primary tooth must be at least 4 mm (Cameron & Widmer, 2021; Camp, 2006; AAPD, 2022; Alaçam, 2000).

Root canal treatment is contraindicated in primary teeth in the following cases; presence of irreversible devastated primary teeth, presence of caries and/or mechanically formed perforation at the pulp base, severe mobility accompanied by severe loss of bone support and severe periodontal attachment loss in the involved tooth, internal and/or external root resorption that can be seen in the radiographic examination, the primary tooth with pathological root resorption and lesion exceeding 1/3 of the root, presence of periradicular pathology and radiolucency including the successive permanent tooth follicle, presence of dentigerous and/or follicular cysts, long-term corticosteroid treatment, and advanced systemic diseases such as leukemia, congenital heart disease (Allen, 1979; Camp, 2006; Moskovitz & Tickotsky, 2016; Alaçam, 2000).

EXTIRPATION CANAL PULP IN PRIMARY TEETH

The tooth for which root canal treatment is planned should be isolated with a rubber cover after the application of local anesthesia. The relatively uncomplicated nature of canal systems makes root canal access and canal instrumentation simpler in anterior primary teeth compared to primary molars. The access cavity is formed like the morphology of the entrance cavity in permanent teeth, which includes the entire pulp chamber in anterior deciduous teeth. After removing the pulp tissue from the root canal system, anterior primary teeth are instrumented in the same way as permanent anterior teeth (Allen, 1979; Moskovitz & Tickotsky, 2016; Alaçam, 2000; Goerig & Camp, 1983).

In posterior region primary teeth, after anesthesia and rubber cover application, the entrance opening to the pulp chamber is provided, like permanent molars. The pulp chamber height is shorter in primary molars. For this reason, care should be taken not to perforate the pulp base during the creation of the access cavity. The goal of a successful access cavity is to remove the dentin protrusions that impede direct access to the canal orifices and to provide full access to the pulp chamber. After the access cavity, the area is washed with sterile saline solution to remove the macroscopic pulp residues and debris in the pulp chamber, and the canal openings are determined. Before instrumentation, the pulp chamber should be washed with plenty of sodium hypochlorite (Allen, 1979; Moskovitz & Tickotsky, 2016; Alaçam, 2000; Goerig & Camp, 1983).

Root canal treatment in primary teeth continues with the use of appropriately sized tines for root pulp extraction. Since the root canals of prima-

ry molar teeth are divergent, tirnerf should be used by giving a slope, and the root pulp should be removed by placing it inclined to the canals. The softer dentin of deciduous teeth compared to permanent teeth increases the possibility of insertion of the incisors into the canal walls. During use, care should be taken not to allow the tirnerf to meet the side walls of the canal (Moskovitz & Tickotsky, 2016; Alaçam, 2000).

DETECTION OF WORKING CANAL LENGTH IN PRIMARY TEETH

Endodontic files are used to determine the working length of the canal in primary teeth. Files are used with vertical angulation in primary incisors because they are positioned more vertically in the alveolar socket. Files originating from the divergent root form of deciduous molars should be placed in the canals by giving an anterior slope in accordance with the curvature. In primary teeth, there is no definite judgment on the endpoint of root canal treatment in the apical region; accurate determination of working length is important for the prognosis of treatment (Moskovitz & Tickotsky, 2016; Goerig & Camp, 1983; Zehnder, 2006). Excess root canal filling in primary teeth shows a greater risk of failure in terms of treatment prognosis and preservation of the successive permanent tooth germ compared to incomplete filling (Ricucci & Langeland, 1998).

The ongoing physiological root resorption in primary teeth causes the apical foramen position and width to constantly change, the anatomical apex and apical opening due to oblique development of resorption not being at the same point, and the loss of the apical narrowing region. This makes it difficult to determine the working length and increases the risk of flooding (Camp, 2006; Camp, 1984). Camp (Camp, 1984) reported that the working length could be determined as 1-2 mm behind the radiological apex in primary teeth with no resorption started, and 2-3 mm behind the radiological apex in primary teeth with physiological and/or pathological root resorption. Garcia-Godoy (Garcia-Godoy, 1987), on the other hand, includes the inclusion of the entire root length of the primary tooth in the treatment in cases where the successive permanent tooth germs are in the bone under the deciduous molar roots; reported that treatment should be terminated at the occlusal level of the successive tooth germ in cases where it is in the furcation region.

In addition to the radiological examinations with endodontic files, various apex locators were used for working length determination. However, it has been stated that the determination of working length with apex locators will not provide reliable and positive results due to the presence of lateral and accessory canals, and the widening and changing apical opening. For this reason, it has been reported that the apex locator and endodontic

files should be used in combination (Camp, 2006; Enes *et al*., 2011; Zeren & Sarı, 2014)

MECHANICAL CLEANING OF ROOT CANALS IN PRIMARY TEETH

Another stage of root canal treatment is to prepare the canal walls. Mechanical instrumentation of root canals has traditionally been performed with K-type and H-type hand instruments. (Alaçam, 2000; Yaman, 2002).

It is recommended to enlarge the file up to 3-4 instruments larger than the first inserted and jammed file in the preparation with hand tools in deciduous teeth and to prepare canals up to 35-50 handpieces for deciduous teeth and 30-35 for deciduous molars. It is recommended to use short instruments with a length of 19-21 mm to reduce the risk of flood instrumentation (Alaçam, 2000; Goerig & Camp, 1983). Characterized by higher flexibility in bending compared to traditional stainless steel instruments, reducing time consumption, helping to preserve the original canal anatomy, low elastic modulus, high resilience, and corrosion resistance, to avoid the unwanted shaping effects of traditional files and excessive tissue elimination from the inside of curved canals, Thanks to its advantages such as shape memory, Nickel-Titanium (Ni-Ti) systems, which provide more successful results in root canal treatment and have good fracture resistance, have become an alternative to traditional instruments (Nagaratna *et al*., 2006; Silva *et al.,* 2004; Arıkan *et al.,* 2010).

IRRIGATION OF ROOT CANALS IN PRIMARY TEETH

Current endodontic instrumentation techniques result in the formation of a two-layered layer, called the smear layer, which covers the root canal walls and dentinal tubules, with a 1-2 μm superficial layer and a 40 μm deep layer that adheres loosely to the tubules. This layer contains components such as inorganic dentin chips and organic necrotic pulp tissues, odontoblast residues, and microorganisms (Demiray *et al*., 2016; Pintor *et al.,* 2016; Gupta *et al.,* 2015). It is claimed that during the preparation the smear layer formed should be removed from the root canal system for successful root canal treatment. To ensure irrigation of the canal, the smear layer must be removed from the root canal system. (Demiray *et al*., 2016; Pintor *et al.,* 2016; Gupta *et al.,* 2015; Vora *et al.,* 2017). Physiological saline, sterile water, sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), citric acid (CA) and chlorhexidine are materials that have been used as irrigation agents in the endodontic treatment of deciduous teeth (Moskovitz & Tickotsky, 2016; Zehnder, 2006; Pintor *et al.,* 2016; Vora *et al.,* 2017*;* Haapasalo *et al*., 2005). Sodium hypochlorite (NaOCl), a solvent for organic tissues such as pulp tissue residues and collagen, high

antibacterial properties, and effectiveness in removing the organic part of the smear layer has been the most frequently used irrigation agent. It is recommended to be used in primary teeth at a concentration of 1% (Moskovitz & Tickotsky, 2016; Zehnder, 2006; Silva *et al.,* 2004; Vora *et al.,* 2017*;* Haapasalo *et al.*, 2005). EDTA is a solution that has an effective pH range of 6-10, affects the inorganic and organic parts of the smear layer, and has found use at 17% concentrations because it increases dentin demineralization at high concentrations. The material is an inorganic tissue solvent effective solution. Citric acid has been used in various concentrations from 1% to 50%, but at low concentrations, it has found more widespread use at 10% concentration, as it affects removing the inorganic content of the smear layer. They effectively dissolve inorganic components and smear layer with little or no effect on organic tissue (Demiray *et al*., 2016; Vora *et al.,* 2017; Dechichi & Moura, 2006; Pérez-Heredia *et al.,* 2006; Hariharan *et al.,* 2010) . Another agent that has found use in primary tooth root canal treatments is chlorhexidine material, which has high antibacterial and antifungal effects, is biocompatible, and has a less caustic effect compared to sodium hypochlorite. However, its use as the main irrigation agent is limited in primary teeth due to its insufficient activity in dissolving organic tissue and its low effectiveness on gram (-) microorganisms (Zehnder, 2006; Haapasalo *et al.*, 2005). Different irrigation agents such as MTAD (Mixture of doxycycline, citric acid, and detergent), tetraclean, carisolv, electrochemically activated agents, ozonated water, and herbal agents are also agents studied for use in deciduous tooth root canal treatment (Vora *et al.,* 2017). After the irrigation process, the root canals are dried with the help of sterile paper cones and the filling process is started (Moskovitz & Tickotsky, 2016).

FILLING ROOT CANALS IN PRIMARY TEETH

Filling the root canals completely with a high-coverage canal filling paste in the corona-apical direction is the last step in completing the root canal treatment in primary teeth. (Ölmez *et al.,*1996; Reddy & Ramakrishna, 2007). Filling materials applied to permanent teeth are different from the filling materials used in primary teeth. The ideal canal filling material; should not damage all of the periapical tissues and the successor permanent tooth germ, should follow the physiological root resorption with the primary tooth root and be resorbed at the same rate and rate as the root, should be resorbed quickly and easily when it overflows into the periapical tissues, should be easy to apply to the root canals and remove from the canals when necessary, should have good adaptation and adhesion to the walls, should not shrink or show minimum shrinkage when hardening, be radiopaque so that the quality of canal filling can be evaluated, have strong antiseptic and antimicrobial properties, be tissue-friendly, should not be discolored after

treatment, not be affected by tissue fluids, and should not dissolve easily from canals. should not completely harden and block the eruption path of successive permanent teeth (Moskovitz & Tickotsky, 2016; Ölmez *et al.,*1996; Kınoğlu, 1964).

Canal sealers for root canal filling; can be applied to the canal with different techniques such as injection technique with pressure syringe, incremental technique, and lentil spiral technique. (Aminabadi *et al.,* 2020; Bawazir *et al.,* 2007). After the root canals are hermetically filled, the excess paste residues in the pulp chamber are cleaned using an excavator or moist cotton pellets. Afterward, to complete the treatment, it is necessary to restore the relevant tooth with appropriate materials and perform a radiographic control to evaluate the treatment (Moskovitz & Tickotsky, 2016; Alaçam, 2000).

CANAL FILLING MATERIALS USED IN PRIMARY ROOT CANAL TREATMENT

The commonly used pastes in root canal treatments of primary teeth are pastes containing zinc oxide eugenol (ZOE), calcium hydroxide ($Ca(OH)_2$), and iodoform or combinations of these materials (Ölmez *et al.,*1996).

Zinc oxide eugenol (ZOE), which was first used as a primary tooth root canal filling paste by Sweet (Sweet, 1930) in 1930, ZOE can be used alone or combined with some fixative agents such as paraformaldehyde, formocresol, formaldehyde, camphor, monochlorophenol, and menthol (Holan & Fuks, 1993; Vakil *et al.,*2019). The negative features of zinc oxide eugenol are that it is a slow-resorbing material that does not choke with physiological root resorption, and that it causes a foreign body reaction after overflowing into the periapical tissues (Holan & Fuks, 1993; Mass & Zilberman, 1989; Bramante & Berbert, 1987; Meryon *et al.,* 1988).

Iodoform (triiodomethane) is an organoiodine compound with a lemon-yellow color, consisting of hexagonal bright crystals and a sharp odor (Estrela *et al.,* 2006). KRI paste is the most widely used iodoform root canal sealer and contains 4.86% camphor, 1.215 % menthol, 2.025 % para chlorophenol (PCP), and 80.8% iodoform. Iodoform paste, which is frequently used for root canal filling in primary teeth due to its antiseptic feature, is resorbed in harmony with the roots of primary teeth. In addition, it does not harm the successive permanent tooth germ (Holan & Fuks, 1993). In studies, clinical success rates after treatment with CRI paste were found to be 84% to 100% (Holan & Fuks, 1993).

Maisto paste; It is a lice paste containing 42 grams of iodoform, 14 grams of zinc oxide, 42 grams of thymol, 3 cc of chlorophenol camphor,

and 0.5 grams of lanolin, designed to prevent the resorption of KRI paste in the root canals (Mass & Zilberman, 1989). It has been stated that Maisto paste is effective in providing bone regeneration, supports the healing of pathologies occurring in the furcation region. In addition, the paste has a strong antimicrobial effect (Reddy, 1996).

Endoflas with powder containing tri-iodomethane and iodine dibutylorthocresol (40.6%), calcium hydroxide (1.07%), zinc oxide (56.5%), barium sulfate (1.63%); Eugenol is obtained by mixing the liquid consisting of paramonochlorophenol materials. While Endoflas can be resorbed if it overflows into the periapical tissues; With its zinc oxide eugenol content, it can remain without resorption in the root canals (Moskovitz *et al.,* 2005; Pandranki *et al.,* 2017). With its hydrophilic feature, Endoflas can be applied to moist root canals. However, with its broad antimicrobial spectrum, the paste can disinfect the deep surfaces of the dentinal tubules and hard-to-reach areas that are mechanically prepared. With its successful adaptation to the root canal walls, it forms a good seal in the canals and has high biocompatibility (Fuks *et al.,* 2003).

Calcium hydroxide material ($Ca(OH)_2$) is an odorless white powder. The solubility of this material, which has a relatively low water solubility, declines as the temperature increases. It is insoluble in alcohol and has a high pH (12.5-12.8) (Farhad & Mohammadi, 2005). The paste has a bactericidal or bacteriostatic effect depending on the pH concentration. In addition, it is a biocompatible material that can be easily resorbed from the tissue after overflow to the periapical tissues, does not harm the successive permanent tooth germ, and provides remineralization. (Reddy *et al.,* 2020; Foreman & Barnes, 1990). $Ca(OH)_2$, which has been used as a canal filling paste in the endodontic treatment of primary teeth with its low toxicity, healing of periapical lesions, resorption, and antibacterial properties in recent years, has provided successful endodontic treatments (Chawla *et al.,* 1998; Hendry *et al.,* 1982). The major disadvantage of the material is that, despite its antiseptic and osteoconductive properties, it tends to be resorbed from the canals before physiological root resorption (Rajsheker *et al.,* 2018).

Calcium hydroxide paste with iodoform, which was created by combining calcium hydroxide and iodoform was first produced and used in Japan as its trade name Vitapex. Vitapex is available as a ready-to-use mixture in syringe form as a viscous mixture of iodoform-calcium hydroxide, containing 30.3% $Ca(OH)_2$, 22.4% silicone oil, and 40.4% iodoform (Nurko & Garcia-Godoy, 1999). The addition of silicone oil to Vitapex increases the fluidity and permeability of the material (Hunter *et al.,* 1998). It has been reported that the material is easy to apply, resorbs slightly faster than the roots that have completed endodontic treatment and does not cause

any harmful effects in case of protrusion of the successive permanent tooth germ into the apical tissues (Nurko & Garcia-Godoy, 1999; Nurko *et al.,* 2000; Nakornchai *et al.,* 2010). It has been stated that the iodoform-calcium hydroxide paste is the paste that most meet the ideal canal filling paste characteristics (Nurko & Garcia-Godoy, 1999). Researchers have reported that iodoform calcium hydroxide paste is resorbed from the canal early, but this does not harm the success of root canal treatment (Nurko *et al.,* 2000; Nakornchai *et al.,* 2010; Ozalp *et al.,* 2005).

EVALUATION OF SUCCESS OF CANAL TREATMENT IN PRIMARY TEETH

Root canal treatment success is evaluated by clinical and radiographic controls. Root canal treatment is considered successful in clinical evaluation if there is no fistula, mobility, sensitivity on percussion, pain, and similar signs of infection, and if the physiological root resorption is maintained in a healthy way (Dummet & Kopel, 2002). There is no consensus among researchers on the issue of radiographic success. Garcia-Godoy considered the treatment successful when the radiolucency resolved and/or the lesion did not progress. Dummett et al. On the other hand, they reported that for the treatment to be considered successful, complete healing of the lesion or reduction in lesion size should be observed (Garcia-Godoy, 1987; Dummet & Kopel, 2002).

In the radiographic evaluation, it is expected that pathological root resorption associated with the bone will not be seen, the lesions in the apical and furcation areas will shrink and disappear over time, and no new lesions will be observed (Moskovitz & Tickotsky, 2016; Alaçam, 2000; Dummet & Kopel, 2002). It should be underlined that the lesions should be evaluated correctly in the follow-up sessions, with the prediction that the lesions observed radiographically will heal within 6 months after the completion of the root canal treatment procedure. Root canal treatments are expected to ensure the survival of primary teeth in the mouth until the time of physiological exfoliation. Annual clinical and radiological follow-ups during the physiological decline are important for the management of the process (Moskovitz & Tickotsky, 2016; Dummet & Kopel, 2002).

CONCLUSION

Root canal treatment applied in primary teeth is a treatment protocol that allows the teeth to be kept in the mouth until the physiological fall. The process steps in root canal treatments of primary teeth are similar to root canal treatments applied to permanent teeth. However; Physiological root resorption observed in primary teeth as well as secondary dentin deposition and changing root canal morphologies lead to some differences in

the way the treatment is applied. Success rates in root canal treatments may vary due to parameters such as the health status of the tooth, its restorability, the patient's dental age, the differences in the preparation and irrigation protocols applied to the canals, the changes in the canal filling materials used and the application methods of the materials, and the differences in the restoration of the related tooth after the root canal treatment. However, high success results are achieved by performing the treatment under appropriate indications and conditions, making it a preferred treatment option in pediatric dentistry.

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CHAPTER 8

EFFECTS OF MELATONIN ON CANCER-RELATED SIGNALLING PATHWAYS

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1. Melatonin as an anticarcinogen

The prevalence and fatality of cancer, a foremost contributor to global mortality, are experiencing a precipitous escalation. In the year 2020, a staggering 19.3 million novel instances of cancer emerged, concomitant with an approximate 10 million demises were attributed to the malignancy (Sung et al., 2020). Cancer treatment typically involves a multifaceted approach, commonly incorporating surgical procedures alongside chemotherapy and/or radiation therapy. However, low survival rate and poor prognosis do not fully provide the expected benefit from these treatments. Prevention of cancer and the development of treatment methods are the top priority to protect human health from this serious threat and to improve patients struggling with cancer (Wang, Wang & Choi, 2022).

Melatonin, a hormone found in nature that undertakes a central function in overseeing the circadian rhythm of sleep and wakefulness, has captured the interest of researchers owing to its observed anti-cancer properties. A cohort of researchers, dedicated to enhancing the effectiveness of prevailing cancer interventions while concurrently pioneering novel therapeutic modalities, manifests a keen interest in the potential of melatonin. Melatonin, scientifically termed N-acetyl-5-methoxy tryptamine, stands as an exceedingly multifaceted and adaptable molecular entity (Yılmaz et al., 2020). Originally presumed to be exclusive to the pineal gland, contemporary understanding recognizes its ubiquitous synthesis across numerous organs and potentially within the mitochondria of every cell. Melatonin transcends species boundaries, being present not solely in vertebrates but also in invertebrates and the botanical realm. Acknowledged as a 'smart' molecule, its commendable capacity for modulating molecular physiology at the cellular level is well-documented (Mir et al., 2022; Sagrillo-Fagundes, Bienvenue-Pariseault & Vaillancourt, 2019; Yılmaz et al., 2021). In this review, we tried to summarise the effect of melatonin on different cancer-related signalling pathways.

2. PI3K/Akt/mTOR signaling pathway

The intracellular signaling pathways, Phosphatidylinositol 3-kinase (PI3K)-Akt and mammalian target of rapamycin (mTOR), constitute pivotal elements governing diverse facets of cellular functions. These functions are integral to both normal physiological states and the progression of various pathological disorders, notably impacting processes such as cell survival and growth, with particular relevance to conditions like cancer. The closely interconnection between these two pathways is so pronounced that, in certain instances, they are regarded as an indivisible entity essential for orchestrating the regulatory dynamics of the cell cycle (Tewari, Patni, Bishayee, Sah & Bishayee, 2022).

The central function of maintaining cell survival during challenging circumstances is attributed to the PI3K-Akt pathway. Anomalies in the initiation of this pathway have been linked to diverse forms of human malignancies, emphasizing its critical significance in exploring compounds with anti-tumor attributes (Porta and Figlin, 2009). Given the indispensable contribution of the PI3K-Akt-mTOR pathways to both normal cellular physiology and pathological transformations, a heightened frequency of mutations within this pathway is prevalent in various cancer types (Bartholomeusz and Gonzalez-Angulo, 2012).

The process commences with the activation of PI3K, initiating the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2). This, in turn, gives rise to the creation of phosphatidylinositol 3,4,5-trisphosphate (PIP3). This event triggers downstream signaling cascades. PIP3 orchestrates the recruitment of Akt to the cellular membrane, where it undergoes activation through phosphorylation. Upon activation, Akt assumes a critical function in fostering cellular survival, impeding programmed cell death, and overseeing the progression of the cell cycle. Subsequent Akt activation induces the activation of mTOR, a pivotal regulator of cellular growth and protein synthesis. mTOR manifests in two distinct complexes, mTORC1 and mTORC2. While mTORC1 facilitates protein synthesis and cellular growth, mTORC2 regulates Akt and other signaling molecules. Irregularities in the activation of the PI3K/Akt/mTOR pathway are a common occurrence in diverse types of cancer. Genetic alterations, such as mutations or amplifications in genes encoding pathway proteins like PIK3CA (PI3K), PTEN (phosphatase and tensin homolog), and Akt, can precipitate hyperactivation of the pathway (Tewari et al., 2022; Chen et al., 2021) (Figure 1).

Anomalous activation of the PI3K-Akt-mTOR signaling pathway has been consistently documented across diverse malignant tumors (Aziz, Farid, Qin, Wang & Liu, 2018). In their 2021 research, Chen and colleagues delved into the influence of melatonin on gallbladder cancer, examining its anti-tumor properties through a blend of laboratory experiments and studies on living organisms. The results indicated that melatonin demonstrates restraining impacts on the proliferation, movement, and infiltration of gallbladder cancer cells by suppressing the phosphorylation of PI3K, Akt, and mTOR (Chen et al., 2021). Dysregulated PI3K mutations have been observed in diverse cancer types, encompassing renal cell, bladder and breast cancers. Akt and mTOR, positioned as downstream targets of PI3K, are frequently implicated in the uncontrolled proliferation of tumor cells when abnormally activated (Guo et al., 2015; Ellis and Ma, 2019).

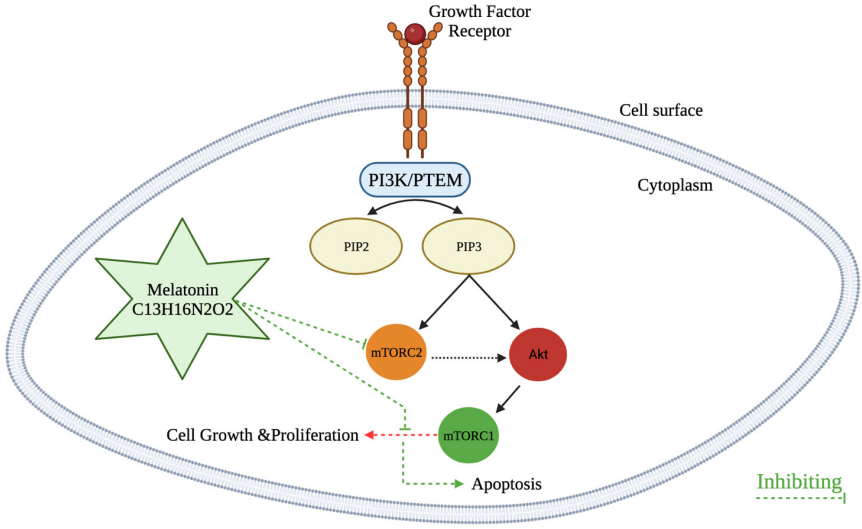

Figure 1: *Representation of PI3K/Akt/mTOR signaling pathway in cancer cell. Figure was created from free trial version Biorender (Biorender.com).*

3. Wnt/β-katenin Signaling Pathway

This pathway constitutes an intricate cascade of protein regulatory networks intricately linked to diverse biological phenomena, encompassing embryonic development, tissue formation and regeneration, as well as the initiation and advancement of cancer. Deviations from normalcy in Wnt signaling activation can instigate various pathological processes, including but not limited to cancer, inflammatory and immune disorders, and metabolic irregularities (Nusse and Clevers, 2017; Zhang and Yu, 2023). Wnt signaling is triggered by the interaction of Wnt family ligands with specific membrane receptors. These receptors comprise the Frizzled (FZD) family, low-density lipoprotein receptor-related protein 5/6 (LRP5/6), and receptor tyrosine kinase-like orphan receptors (ROR1/2). Following the initiation of Wnt signaling, a series of transcriptional events is initiated, engaging numerous downstream effectors. Incoming signals from the external environment initiate communication with the interior of the cell by activating the intracellular regions of receptors located on the cell membrane. Subsequently, this activation sets off Wnt signaling cascades, whether dependent on β-catenin or independent of it (Clevers and Nusse, 2012).

Different Wnt pathways, particularly the canonical (β-catenin-dependent) and non-canonical signaling pathways (Wnt/Ca2+ [calcium] and Wnt/PCP [planar cell polarity]), display unique characteristics and the possibility of mutual interference. This intricacy highlights the complex

regulatory networks inherent in Wnt signaling, as extensively discussed by Clevers and Nusse (2012), Nusse and Clevers (2017), and Zhang and Yu (2023).

The Wnt/β-catenin pathway is defined by the interaction of Wnt with the core receptor complex, comprising either LRP5 or LRP6 and ten members from the FZD protein family (Shi, Li, Luo, Wei & Liu, 2017). Under typical circumstances without the presence of Wnt ligands, the cytoplasmic β-catenin experiences phosphorylation orchestrated by a molecular complex that includes glycogen synthase kinase 3β (GSK3β), casein kinase I (CK I), Axin, and adenomatous polyposis (APC). Axin assumes pivotal function in promoting the assembly of a complex with GSK3β and APC. In this intricate association, GSK3β facilitates the cytoplasmic phosphorylation of β-catenin, while APC coordinates the linking of phosphorylated β-catenin with the ubiquitin-mediated proteolytic pathway in the cellular cytoplasm (Nusse and Clevers, 2017). When Wnt protein ligands interact, their connection with the central receptor complex initiates the onset of Wnt signaling. This induction entails the enlistment of cytosolic Dishevelled (Dvl) protein, which subsequently impedes the formation of the Axin/GSK3/APC complex. Consequently, the breakdown of β-catenin is impeded, causing the accumulation of β-catenin within the cytoplasm. Accumulated cytoplasmic β-catenin then migrates to the nucleus, forming a collaborative bond with the transcription factor T cell factor/lymph enhancer factor 1 (TCF/LEF1). This synergistic interaction serves as the impetus for triggering Wnt target genes (Bugter, Fenderico & Maurice, 2021; Disoma, Zhou, Li, Peng & Xia, 2022) (Figure 2).

The Wnt/β-catenin pathway assumes a critical role in overseeing fundamental cellular operations, dictating cell fate, growth, sustainability, and differentiation. It intricately oversees embryonic growth, cellular proliferation, differentiation, apoptosis, and the advancement of inflammation-associated cancers, as extensively elucidated by Tewari et al. (Tewari, Bawari, Sharma, DeLiberto & Bishayee, 2021). Functioning as the primary organizer pathway, Wnt/β-catenin signaling intricately engages with various signaling cascades. In the context of breast tumor and intestinal tumor, a synergistic partnership arises between Wnt/β-catenin and TGFβ at the transcriptional grade, promoting the occurrence of epithelial-mesenchymal transition (EMT) and fibrosis. Similarly, during both developmental processes and tumorigenesis, Wnt/β-catenin engages in synergistic interactions with the Notch pathway, as detailed by Kwon et al. (Kwon et al., 2011). The Wnt/β-catenin pathway exercises precise regulation over the division of cells in the intestinal crypt, along with ensuring the survival and upkeep of the stem cell environment. Primarily, mutations that activate the Wnt/β-catenin pathway are implicated in the origin of the majority of col-

orectal cancers. Boosting Wnt signaling, enabled by coiled-coil receptors and LRP5/LRP6 co-receptors, results in heightened nuclear β-catenin concentrations by modulating the function of GSK3β, as elucidated by Raisch et al. (Raisch, Côté-Biron, Langlois, Leblanc & Rivard, 2021).

Sokolov et al. (2022) observed that the simultaneous application of melatonin and andrographolide demonstrated inhibitory impacts on colospheroids (CSC), which are spheroids originating from colon cancer cells, thereby impeding the proliferation of cancer cells. Their observations revealed that the melatonin+andrographolide treatment induced apoptosis and impeded the growth of CSCs by down-regulating Wnt/β-catenin signaling pathways (Sokolov et al., 2022). At the another study, the effect of melatonin on the resistance of nasopharyngeal carcinoma cell lines to cisplatin and the possible mechanisms driving chemoresistance were investigated both in the laboratory and in living organisms. The study showed that the Wnt/β-catenin signaling pathway mediated cisplatin chemoresistance in nasopharyngeal carcinoma cells. Melatonin was discovered to not only overturn cisplatin resistance but also boost cisplatin's effectiveness against tumors by inhibiting the movement of β-catenin into the cell nucleus and diminishing the expression of genes responsive to Wnt/β-catenin signaling in nasopharyngeal carcinoma cells. In live subjects, the concurrent application of cisplatin and melatonin led to a more pronounced reduction in tumor load in a model involving mice with orthotopically implanted xenografts, surpassing the outcomes achieved with individual drug administrations (Zhang et al., 2020). Li et al. stated that the overexpression of long non-coding RNA (lncRNA) JPX in osteosarcoma (OS) cell lines promoted cell viability, proliferation, and metastasis. Furthermore, they found that melatonin impeded OS progression by regulating the Wnt/β-catenin pathway, specifically by suppressing the expression of lncRNA JPX (Li, Zou & Li, 2021).

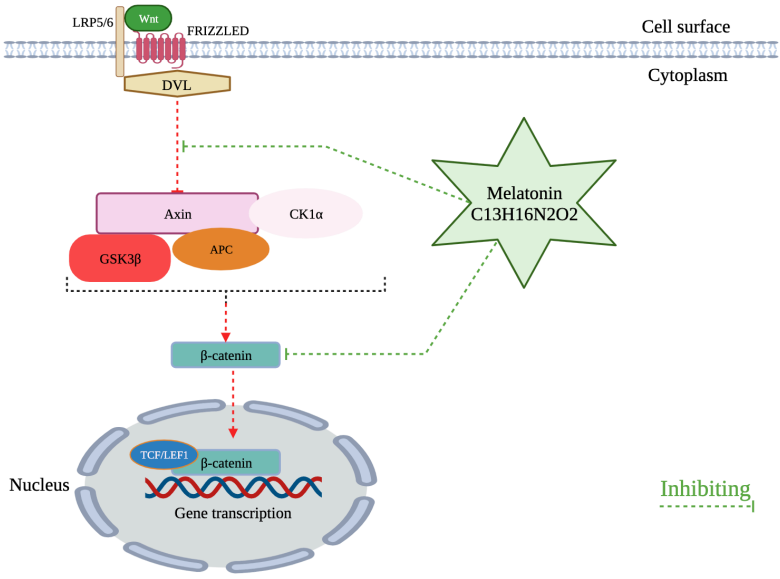

Figure 2: *Representation of Wnt/β-katenin Signaling Pathway in cancer cell. Figure was created from free trial version Biorender (Biorender.com).*

4. JAK-STAT signaling pathway

The communication route of Janus kinase (JAK) and signal transducer and activator of transcription (STAT) represents a broadly conserved sequence crucial for a variety of physiological mechanisms. These include the creation of blood cells, cellular differentiation, metabolic functions, and the management of the immune system. Regarding its composition, the JAK-STAT pathway comprises receptors situated on the cellular membrane, cytoplasmic tyrosine kinases linked to these receptors (JAKs), and signal transducers along with activators of transcription (STATs) (Barrat, Crow & Ivashkiv, 2019; Xue et al., 2023). The JAK protein family is composed of four unique members: JAK1, JAK2, JAK3, and TYK2. Similarly, the STAT family consists of seven proteins: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 (Meraz et al., 1996).

The JAK-STAT pathway acts as a signaling cascade initiated by cytokine activation, enabling the transmission of external signals from the cellular membrane to the nucleus, thereby prompting changes in DNA transcription (O'Shea et al., 2015). Within this pathway, cytokines initially bind to receptors, leading to receptor dimerization. Following this, Janus kinases (JAKs) experience reciprocal phosphorylation, subsequently leading to the phosphorylation of the receptor by JAK, resulting in the formation of phosphotyrosine binding sites for the SH2 domain of STAT. When connected to the receptor, JAK triggers the phosphorylation of STAT, caus-

ing a structural alteration in STAT that induces its liberation. The phosphorylated STAT separates from the receptor, creates dimers, and moves into the cell nucleus. Once situated within the nucleus, the phosphorylated STAT attaches to DNA, ultimately initiating alterations in the transcription of genetic material (Bose et al., 2020) (Figure 3).

The JAK-STAT signaling pathway oversees numerous cellular functions, encompassing cellular expansion, mobility, differentiation, and programmed cell demise. Its critical regulatory role in immune processes has been emphasized in research conducted by Fahmideh et al. and Banerjee et al. (Fahmideh et al.,2022; Banerjee, Biehl, Gadina, Hasni & Schwartz, 2017). Current discoveries propose a dual function for the activation of JAK-STAT in illnesses. The excessive activation of the JAK-STAT pathway has been associated with unfavorable consequences in diverse health conditions, such as melanomas, glioblastomas, and malignancies impacting the head, neck, lungs, pancreas, breasts, rectum, and prostate. On the flip side, the regulatory functions of the JAK-STAT pathway have exhibited positive results in scenarios like head and neck squamous cell carcinomas, along with prostate and colorectal cancers, as supported by evidence (Thomas, Snowden, Zeidler & Danson, 2015; Baratchian et al., 2022). In the realm of oncology research, JAK/STAT is gaining prominence, with an increasing body of evidence indicating that STAT3 is constitutively activated in tumors, playing a pivotal role in cellular carcinogenesis (Teng, Ross & Cowell, 2014; Jin, 2020).

Recent studies, particularly the work by Mihanfar et al. (2022), suggest a significant interaction between melatonin and the JAK/STAT signaling pathway, particularly in the context of immune cells, notably macrophages (Mihanfar, Yousefi, Azizzadeh & Majidinia, 2022). Melatonin has demonstrated the ability to influence signaling pathways such as JAK/STAT and NF-κB in macrophages. Therefore, it can be deduced that melatonin assumes a regulatory function in the progression of diverse diseases linked to macrophages, including cancer, as suggested (Xia et al., 2019). Furthermore, melatonin exhibits a protective effect against angiotensin II-related damage and apoptosis of podocytes in diabetic nephropathy by inhibiting the JAK/STAT signaling pathway (Ji and Xu, 2016).

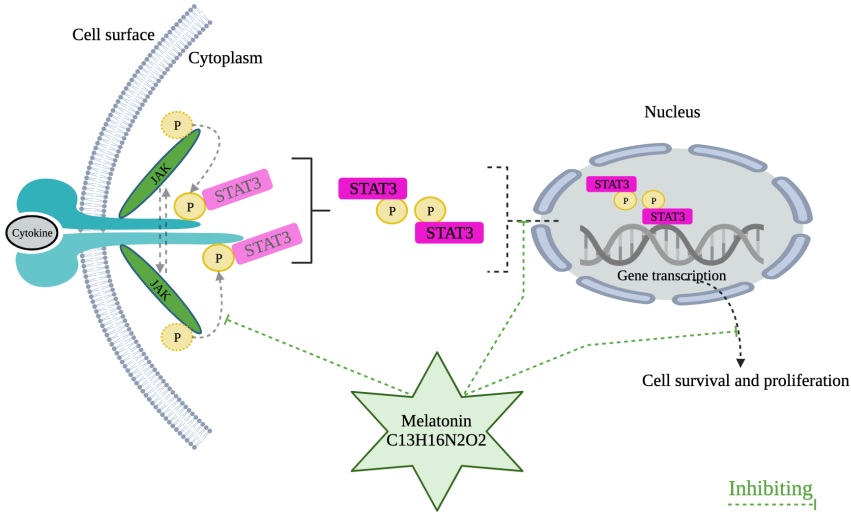

Figure 3: *Representation of JAK/STAT signaling pathway in cancer cell. Figure was created from free trial version Biorender (Biorender.com).*

5. MAPK/ERK signaling pathway

The mitogen-activated protein kinase (MAPK) cascade stands as a pivotal pathway influencing the survival, invasion, and resistance to drug therapy in human cancer cells. Consisting of four separate signaling families, specifically the MAPK/ERK family or classical pathway, Big MAP kinase-1 (BMK-1), c-Jun N-terminal kinase (JNK), and p38 signaling pathways, this complex network holds a pivotal function in cellular reactions (Cossa et al., 2013; Burotto, Chiou, Lee & Kohn, 2014).

The cellular process termed mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) plays a crucial role in supervising a range of cellular functions such as cell multiplication, specialization, movement, aging, and programmed cell demise (Sun et al. ,2015). The canonical MAPK/ERK pathway comprises three types of MAPKKKs, namely A-RAF, B-RAF, and RAF-1 or C-RAF kinases. Noteworthy is the prevalence of mutations in the BRAF gene at this particular level in human cancers. Progressing downstream to the subsequent tier, we find the MAPKKs, which include MEK1 and MEK2. Positioned ultimately at a lower stratum are ERK1 and ERK2, acting as the final executors of the MAPK pathway (Robinson and Cobb 1997; Burotto et al., 2014).

The initiation of the pathway occurs through the activation of cell surface receptors, typically triggered by external signals like growth factors or mitogens. These activated receptors set in motion the activation of RAS

proteins, pivotal GTPases facilitating signal transmission within the cell. The activated RAS, in turn, initiates the activation of RAF (Rapidly Accelerated Fibrosarcoma) kinases, crucial components that propagate the signal downstream (Sigoillot, Evans & Guy, 2002). RAF kinases activate MEK (Mitogen-Activated Protein Kinase Kinase) through phosphorylation. Following this, MEK initiates phosphorylation, activating ERK. The activated ERK then moves into the cell nucleus, where it initiates phosphorylation of diverse transcription factors. This nuclear translocation exerts influence on gene expression, thereby regulating cellular responses. ERK that is in an active state within the nucleus triggers the phosphorylation and activation of numerous transcription factors. This includes carbamoyl phosphate synthetase II (CPS II), which attaches to DNA, or p90RSK, stimulating the advancement of the cell cycle (Sigoillot et al., 2002; Zassadowski, Rochette-Egly, Chomienne & Cassinat, 2012) (Figure 4). The progression of these consecutive occurrences collectively adds to the stimulation of cell proliferation by MEK/ERK. In immune cells, the activated state of ERK contributes to the innate response across various phases of the inflammatory cascade, amplifying the manifestation of tumor necrosis factor alpha (TNF-α) and inducible nitric oxide synthase (iNOS) (Arthur and Ley, 2013).

Phosphorylated transcription factors within the nucleus exert a regulatory influence on the expression of specific genes, which, in turn, participate in the modulation of cell cycle progression, cell proliferation, survival, and various cellular responses. A predominant consequence of the activated MAPK/ERK pathway in cancer is the facilitation of cell proliferation. Unrestrained cell growth, a hallmark of cancer, is significantly influenced by this pathway, contributing prominently to this pathological process. Concurrently, the pathway plays a pivotal role in promoting angiogenesis, the formation of novel blood vessels. This mechanism is indispensable for supplying nutrients and oxygen to the expanding tumor. Furthermore, the initiation of the MAPK/ERK pathway is intricately connected to increased invasiveness and the metastatic capability of cancer cells (Asl et al., 2021; Czyz, 2019).

A thorough grasp of these phases is essential for the formulation of targeted treatments designed to regulate the MAPK/ERK pathway in the context of cancer. Ongoing research actively investigates and utilizes inhibitors targeting various components of this pathway as a strategy in cancer treatment to disrupt aberrant signaling and impede tumor growth. Due to its intimate connection with cellular growth and viability, the disruption of the MAPK/ERK pathway is a widespread feature in numerous types of cancers. Melatonin has exhibited promising anticancer properties, partially attributed to its regulatory impact on the MAPK/ERK pathway.

Melatonin holds the capacity to prompt a halt in the cell cycle and trigger programmed cell death in cancerous cells by obstructing the ERK pathway. Consequently, melatonin emerges as an intriguing candidate for adjuvant cancer therapy, as supported by the studies conducted by researchers (Pan and Niles, 2015; Mayo et al., 2017; Nikolaev, Robeva & Konakchieva, 2021).

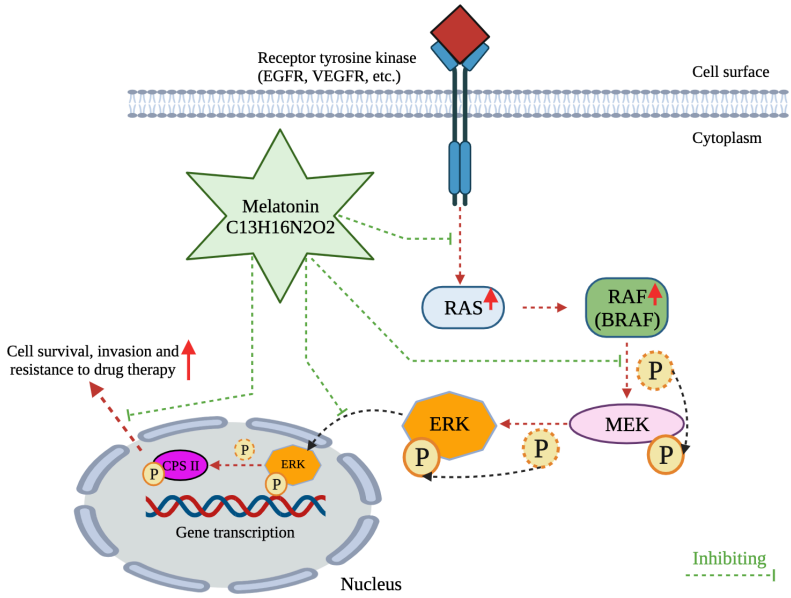

Figure 4: *Representation of MAPK/ERK signaling pathway in cancer cell. Figure was created from free trial version Biorender (Biorender.com).*

Conclusion

While there is encouraging research indicating the potential anti-cancer properties of melatonin, it is essential to emphasize that the clinical implementation of melatonin in cancer treatment remains a subject of continuous investigation and study. Melatonin supplements are sometimes used as complementary therapies alongside conventional cancer treatments, but their efficacy and safety in specific cancer types and at various stages of cancer are still under investigation. In this review, we investigated the anticancer properties of melatonin through different signalling pathways. We hope that this review will make important contributions to the literature.

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CHAPTER 9

REFLECTIONS OF SENESCENCE AS AN INEVITABLE PROCESS ON PHYSIOLOGICAL SYSTEMS

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Introduction

As in every living thing, after the maturation process is completed in humans, this process in which physiological reserve capacities and cellular regeneration rate gradually decrease and life functions are disrupted accordingly is referred to as old age. It is an inevitable fact that changes in physiological systems occur with aging. However, because chronological age and physiological age can differ, age-related changes don't always occur in the same ways in different people. Endogenous or exogenous factors are effective in the formation of this difference. These factors include genetic tendency, hereditary diseases, nutritional disorders, exposure to toxic and harmful substances and sedentary lifestyle. Considering the factors listed above, the aging process is a complex set of events involving biological, molecular, cellular, genetic and physiological changes. In this section, we will focus on the changes that occur in physiological systems during the aging process.

1. Senescence and the cardiovascular system

With ageing weakening of the immune system, increased inflammation, increased susceptibility to infectious diseases, oxidative stress, mitochondrial dysfunction, dysregulated autophagy and energy deficiency can lead to cardiovascular system dysfunctions. Cardiovascular disorders, whose prevalence increases with aging, are one among the primary causes of morbidity and mortality around the world (1,2). These comprise valvular heart disease, myocardial infarction, atherosclerosis, coronary artery stenosis, thoracic aortic aneurysm, and heart failure.

Hematopoietic stem cells are the source of immune system-dominant cells like T, B, dendritic, and macrophage cells. However, it is an undeniable fact that loss of regenerative potential and decrease in proliferative capacity occur in these stem cells with aging. In addition, it is stated that a rise in the amount of adipocytes occurs with aging and this increase may be accompanying with a raise in proinflammatory cytokine levels (TNF-α, IL-6, IL-1) (3,4). In study involving participants aged 86-94 years, this was discovered that their levels of pro-inflammatory cytokines IL-6 and soluble intercellular adhesion molecule-1 increased, while IL-10 levels did not change. It has been suggested that this change in cytokine profile with aging may negatively affect the immune system and cause deterioration of health (5). It has been claimed that these cytokines, which are effective in the initiation of inflammation, may cause an increase in platelet production and this increase may contribute to thrombosis. (6). In addition, it is known that the change in antioxidant capacity in endothelial cells with aging and susceptibility to oxidative stress, increased collagen accumulation, fibrosis and functional changes trigger the development and progression of car-

diovascular diseases (7). Endothelial cell senescence causes a rise in the production of CD44, one of the cell surface glycoproteins, and accordingly monocytes exhibit increased endothelial cell adhesion and may cause vascular inflammation and atherosclerosis formation and/or progression (8,9). In short, although atherosclerosis is considered to be a disease of aging associated with increasing age, it is actually a pathological situation that occurs in both organismal aging and cellular aging. It has been reported that cell senescence markers, which are characteristics of cell senescence, increased tendency to cell death and DNA injury, as well as excessive telomere shortening and dysfunction were identified in the cells of atherosclerotic plaques (10). It has been stated that Klotho gene, which is known as an aging suppressor gene, increases klotho protein expression when overexpressed, thus prolonging life span and accelerating aging-like phenotypes when disrupted in mice. Additionally, it was determined that increasing the expression of this anti-aging protein shows vascular protective effects and may be an important step for new treatment protocol (11,12). In another study, that was suggested that apoptosis and aging in vascular endothelial cells have a strong connection to the development of atherosclerosis and that Klotho protein may be a humoral factor that decreases oxidative stress-induced apoptosis and cell senescence in blood vessel cells (13). The primary fibrinolytic system inhibitor, plasminogen activator inhibitor-1 (PAI-1), induces a hypofibrinolytic, prothrombotic state which can contribute to the emergence of cardiovascular disorders. PAI-1 has also been found to be overexpressed in various cell types related to plaques of atherosclerosis in human coronary vessels, suggesting that this biochemical marker, which is also effective in the aging process, mediates pathways that are effective in both cellular aging and organismal aging (14,15). Morphological and molecular changes and cell death are expected to be observed in atherosclerosis and apoptosis is the most emphasised and investigated pathway in this process. In advanced stages of atherosclerosis, it was recently defined that endoplasmic reticulum (ER) stress occurs in atherosclerotic plaques and apoptosis is induced accordingly. As a result of defects in ER function, it has been demonstrated that ER-resident proteins - inositol requiring protein-1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor-6 (ATF6) - cause ER stress. Activation of these proteins initially sets off a sequence of corrective actions. However, when the increased function exceeds the level to compensate for the impairments, these proteins have been reported to trigger apoptosis through C/EBP-homologous protein (CHOP) or c-Jun N-terminal kinase (JNK). ER stress is effective in activating IRE1 by auto-phosphorylation, IRE1 is involved in the activation of JNK, JNK stimulates the expression of the proapoptotic protein BIM and deactivates the antiapoptotic protein BCL2 (16,17).

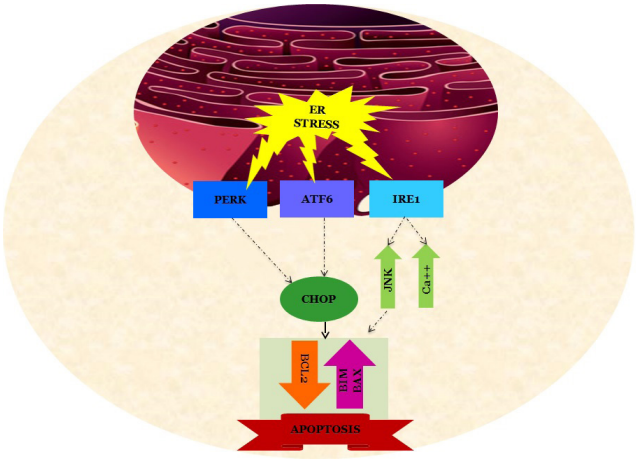

Figure 1. *IRE1/PERK/ATF6 pathway in endoplasmic reticulum stress*

According to a different study, a number of markers, including homocysteine, oxidized low density lipoprotein, reactive oxygen species, angiotensin II, tumour necrosis factor-α, and endothelial cell apoptosis, induce endothelial cell apoptosis. It is important to consider this early event in the pathology of atherosclerosis when creating new treatment methods (18).

Only taking endothelial cell development into account when discussing atherosclerosis development would be incorrect. Macrophages and vascular smooth muscle cells (VMSCs) play a critical role in the formation of atherosclerosis, in addition to endothelial cells. Damage to endothelial cells leads to the accumulation of low-density lipoproteins (LDL) in the blood vessel wall, which are then transformed into ox-LDL. Subsequently, monocytes attach themselves to the injured endothelial cells, penetrate the subendothelial intima, and mature into macrophages. Macrophages phagocytise ox-LDL and transform into foam cells, leading to the formation of early atherosclerotic lesions. Additionally, inflammatory markers such as cytokines are produced in excess by impaired and foam cells, contributing to inflammation. These markers cause VMSCs to proliferate from the media into the intima and surround the plaque, stabilising it and giving it a fibrous structure. Cell death, the rupture of atherosclerotic plaques, and thrombosis are caused by progressive inflammation and a hyperinflammatory response (18,19,20). Thrombosis formation is frequently observed with aging due to changes in both haemostatic system and vascular structure as mentioned above. With advancing age, the so-called Virchow triad of 1. intravascular vessel wall damage, 2. flow arrest, 3. hypercoagulability is frequently encountered and as a result, inevitable thrombosis situation occurs (21,22). Considering the changes in the haemostatic system, it has been stated that the levels of coagulation factors FI, VII, VIII:C,

X, HMW-kininogen and prekallikrein increase and antithrombin III levels decrease with ageing, which will lead to increased fibrin formation and/or delayed fibrinolysis in the elderly. This situation is known to be an important mechanism responsible for the increased risk of thromboembolic disorders in the elderly (23,24). Another study found that when elderly patients were compared to younger patients following heart surgery, there was an increase in fibrinolysis and platelet activation; in other words, the elderly patients' haemostatic system was more activated (25).

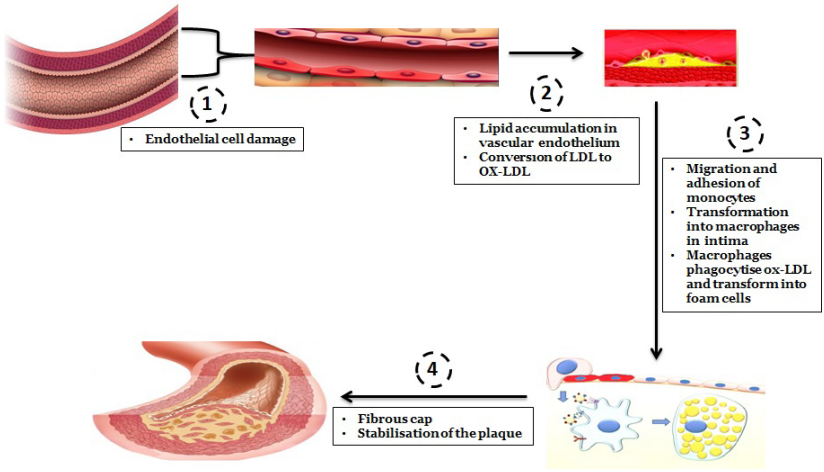

Figure 2. *From endothelial damage - to the development of atherosclerosis*

Another significant event in the aging process is the shortening of telomeres at the ends of eukaryotic chromosomes, which protect chromosomes of end-to-end joining and random double-stranded DNA breaks. Telomere length in body cells provides information about replication history, and shortening of telomeres in cells causes chromosomal end-to-end fusions, DNA damage, replicative senescence and apoptosis (26,27). There have been claims that telomere shortening and endothelial cell dysfunction are closely related, and that telomere shortening-induced replicative ageing contributes to the formation of atherosclerosis plaques in vessels (28). Minamino et al. (29) found that vascular endothelial cells with aging-related phenotypes were present in human atherosclerotic lesions and concluded that endothelial cell senescence induced by telomere shortening may play an important role in the pathophysiology of atherosclerosis. In another research, it was reported that telomerase expression, which is effective in preserving telomere length after myocardial infarction, increased in cardiomyocytes and endothelial cells and may play a role in mechanisms regulating tissue repair after myocardial infarction (30). Bhupatiraju et al. (31) compared hypertensive individuals with healthy individuals in the Indian population and found that hypertensive individuals had shorter telomere length.

With aging, not only the scane we call arteriosclerosis occurs, but also very serious conditions affecting the circulatory system such as increased myocardial stiffness, decreased myocardial relaxation, increased left ventricular mass, and hypertension. Cellular senescence is often associated with ageing and cells are metabolically active even as they age. An exemplary case in point is the secretory phenotype associated with ageing (SASP), which is intimately linked to the release of cytokines that are proinflammatory (IL-1α, IL-6, and TNF-α), which are important contributors to inflammation (32,33). With ageing, changes such as endothelial dysfunction, wall thickening, arterial hardening and loss of elasticity occur, due to these changes, vasoconstriction-vasodilatation mechanisms are disrupted and hypertension is triggered. Of course, hypertension is observed in both young and elderly people, and early vascular ageing is mentioned in these people (34). Vascular structures have the flexibility to expand under the influence of blood pressure, but have the tension and resistance to prevent damage to the vessel. The extracellular matrix (ECM) in vascular structures is composed of special proteins such as collagen and elastin, fibronectin and proteoglycans and therefore has both a structural and regulatory role (34,35). Increasing functional and structural changes due to age (changing cytokine levels, inflammation, oxidative stress, SASP factors) lead to changes in the ECM and the ECM is degraded and remodelled. Enzymes such as matrix metalloproteinases, elastases and lysyl oxidases play an active role in this remodelling process (36,37). Changes in the ECM, which is one of the first steps of vasculer reorganisation in hypertensive conditions, are carried out by matrix metalloproteinases, serine and cysteine proteases. Mitogen-activated protein kinases (MAPK) and nuclear factor-κB (NF-κB), which are crucial for the pathophysiology of inflammation, have been found to be the most significant factors in the activation of these proteinases. After the disruption in the ECM, proteins are re-synthesised and new bonds are formed between matrix proteins; moreover, hypertrophy, hyperplasia and calcification processes start in vascular smooth muscle cells and vascular hardening is observed (37).

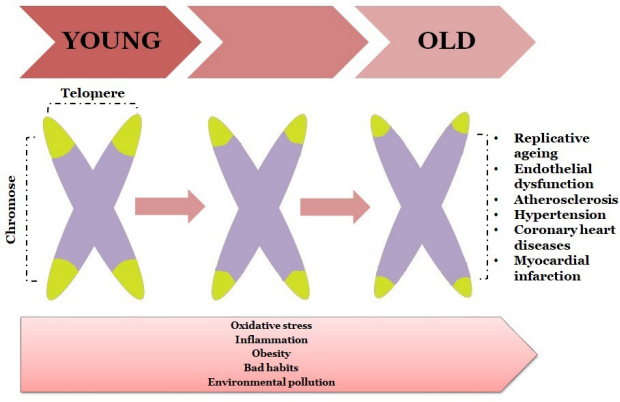

Figure 3. *Factors affecting telomere shortening and effects on cardiovascular system*

Disorders in the cardiovascular system are like links in a chain and trigger each other. One of these disorders is coronary heart disease, and there is a positive relationship between ageing and the increasing prevalence of coronary heart disease. Myocardial infarction (MI) occurs when a thrombus formed by the breakdown of coronary atherosclerotic plaque blocks the coronary artery and blood flow stops (9). In a study, it has been reported that Nrf2 activity decreases after MI and oxidative stress that will cause aging in infarcted heart cells occurs. Nrf2 has been appears to be an important mediator in suppressing factors associated with cellular senescence in infarcted hearts and therefore may be a new therapeutic target (38). Damy et al. (39) found that inhibition of NOS1, one of the NOS isoforms, increased left ventricular dysfunction after MI, morever increased NOS1-induced NO production was a marker that played an active role in the regulation of myocardial contractility after MI. It is stated that one of the factors that are effective in the pathogenesis of MI may be senescent T cells in which both telomere shortening occurs and these cells show highly proinflammatory activity and trigger inflammation by stimulating IFN-γ and TNF-α expression (40,41). In a study investigating the ability of the age of mesenchymal stem cell (MSC) donors to repair aging heart tissue, It has been reported that the ability to repair was influenced by the donors' age and that using young, healthy MSCs, old infarcted myocardium could be repaired (42).

2. Senescence and the respiratory system

Changes occurring in the whole organism with ageing also affect the respiratory system. In number ofthis process, impairments in respiratory mechanics-ventilation, transport of blood gases and gas exchange may cause hypoxia conditions to occur (43). Alder et al. (44) founded after telomere dysfunction in type 2 alveolar epithelial cells, a population containing stem cells, the cells survived but cellular aging was observed. It has been suggested that this dysfunction stimulates the immune system and subsequently triggers inflammation in the lung through cytokines. There is a rise in the frequency of chronic respiratory system diseases due to ageing. One of these diseases is chronic obstructive pulmonary disease (COPD). COPD is a disease characterised by restriction of airflow due to chronic bronchitis and emphysema caused by chronic bronchitis and emphysema caused by excessive mucus production and mucus plugs due to irritation of bronchi and bronchioles by air pollution, smoke, irritant gases, etc (45). It has been reported that the development of COPD does not only occur in response to agents that irritate the respiratory system, but that alpha-1-protease inhibitor deficiency is also an important factor. Deficiency and/or absence of this enzyme has been found to cause the development of severe emphysema (46). Chronic airflow limitation triggers the development

of inflammation and/or abnormal inflammatory response (47). It has been stated that COPD disrupts the balance between oxidants and antioxidants and triggers the formation of oxidative stress, and the increase in oxidative stress causes epithelial damage, mucus hypersecretion, migration of neutrophils into lung tissue and increased expression of proinflammatory markers (48). Although it has been demonstrated that cytokines play an essential part in the regulation of inflammation and many cytokines play an active role in the inflammatory process in COPD, their role in the pathophysiology of COPD has not been fully elucidated (49). In another study, it was found that the percentage of CD8+ and TC2 cells increased in the lungs of patients with COPD, moreover, the number of cells expressing cytokines such as IFN-y, IL-4, TNF-a increased (50). Calabrese et al. (51) found that there was overexpression of IL-32 in the periphery of the lung in surgically resected specimens of smokers with COPD, and that there was a positive correlation between IL-32 level and the presence of TNF-a with a level of obstruction in the airway. Many factors, including protease/antiprotease imbalance, abnormal inflammatory response and impaired oxidant-antioxidant balance, which are effective in the development of COPD, are reported to promote apoptosis (52). Imai et al. (53) found that apoptosis markers such as Bax and Bad and cell proliferation increased in emphysematous lungs. Although both apoptosis and proliferation have been reported to increase in emphysema, it has been shown that there is no balance between them and a decrease in lung surface area occurs. Zhang et al. (54) reported that inhibition of PI3K/AKT/mTOR pathway suppressed autophagy but induced apoptosis in alveolar epithelial cells in COPD. In another study, it was stated that CD8/CD28null T cells increased in both the pulmonary tissue and blood of mouses who breathed smoke from cigarettes, and that the expression of molecules stimulated by these cells could have a role in inflammation and/or autoimmune reactions in COPD (55).

Idiopathic pulmonary fibrosis is one of the chronic lung diseases in which a decrease in lung compliance occurs as a result of altered cellular composition and excessive accumulation of extracellular matrix in the lung and severe loss of lung function is observed (46,56). It has been reported that micro-injuries in lung alveolar epithelia may be the trigger of pathophysiological changes in idiopathic pulmonary fibrosis. As a result of these micro-injuries, disruption and destruction of the alveolar epithelial layer occurs, which then leads to activation of the coagulation cascade and an imbalance between proteases and antiproteases leading to increased accumulation of extracellular matrix (56). Zhang et al. (57) found that IL-18 strongly stimulates lung fibroblast senescence and SASP by blocking the Klotho pathway and IL-18 binding protein can neutralise IL-18 and suppress lung fibroblast senescence and show antifibrotic effect. Therefore,

they suggested that IL-18 may be an important target in new treatment protocols developed for pulmonary fibrosis. Rana et al. (58) found that aging of alveolar type II (ATII) cells has important effects on the pathogenesis of idiopathic pulmonary fibrosis and that these cells stimulate profibrotic gene expression in alveolar macrophages via cytokines such as IL-4 and IL-13. In addition, in this study, it was stated that TGF-β1, a strong profibrotic cytokine, contributed to the development of lung fibrosis by inducing PAI-1 and p16. In another study, it was found that PTEN levels decreased, NF-κB increased, and aging biomarkers such as P21WAF1, P16ink4a and SA-β-gal increased in aging alveolar epithelial cells. It has also been stated that collagen accumulation is facilitated in the fibroblasts of aging cells under the influence of the PTEN/NF-κB pathway, and lung fibrosis develops accordingly (59). Sanders et al. (60) pointed out that cellular aging associated with variable apoptosis sensitivity is effective in the pathogenesis of aging and age-related diseases, and although the exact mechanism has not been elucidated, it has been pointed out that there is a relationship between fibroblast aging and apoptosis resistance. They found that compared to control fibroblasts, aging human diploid fibroblasts expressed more Bcl-2 and less Bax, which increased their ability to resist oxidative stress-related cell death.

Cancer, now recognised as the plague of the age, has become among the most prevalent leads to of death worldwide. The role of lung cancer in these deaths is too high to be ignored. It has been reported that the prevalence of lung cancer increases with age, especially after the age of 60 years (61). It is an inevitable fact that lung cancer, which has a close relationship with age, will continue to be a very common public health problem in the future as the geriatric population increases (62). According to some reports, it can be challenging to diagnose lung cancer early, particularly in older patients who have a history of chronic respiratory conditions. There is also a greater chance of missing symptoms associated with the disease (63). The age of human cells, tissues, and organs has allegedly been measured by biomarkers called “epigenetic clocks” according to DNA methylation (DNAm) levels. It has also been suggested that by measuring the acceleration of epigenetic age, one can determine whether one will be at an elevated risk for getting cancer of the lungs (64,65). It has been reported that tissue damage caused by chronic infection and inflammation in the lungs may be related to the occurrence of lung cancer. It has been suggested that elevated C-reactive protein, one of the markers of inflammation, is connected with increased likelihood of lung cancer, and the chemopreventive feature of chronic NSAID administration is due to COX inhibitor activities (66,67). According to reports, there is a link between COPD

and lung cancer because of their shared etiology, and COPD patients have twice a possibility of developing lung cancer (68,69).

3. Senescence and the nervous system

Since 1961, when the concept of cellular ageing was introduced by Leonard Hayflick and Paul Moorhead, a great deal of research has been carried out and is still being carried out, especially on how to prevent ageing or how to reduce the negative effects of ageing (70). Although advancing age leads to deterioration of many systems and related mechanisms, it perhaps affects the brain and nervous system the most. As a matter of fact, with advancing age, there is an accumulation of cells that have lost their ability to divide, but have not undergone cell death and are called senescent cells (71). Age-related increases in reactive oxygen species generated by neurons and increased blood brain barrier permeabilization result in a decline in cells' defense mechanisms and an increased susceptibility to damage. In addition, it causes a decrease in phagocytic activity in microglia, astrocytes to be more sensitive to reactive oxygen species, increased inflammatory signals, and slowdowns in myelination, deterioration and breakage of myelin sheaths in oligodentrocytes (71,72). Studies have shown that neurogenesis, subventricular zone and dentate gyrus significantly decrease with age in mice (73,74). A study showed that old mice have more olfactory interneurons in total than young mice, however, they do not differ from young mice in their ability to discriminate two different odours. However, it was determined that old mice had significantly poor discrimination capacity in distinguishing similar odours that require fine discrimination (74). In a study conducted on human subjects aged between 14 and 79 years, it was reported that neurogenesis in hippocampal brain tissue continues in the elderly brain, but elderly people have less angiogenesis and neuroplasticity, especially in the anterior dentate gyrus, and have a smaller and immobile neuron pool compared to younger people (75). As a result, although neurogenesis continues in old age, it is known to decrease with age (76). Most DNA polymerase enzymes are incapable of replicating the terminal ends of linear DNA in any way. The telomerase enzyme is the only one that fully possesses this characteristic. Telomerase is not expressed by the majority of mammalian cells, though. As a result, the absence of this telomere-protecting factor leads to telomere shortening and restriction of cell proliferation and ultimately telomere depletion, interrupting the cell cycle and leading to cell senescence (77,78). In biological aging, which is defined as the progression of age, the role of compensatory mechanisms such as oxidative damage and autophagy is very important in addition to telomere shortening. Because mitochondria play a part in the production of reactive oxygen species, researchers have recently directed more of their attention toward autophagy disorders in aging and

mitophagy. Autophagy and mitophagy are involved in the pathophysiology of numerous age-related disorders, including Parkinson's and Alzheimer's disease (79).

In the brain, cells that should normally be subjected to cell death but escape this process and accumulate can often cause chronic inflammation and neurodegenerative diseases like Parkinson's and Alzheimer's (71). With age, the risk of developing a variety of pathological conditions, including neurological disorders, raises significantly. Higher life expectancy as a result of medical advances increases the number of people struggling with geriatric neurodegenerative disorders like Parkinson's and Alzheimer's (80). The World Health Organization (WHO) estimates that over 55 million people worldwide suffer from dementia, with 10 million new people added each year. While there are many conditions that cause dementia, Alzheimer's disease accounts for 60 to 70 per cent of all cases (81). There are also types of dementia such as Parkinson's disease, Vascular dementia, Lewy body dementia, Frontotemporal dementia, Huntington's disease, Creutzfeldt-Jakob disease, Hydrocephalus, posterior cortical atrophy, Korsakoff syndrome and Mixed dementia (82). It should not be forgotten that dementia is the world's seventh leading reason for passing away (81).

The 51-year-old patient named Auguste D, who was examined by the famous scientist Alois Alzheimer in 1901, went down in history as the first person to be diagnosed with the disease bearing the same name as the scientist. The symptoms of the disease first manifested themselves in the form of strong feelings of jealousy, increasing memory disorders, confusion, and the feeling that someone would harm himself. After 4.5 years of illness, he died in 1906 from septicaemia caused by decubitus ulcers in the sacral and left trochanteric regions. Microscopic examination of the brain revealed moderate hydrocephalus, cerebral atrophy and suspected atherosclerosis of small cerebral vessels (83). Alzheimer shared this case with the scientific world 1 year after he presented it at the congress in 1906 and it has been known by his name since then (84).

Many changes occur in brain tissue with ageing. It is reported that the normal brain weight is 1.2 - 1.4 kg, the weight starts to decrease from the ages of 45 - 50 and reaches its lowest level at the age of 86. It has been demonstrated that a decrease in gyrus, a decline in white matter quantity, and a decrease in the number of neurons are all related to a decreasing brain weight as one ages. It has also been reported that the dura mater thickens and the arachnoid membrane becomes cloudy and thickened (84). Currently, Alzheimer's disease is recognised as a progressive neurodegenerative disorder and is considered to be one of the major causes of dementia in old age. Numerous theories, including the cholinergic, tau, Aβ, and inflammatory hypotheses, have been put forth to explain this multifactorial disorder

(85). Alzheimer's disease is morphologically characterised by brain atrophy and cerebral ventriculomegaly due to accumulation of cerebrospinal fluid or enlargement of fluid-filled spaces. Biochemically, it is characterised by a decrease in choline acetyltransferase levels. Pathophysiologically, it is associated with intracellular accumulation of amyloid β plaques and neurofibrillary tangles, inflammation and oxidative damage in neurons (86). Autophagy is the main cellular mechanism responsible for the removal of these Aβ plaques and tau neurofibrillary tangles accumulated in the brain. However, the accumulation of immature autophagic vacuoles in dystrophic neurites in the brains of Alzheimer's patients suggests that the autophagy process is impaired in this disease (87). Studies have shown that the autophagy mechanism is much more effective in young neurons than in old ones. This is due to the fact that as we age, autophagy-associated proteins like Atg5, Atg7, and beclin-1 will decrease, which will probably hasten the beginning of neurodegenerative disorders like Alzheimer's and Parkinson's (88,89). Numerous studies have also connected leukocyte telomere length to Alzheimer's along with dementia (90,91,92).

Nowadays, it is well known that not only the diet but also the cooking procedures in the preparation of food affect the occurrence of many diseases. In particular, the accumulation of toxins, especially advanced glycation end products, which occur during different cooking techniques during the course of aging may result in the development or exacerbation of neurodegenerative illness like Alzheimer's (93). It is also emphasised that the consumption of vegetables, fruits and fish, xanthophyll carotenoids and omega-3 fatty acids can optimise cognition and reduce the risk of developing Alzheimer's disease, especially in the aging process (94). It is thought that former smoking history, as well as active smoking, promotes Alzheimer's disease pathology through cerebral oxidative stress and that a reduction in smoking prevalence will reduce the global prevalence of Alzheimer's disease in later life (95). Contradictory results emerge in alcohol use. Actually, low-dose alcohol consumption lowers the prevalence of Alzheimer's illness; moderate alcohol consumption may protect against Aβ accumulation; and heavy alcohol consumption raises the risk of the disease, according to epidemiological studies (96,97). Indeed, there is still disagreement over whether alcohol consumption decreases the risk of developing Alzheimer's and, if it does, how much alcohol causes different effects.

There have been many drug trials over the treatment of Alzheimer's as well as the main drug that has demonstrated substantial enhancements in the cognitive function of patients is cholinesterase inhibitors (98,99). In addition, NMDA receptor antagonists such as memantine are another drug utilized for symptomatic therapy of severe Alzheimer's (99). Nevertheless, the neuronal degeneration associated with the disease persists (98). The most prevalent type of dementia, accounting for 60% to 70% of cases, is

Alzheimer's. According to March 2023 WHO data, the amount of Alzheimer's patients in worldwide is around 33-38.5 million. Unfortunately, this number continues to increase day by day (99). Considering that there's no curative therapy for this illness yet and the existing treatment methods are mostly aimed at suppressing the symptoms, it is obvious that additional investigation is required on the subject.

James Parkinson published the paper "An Essay on the Shaking Palsy" in 1817, marking the first time the illness was identified. James Parkinson described the disease, which is known by his name, as the patient's involuntary tremors with decreased muscle strength even when immobilised or even supported, a propensity to tilt his body ahead and to switch from walking to running, as well as the absence of any impairment in mental health and senses. Parkinson named this syndrome he discovered as 'Shaking Palsy' or 'Paralysis Agitans' (100). Currently, Parkinson's illness is the 2nd most common neurodegenerative illness after Alzheimer's illness and it is the most widespread neurodegenerative movement system disorder (101). While tremor, muscle stiffness, bradykinesia-akinesis, and postural instability are the main motor symptoms (102), it is now widely acknowledged that non-motor symptoms can also occur in all stages of the illness and can significantly lower the standard of life. These symptoms include sleep disturbances, cognitive dysfunction, emotional disturbances, autonomic dysfunction, and pain (103). The loss of dopaminergic neurons, which are primarily found in the substantia nigra, and the buildup of misfolded alpha-synuclein in Lewy bodies in the brainstem are the hallmarks of Parkinson's disease. When 60% to 80% of dopaminergic neurons are lost, motor symptoms become prominent (104,105,106).

Recent data suggest that 6 to 7 million people worldwide suffer from Parkinson's disease. Only 4 per cent of people with Parkinson's disease are under 50 years of age. However, its prevalence rises to 41 per 100,000 at the age of 50 and to 1900 per 100,000 in people aged 80 and over. In addition, the prevalence rate is 1.5 times higher in men than in women at all ages. These results show that the prevalence of the disease increases significantly with age and age is the primary factor in the aetiology of the disease (107,108).

Recent research demonstrated that 183 individuals suffering from Parkinson's disease with a baseline age of 59.7 years enjoyed considerably greater amounts of neutrophils, monocytes and high-sensitivity C-reactive protein (Hs-CRP) and a decreased number of lymphocytes in comparison with healthy people of similar age group. These results suggest that Hs-CRP may be a marker for Parkinson's disease progression and treatment response (109). Vitamin D deficiency in Parkinson's disease is also a remarkable conditionIndeed, many cross-sectional investigation showed that

serum vitamin D level is correlated with the motor part of the Unified Parkinson's Disease Rating Scale and Hoehn and Yahr stage (110,111,112). According to studies, PARP-1 both promotes cell death or functional decline in old age or pathological conditions, as well as shields cells against aging under physiological circumstances. NAD+ is decreased, SIRT1 is suppressed, and telomere shortening is aided by activated PARP1 (113). There is ongoing debate regarding PARP1's impact on telomere shortening or elongation induction. (114). Nonetheless, PARP1 plays a crucial part in the repair of DNA damage. In primary cultures of cells derived from naked mole rats—a rodent species known for its long lifespan and resistance to tumors—it was found that PARP1 was abundantly expressed (115,116).

Many researchers agree that dietary habits are related to the development of Parkinson's illness. Many researchers have suggested that phytochemicals in fruits and vegetables may slow the progression of Parkinson's disease by reducing the functional decline associated with ageing, in addition to their general health benefits (117,118). It has also been reported that the risk of Parkinson's disease is reduced in men and women who consume vegetables such as tomatoes, potatoes and peppers containing nicotine and who have never smoked (119). Some studies have shown that genistein, a soya bean isoflavone, could lower the chance of getting Parkinson's in postmenopausal women (120), while others have reported that genistein treatment in Parkinson's disease protects dopaminergic neurons against lipopolysaccharide-induced damage by inhibition of microglia activation (121). It has also been reported that caffeine (122) and tea (*Camellia sinensis*) (123) have neuroprotective effects and may have protective effects against Parkinson's disease. However, there are highly contradictory results between eating a fatty diet (124,125), consumption of meat and meat products (126,127), carbohydrate consumption (128,129), alcohol consumption (125,130) and Parkinson's disease.

For the symptomatic treatment of Parkinson's disease, levodopa, dopamine agonists, COMT inhibitors, MAO-B inhibitors, amantadine, and anticholinergic medications are commonly used. In addition, surgical procedures may be used in some cases. In addition, physical and mental exercises, physiotherapies and special diets are often recommended due to their complementary effect. Despite all these methods, there is still no cure for Parkinson's disease (131,132). None of the drugs currently developed are successful in stopping or delaying the degeneration of dopaminergic neurones (104,105,106). As in many neurodegenerative diseases that cause dementia, the lack of an effective treatment method in Parkinson's disease makes research on the subject much more valuable.

Conclusion

As in all living organisms, it is inevitable for humans to enter a process in which physiological reserves and cellular regeneration rate gradually decrease and consequently vital activities deteriorate after the growth and subsequent maturation process. In this process, scientifically called aging, the cardiovascular system, respiratory system and, of course, the nervous system are among the most adversely affected physiological systems. In today's conditions, it does not seem possible to prevent cellular ageing, and the common opinion of scientists is to ensure healthy ageing.

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CHAPTER 10

THE EFFECT OF THE MIGRAINE DISORDER WITHOUT AURA ON RETINA AND OPTIC NERVE

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INTRODUCTION

Migraine is a chronic, multiphasic, common, familial, primary headache disorder which interferes with personal daily activities and it is associated with a number of neurological symptoms. The prevalence of migraine disorder, which is more common in women, is 15-18% among the general public. In the Global Burden of Disease Study 2010 (GBD2010), it was ranked as the third most common disease in the world. Moreover, it was anked as the third highest cause of disability in men and women under 50 in GBD2015.[1,2]

Migraine is a primary headache that lasts between 4-72 hours, increases with physical activity, and it is usually localized on one side of the head. It consists of four successive phases. The first stage, known as the pre-warning phase, is the autonomic symptoms that occur hours/days before the headache begins. Frequent urination, fatigue, poor concentration, and mood changes can be counted among these symptoms. The second stage, observed in approximately one third of migraine patients, is called 'aura', a series of sensory distractions and disturbances, which occurs just before the migraine attacks. The third stage is the phase in which a throbbing headache occurs and it is known to occur as a result of the activation of the trigeminal sensory pathways. Headache gradually increases and negatively affects daily activity. Headache may be accompanied by photophobia, phonophobia, osmophobia, nausea and vomiting. In the fourth stage, which is called poststrom, weakness, fatigue, intolerance to noise and lack of concentration are observed, and the symptoms increase in direct proportion to the duration of the pain and can last up to 48 hours.[1,3,4]

Migraine pain usually occurs on the frontotemporal parts of the head. However, in individuals diagnosed with migraine under the age of 18, unlike adults, the pain is usually bilateral. As opposed to adults, localization to one side is seen in young adolescence and young adulthood.[1,2]

Migraine is handled under two main headings; with aura and without aura: Migraine without aura is characterized by headache with specific characteristics and associated symptoms. In order to diagnose migraine without aura, the following features must be present.

A. Having at least five attacks that meet criteria B-D is sufficient to diagnose migraine without aura.

B. Treatment-resistant headache attacks lasting 4-72 hours.

C. The headache must have at least two of the following features:

1. Unilateral location
2. Pulsating quality

3. Moderate or severe pain
4. Pain intensity increases with physical activity

D. Presence of at least one of the following symptoms during the headache:

1. Nausea and/or vomiting
2. Photophobia and phonophobia[5,6,7]

As for migraine with aura, it usually progresses with focal neurological symptoms that occur before the headache. Usually slowly developing headaches are accompanied by specific migraine symptoms. Recurrent attacks of reversible visual, sensory, and/or other central nervous system symptoms occur. In order to diagnose migraine with aura, the following features must be present:

A. Having at least two attacks that meet criteria B, C is sufficient to diagnose migraine with aura.

B. Presence of at least one of the following reversible auras;

1. visual
2. sensory
3. lingual
4. physico motor
5. medulla spinals
6. retinal

C. Having at least three of the following features:

1. At least one aura symptom that develops slowly within 5 minutes
2. The consecutive appearance of at least two aura symptoms
3. Each aura symptom lasts 5-60 minutes
4. At least one aura symptom is unilateral
5. At least one positive aura symptom
6. Headache accompanies the aura or follows it within 60 minutes[8,9]

Although the pathogenesis of migraine is unclear, there is increasing evidence that the neurovascular system is involved in the development of migraine. It has been proven that the headache phase occurs with the activation of meningeal nociceptors at the origin of 'the trigeminovascular system' (TGVS). TGVS consists of the trigeminal nucleus, trigeminal ganglion, trigeminal nerve, and the meningeal and ocular vascular networks innervated by the nerve.[10] It is known that visual aura is seen in 1/3 of

migraine with aura patients. It is thought that meningeal nociceptors play a role in the emergence of aura and signals are transmitted from the dura mater to the cortex, and the most common visual auras in migraine are light flashes, decreased visual acuity and black dots. [11]

Migraine-type photophobia affects approximately 90% of patients, which is the finding that most affects routine daily functions. It has been reported that stimulation of retinal ganglion cells containing melanopsin, which has high sensitivity to blue light, is the cause of photosensitivity in migraine. It has been thought that blue light may be the basis for photophobia in migraine and that the symptom may decrease by blocking blue light.[12,13] It is known that vasodilation and vasoconstriction during migraine attacks cause perfusion disorders and occur in retinal vessels as well as cranial vessels. Although vascular events during migraine attacks are temporary, they cause permanent damage to the brain and retina. In the past, it was thought that migraine disease might be related to retinal vascular diseases. It has also been suggested that it may be a risk factor for ischemic disease occurring in the retina and optic nerve.[14]

It can be demonstrated that all these retinal changes with Spectral Domain - Optical Coherence Tomography (SD-OCT), which is a noninvasive method. SD-OCT is an imaging technology that creates detailed cross-sectional images. This system enables noninvasive optical biopsies of living tissues using infrared light that can penetrate the tissue to a depth of several hundred microns. The device measures with an interferometric setup to reconstruct the depth profile of the sample with backscattered light.[15]

In the study, macula and optic nerve focused measurements were made by means of SD-OCT. The size of macular thickness was evaluated with Macular OCT, ganglion cell damage with 'Ganglion Cell Complex' (GCC), and optic nerve damage with Retinal Nerve Fiber Layer (RNFL). Vascular changes of migraine disorder were taken into consideration by investigating retinal and optic nerve changes between migraine patients and healthy volunteers.

1. METHOD

1.1. Study Design and Patient Specifications

A prospective, single-center, comparative study was conducted with 48 eyes (Group I-study group) of 24 patients who were previously diagnosed with Migraine Without Aura (MWoA) disorder and did not receive any treatment other than migraine attacks, who applied to Niğde Ömer Halisdemir University Hospital Ophthalmology Polyclinic between January 2022 and April 2022. 48 eyes of 24 healthy individuals (Group II-con-

trol group) were included in the study. Full ophthalmological examinations of the patients were performed. Anterior segment examinations were performed with slit lamp biomicroscopy. Posterior segment examinations after pupil dilation were performed with a 90 D Volk lens. Individuals whose ophthalmological examinations did not reveal any pathology were included in the study. Macular thickness, RNFL and GCC (Topcon America, Paramus, NJ, USA) examinations were performed on all participants with SD-OCT and the results were recorded.

Macular section was taken with SD-OCT and central macular thickness was measured.

The peripapillary RNFL optic nerve was imaged in four quadrants (superior, inferior, nasal and temporal) from the internal limiting membrane to the ganglion cell layer.

With GCC, measurements were made in six quadrants (superior, superior temporal, superior nasal, inferior, inferior temporal and inferior nasal) from the internal limiting membrane to the internal plexiform layer. These parameters were calculated automatically by means of the 3D Wide Report protocol.

Inclusion criteria:

- Being between the ages of 18-65,
- For Group I, having previously been diagnosed with migraine disorder without aura,
- Not having any neurological disease other than migraine disorder,
- Not having any ophthalmological disease and not having undergone ocular surgery.

Exclusion criteria:

- Being under the age of 18 or over the age of 65,
- Having any neurological and/or systemic disease other than migraine disorder,
- Having had previous ocular surgery,
- Chronic drug use.

1.2. Statistical analysis

Statistical analysis was performed through Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) version 23. $P<0.05$ value was considered statistically significant. Mean±standard deviation and percentage values were used in the supplementary statistics of the data. The

conformity of the obtained data to normal distribution was evaluated with the Kolmogorov-Smirnov test. Parametric tests for data with regular distribution; Non-parametric tests were applied for data where the distribution was not regular. To evaluate the variables between two independent groups, parametric independent groups t test and nonparametric Mann-Whitney U test were applied.

2. RESULTS

Out of the 48 participants (96 eyes) included in the study, 42 (87.5%) were female and 6 (12.5%) were male. The average age of the participants between the ages of 18-60 is 32.91±9.90. The average age of 24 patients (48 eyes) included in Group 1 was 35.41±11.27; 23 (95.8%) were female and 1 (4.2%) was male. The average age of 24 patients (48 eyes) included in Group 2 was 30.41±7.78 years; 20 (83.3%) were women and 4 (16.7%) were men. There was no statistically significant difference between the groups in terms of age and sex (p>0.05). The demographic characteristics of the participants are given in Table I.

Table I*: Demographic characteristics*

p	Group I	Group II	Total
Age	35.41±11.27	30.41±7.78	32.91±9.90
	P=0.80		
Sex	95.8/4.2	83.3/16.7	87.5/12.5
K/E (%)	*P*=0.16		

The mean macular thickness values of the right eye were determined as 234.66±18.46 in Group I and 235.37±20.15 in Group II. No statistically significant difference was detected between the two groups (p=0.9). The mean values of left eye macular thickness were determined as 236.33±18.01 in Group I and 236.66±22.98 in Group II. No statistically significant difference was detected between the two groups (p=0.9).

The five values in the RNFL analysis were considered separately. The mean value of right eye RNFL was found to be 91.70±8.74 in Group 1 and 99.91±6.94 in Group II, and a statistically significant difference was found between the two groups (p=0.001). The mean value of left eye RNFL was found to be 91.83±8.95 in Group 1 and 99.75±6.81 in Group II, and a statistically significant difference was found between the two groups (p=0.002). The mean value of the right eye RNFL superior was found to be 108.79±12.70 in Group 1 and 117.62±9.90 in Group II, and a statistically significant difference was found between the two groups (p=0.01).

The mean value of the left eye RNFL superior was found to be 113.25±10.98 in Group 1 and 119.33±12.16 in Group II, and there was no statistically significant difference between the two groups (p=0.07). The mean value of right eye RNFL inferior was found to be 113.91±11.85 in

Group 1 and 113.25±10.08 in Group II, and no statistically significant difference was found between the two groups (p=0.83). The mean value of left eye RNFL inferior was found to be 114.25±13.34 in Group 1 and 114.00±10.37 in Group II, and no statistically significant difference was found between the two groups (p=0.94). The mean RNFL nasal value of the right eye was found to be 70.66±11.92 in Group 1 and 73.00±12.27 in Group II, and there was no statistically significant difference between the two groups *(*p=0.5).

The mean nasal RNFL value of the left eye was found to be 68.87±16.33 in Group 1 and 67.41±11.26 in Group II, and there was no statistically significant difference between the two groups (p=0.7). The right eye RNFL temporal mean value was found to be 65.04±12.05 in Group 1 and 61.95±7.99 in Group II, and no statistically significant difference was found between the two groups *(*p=0.3). The left eye RNFL temporal mean value was found to be 63.04±8.95 in Group 1 and 63.16±8.44 in Group II, and no statistically significant difference was found between the two groups (p=0.9). The RNFL values of the participants are given in Table II.

Table II: *RNFL values of the participants*

	Group 1	Group II	*P*
R/RNFL average	91.70±8.74	99.91±6.94	0.001*
R/RNFL superior	108.79±12.70	117.62±9.90	0.01*
R/RNFL inferior	113.91±11.85	113.25±10.08	0.83
R/RNFL nasal	70.66±11.92	73.00±12.27	0.5
R/RNFL temporal	65.04±12.05	63.16±8.44	0.9
L/RNFL average	91.83±8.95	99.75±6.81	0.001*
L/RNFL superior	113.25±10.98	119.33±12.16	0.07
L/RNFL inferior	114.25±13.34	114.00±10.37	0.94
L/RNFL nasal	68.87±16.33	67.41±11.26	0.7
L/RNFL temporal	63.04±8.95	63.16±8.44	0.9

**: $p<0.05$*

Six different values were considered in the GCC analysis. The mean value of the right eye GCC superior was found to be 79.08±15.07 in Group 1 and 88.50±5.57 in Group II, and a statistically significant difference was found between the two groups (p=0.006). The mean value of the left eye GCC superior was found to be 77.29±17.51 in Group 1 and 88.45±5.90 in Group II, and a statistically significant difference was found between the two groups (p=0.005). The right eye GCC superiotemporal mean value was found to be 79.75±10.22 in Group 1 and 84.91±5.74 in Group II, and a statistically significant difference was found between the two groups (p=0.03). The left eye GCC superiotemporal mean value was found to be 76.62±16.59 in Group 1 and 84.50±5.95 in Group II, and a statistically

significant difference was found between the two groups (p=0.002).

The right eye GCC superional mean value was found to be 80.45±16.04 in Group 1 and 89.75±5.80 in Group II, and a statistically significant difference was found between the two groups (p=0.01). The left eye GCC superionary mean value was found to be 77.12±19.29 in Group 1 and 90.70±6.14 in Group II, and a statistically significant difference was found between the two groups (p=0.002). The mean value of the right eye GCC inferior was found to be 78.08±15.04 in Group 1 and 88.45±5.29 in Group II, and a statistically significant difference was found between the two groups (p=0.003). The mean value of left eye GCC inferior was found to be 76.20±16.99 in Group 1 and 88.41±5.95 in Group II, and a statistically significant difference was found between the two groups (p=0.002).

The mean GCC inferotemporal value of the right eye was found to be 81.70±9.12 in Group 1 and 86.75±5.30 in Group II, and a statistically significant difference was found between the two groups (p=0.02). The left eye GCC inferotemporal mean value was found to be 78.33±15.79 in Group 1 and 86.54±4.94 in Group II, and a statistically significant difference was found between the two groups (p=0.01). The mean GCC inferonasal value of the right eye was found to be 80.79±15.06 in Group 1 and 90.08±5.57 in Group II, and a statistically significant difference was found between the two groups (p=0.007). The left eye GCC inferonasal mean value was found to be 77.37±17.68 in Group 1 and 89.75±6.16 in Group II, and a statistically significant difference was found between the two groups (p=0.002).

Table III: *GCC values of the participants*

	Group 1	Group II	*P*
R/GCC superior	79.08±15.07	88.50±5.57	0.006*
R/GCC superotemporal	79.75±10.22	84.91±5.74	0.03*
R/GCC superonasal	80.45±16.04	89.75±5.80	0.01*
R/GCC inferior	78.08±15.04	88.45±5.29	0.003*
R/GCC inferotemporal	81.70±9.12	86.75±5.30	0.01*
R/GCC inferonasal	80.79±15.06	90.08±5.57	0.007*
L/GCC superior	77.29±17.51	88.45±5.90	0.005*
L/GCC superotemporal	76.62±16.59	84.50±5.95	0.002*
L/GCC superonasal	77.12±19.29	90.70±6.14	0.002*
L/GCC inferior	76.20±16.99	88.41±5.95	0.002*
L/GCC inferotemporal	78.33±15.79	86.54±4.94	0.01*
L/GCC inferonasal	77.37±17.68	89.75±6.16	0.002*

*: $p<0.05$

Macular thickness, RNFL and GCC values of Group I patients with migraine disorder were compared between right and left. It was found that there was no statistically significant difference between the right and left eyes (p>0.05).

3. DISCUSSUON

In this study, very striking data were obtained from 48 participants (n=48), 24 of whom had migraine disorder and 24 of whom were healthy individuals. Considering whether migraine disorder made a difference according to sex, results supporting women were obtained between both sexes. On the other hand, migraine disease varies depending on age. Data from the study showed that migraine disorder was more common, especially in women. In their review, Aguilar-Shea AL et al. reported that migraine affects 18% of women and 6% of men found that it was more common in women.[16] Moreover, in their review of individuals with migraine disorders, Flynn et al. found the prevalence of migraine to be 19%. In addition, it was found to be more common in women, with a frequency of 23% in women and 12% in men.[17]

According to the data in line with the result of the first research question of the study; when considering the average macular thickness between patients with migraine disorders and healthy participants, it was found that there was significant difference between both groups (p>0.05). In a study conducted by Ozcift et al., in which they compared 38 individuals with migraine disorder and 38 healthy individuals, it was found that there was no significant difference of the mean macular thickness value between the groups.[18]

The data obtained in this study reveal the effect of migraine disorder on the optic nerve through RNFL data. In this context, peripapillary RNFL average and superior thicknesses are tninner in Group I. In a study conducted with 26 studies, Feng YF. et al., in their meta-analysis to evaluate RNFL thickness in migraine disorder, 432 migraine disorder and 288 healthy participants were examined. As a result of the study, it was found that the average RNFL thickness was significantly thinner in those with migraine disorders.[19] In a study collected with the review of 26 studies on 1530 migraine disorder and 1105 healthy individuals by Lin X et al., they investigated RNFL thicknesses. As a result of the study, the mean RNFL thickness was found to be significantly lower in the migraine group.[20] In their study Kurtul et al., 23 pediatric migraine without aura and 23 healthy participants were included. It was found that there were no significant difference values between the two groups in terms of RNFL.[21] Liu Z et al., in their review of 16 studies consisting of 379 migraine with aura, 583 migraine without aura and 658 healthy participants found that peripapillary RNFL values in individuals with migraine and without aura were lower than the healthy group.[22] The most important result obtained in this study shows that all values obtained from GCC are lower in patients with migraine disorder (Group I). For this reason, it is obvious that the study will contribute to the literature in a positive manner. Yulek et al. exam-

ined the changes in RNFL, GCC and macular thickness in a study consisting of 30 participants with migraine disorder with aura, 20 patients with migraine disorder without aura and 50 healthy participants.The average RNFL thickness is thinner in individuals with migraine disorder than in healthy individuals; moreover, they do not detect any significant difference in terms of other findings.[23] Çolak HN. et al., in their study evaluating OCT values of 45 migraine with aura disorder and 45 healthy individuals, superior and inferior quadrant RNFL values were found to be thinner in the migraine group, while no difference was found between GCC values.[24] Reggio E. et al., 21 migraine with aura, 12 without aura. They evaluated OCT values in a study consisting of migraineurs, 44 chronic migraineurs and 42 healthy controls. RNFL and GCC thicknesses were found to be significantly thinner in patients with migraine disorders than in healthy individuals. [25] Kanar ES et al., published a study in which they examined RNFL and GCC values, including 37 participants with migraine without aura, 40 with migraine disorder with aura, and 50 healthy participants. In this study, it was found that superior and inferior quadrant RNFL thicknesses were thinner in the migraine group with and without aura than in the healthy group.

Superior and inferior quadrant GCC thicknesses were also found to be thinner in the migraine with and without aura group.[26] Raga-Martínez et al., compared the possible changes in RNFL, GCC and macular thickness in chronic migraine patients with healthy controls. In the study of 90 chronic migraine disorder and 90 healthy individuals, the average macula, RNFL superior quadrant, and average and GCC thicknesses were found to be thinner in patients with chronic migraine disorder than in healthy individuals.[27]

CONCLUSION

As a conclusion, the strengths of this study are as follows; it reveals that migraine disorder has a negative effect on ganglion cells and optic nerve fibers. It is predicted that neurovaMigrainescular dysfunction seen in migraine disorder may disrupt blood flow in ocular tissues, cause hypoperfusion; moreover, it may cause permanent damage to ocular tissues.

It was considered that limitations of this study included the limited number of patients and the fact that only migraine without aura disorder. There might be some future studies which could be conducted with larger participants as well as including migraine subclasses.

It is obvious that the RNFL and GCC thicknesses are significantly thinner in patients with migraine disorders, and this study will contribute to the literature. Although there are several studies in the literature on this

subject, the results are controversial. It is thought that this study will contribute to supporting ocular tissue hypoperfusion caused by autoregulation disorder seen in migraine disorder.

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CHAPTER 11

ENDOTHELIAL DYSFUNCTION AND RELATED DISORDERS

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1. Introduction

Endothelium is mesoderm-derived single-fold squamous epithelial tissue that covers the inner layer of the vessels in the vascular system (Fig. 1) (Carlomosti et al., 2017; Lakna, 2017; Medina-Leyte et al., 2021) During its early discovery, the epithelium was assumed to be a simple barrier that exchanges water and small molecules and furnishes the inner wall of the vessels (Adler et al., 2000; Kharbanda & Deanfield, 2001). However, by the time, despite its simple organization, epithelium and its components were accepted as a critical tissue functioning in multiple processes such as the maintenance of body homeostasis and vascular tonus, and the regulation of cellular adhesion, inflammation, vascular permeability, and blood coagulation (Fishman, 1982; Muniyappa & Sowers, 2013). This single-layer structure, which consists of endothelial cells (ECs) and covers the circulating system from the heart to the tiniest capillary vessels, functions not only by forming a barrier isolating vascular space and tissues but also by playing critical roles in the regulation of several physiological and pathological processes (Limaye, 2007; Yaylalı, 2011). Endothelial dysfunction (ED) refers to the transition from a still endothelial phenotype into a defense-response status called endothelial activation (Hansson, 2005). Even though the term ED is used to define pathological conditions such as the alterations in anticoagulant and anti-inflammatory features of endothelium and impaired modulation of vascular development, it is commonly defined as the vasorelaxation defect in endothelium due to the loss of nitric oxide (NO) bioactivity on the vascular wall (Urbich, Kuehbacher, & Dimmeler, 2008). Under normal conditions, there is a sensitive balance in aerobic cells between the oxidant and antioxidant levels. On one hand, the oxidant status of a cell is determined by the levels of reactive oxygen species (ROS) produced after a redox reaction between an oxygen molecule and an electron. On the other hand, the antioxidant status is the ability of a cell to fight against the ROS. The imbalance in favor of ROS levels or the insufficient detoxification rate by the antioxidant system results in ROS accumulation within the cell which is defined as oxidative stress (Lund, 2018; Victor et al., 2009). Thereafter, increased ROS levels or insufficient clearance of ROS curbs the bioavailability of NO and causes ED. Elevated oxidative stress is closely associated with cardiovascular risk factors such as diabetes, hypercholesterolemia, renal failure, aging, hypertension, and smoking (Lund, 2018; Victor et al., 2009). ED can play a role in the pathogeneses of atherosclerosis, hypertension, diabetes, hypercholesterolemia, and cardiac insufficiency as well as it can emerge as a result of these pathologies (Munzel, Sinning, Post, Warnholtz, & Schulz, 2008).

2. Structure and Functions of Endothelium

ECs have unique and significant functions in the context of vascular biology due to their location between the blood and vascular walls of the endothelium. ECs play several roles in the circulatory system including; the regulation of blood flow by easing the blood transition throughout the vessels via providing an active antithrombotic surface, fibrinolytic functions, inhibition of thrombocyte aggregation, and regulation of vascular tonus. Together, the endothelium also provides the regulation of thrombocyte adhesion and substance exchange between blood and tissues (Limaye, 2007; Yaylalı, 2011). Inflammation and perturbations taking place in high hydrodynamic shear stress can distort these activities and engage the ECs to form prothrombic and antifibrinolytic microenvironments (Roberts & Porter, 2013). ECs are negatively charged owing to glycoproteins and glycosaminoglycans on their plasma membrane (Fig. 2), in which there are various receptors providing connection with intracellular and hormonal molecules (Medina-Leyte et al., 2021; Muniyappa & Sowers, 2013; Yaylalı, 2011).

2.1. Physiological Functions of Endothelium

Previously, the endothelium was considered to have unspecific and simple functions like transiting water and electrolytes through the vessels however, together with the advancements in the field it is evident that its functions are more complicated (Muniyappa & Sowers, 2013). It spreads throughout the circulating system namely from tiny capillary vessels to the heart's most complicated arteries and functions as a specific barrier providing exchange between blood and tissues as well as host most of the receptor-based signaling pathways (Limaye, 2007; Medina-Leyte et al., 2021; Ross, 1999). The endothelium is also involved in the regulation of hemostasis in the vascular system. Moreover, it regulates several processes e.g., vascular tonus, maintenance of circulation and blood flow, coagulation, and inflammatory responses. The endothelium is also responsible for the synthesis of several vasoactive and connective tissue components (Emre, Öcal, & Şan, 2004; Gonzalez & Selwyn, 2003; Ross, 1999). Including the secretion of vasodilators and vasoconstrictors and the modulation of the vascular wall as a signal modifier by interacting with circulatory factors, the endothelium carries out many vital physiological functions. Among autocrine and paracrine functions of ECs, the syntheses of several vital molecules exist, which sustain vascular tonus, reduce leukocyte migration, control permeability, regulate the migration and proliferation of smooth muscle cells, and regulate thrombocyte aggregation (Sena, Leandro, Azul, Seica, & Perry, 2018).

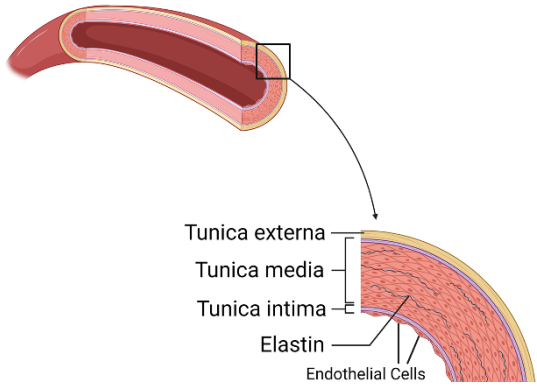

Fig. 1. Structure of endothelium. Endothelium, which covers the inner wall of the vessels, consists of a single layer of ECs. Modified from (Medina-Leyte et al., 2021).

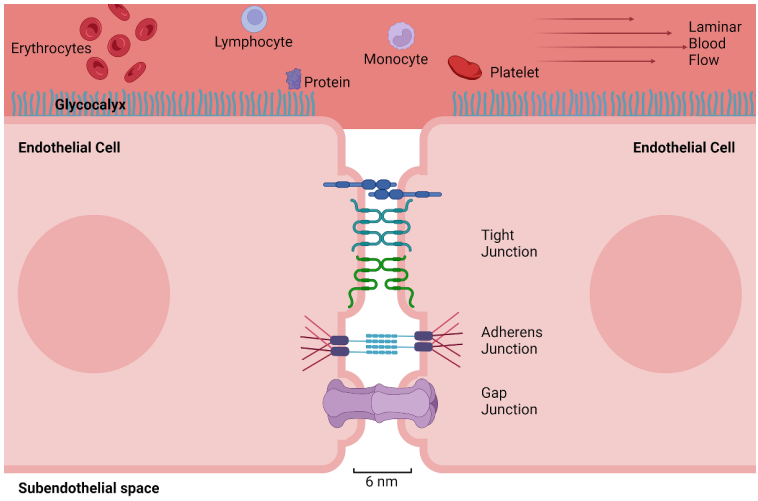

Fig. 2. Endothelial layer in vasculature. The barrier formation by the tight junctions between two ECs and the negatively charged glycoproteins and glycosaminoglycans. Modified from (Medina-Leyte et al., 2021).

The endothelium-specific functions and related mediators are depicted in Fig. 3. In its essence, the endothelium is a paracrine, autocrine, and endocrine tissue that consists of tightly connected ECs and plays a role in several processes like constructing a connection between blood and tissues (Mizuno, Jacob, & Mason, 2011). The wall formed by endothelium between the arterial layer and the blood cells is smooth and thorough (Emre et al., 2004; Gonzalez & Selwyn, 2003; Ross, 1999).

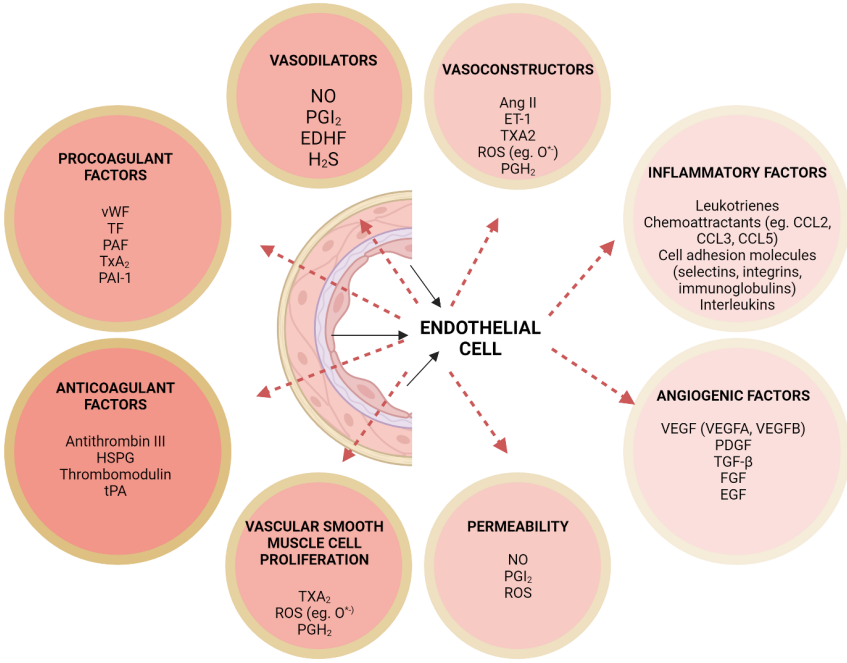

Fig. 3. The primary functions of ECs. Regulation of vascular tonus, control of vascular smooth muscle cell proliferation, inflammation, permeability, angiogenesis, metabolism, and homeostasis. Modified from (Sena et al., 2018).

Owing to its natural location, the endothelium provides signal transduction to its neighboring environment. Thus, it maintains the regular functions of the vascular wall meanwhile providing physical protection (Baykal, Özet, & Kocabalkan, 1998). The functions of ECs as the building blocks of endothelium can be summarized under 5 bullet points:

- ***Endothelium as a Barrier.***

The endothelium is a semi-permeable structure allowing the transition of water and molecules < 6 nm in diameter into the subendothelial space (Medina-Leyte et al., 2021; Mundi et al., 2018). Another critical function of endothelium is the regulation of soluble substances' exchange through the vascular endothelial barrier (Minshall & Malik, 2006). Whereas the substance exchange in circulation takes place mostly in capillaries and pos-capillary venules. Lipophilic and small molecular weighted hydrophilic substances can move easily between the blood and tissues, endothelium builds a semipermeable barrier to macromolecules. This feature is of vital importance for keeping the balance between intra- and extra-vascular fluid (Emre et al., 2004; Medina-Leyte et al., 2021; Özdoğu, 2007). Barrier function is sustained by means of glycocalyx and junction complexes which cover the endothelium luminal surface (Medina-Leyte et al., 2021). Glycocalyx supports the regulation of vascular permeability, provides

a negative charge to ECs, and protects against pathogenic infections by forming a barrier. The net negative charge on the surface repels blood cells which are thrombocytes, erythrocytes, and leukocytes. On the other hand, junction complexes (tight, adherens, and gap junctions) contribute to the barrier function by supporting endothelial maintenance (Medina-Leyte et al., 2021). There are 2 transfer mechanisms in the endothelium; the first is called the transcellular way which transfers large molecules (e.g., plasma proteins like albumin) via the vesicular system (endocytosis, exocytosis), and the second way is the paracellular way that transports proteins through transcytosis (between the cells). The primary objective of transcytosis is to regulate the albumin and immunoglobin distribution, thereof to control the oncotic pressure of tissues and host defense mechanism. The paracellular transfer induced during inflammation takes place through the adherence and tight junctions between ECs (Fung, Fairn, & Lee, 2018; Medina-Leyte et al., 2021; Minshall & Malik, 2006). Albeit the term "vascular permeability" refers to the total structural and functional alterations on the vascular wall, in essence, atherosclerosis-causing transition of macromolecules, liquids, and cells into intima arises from the alterations in endothelial function (Mundi et al., 2018). The binding of neutrophils to endothelium during inflammation causes ED-inducing oxidant formation and permeability elevation. Additionally, thrombin, one of the inflammatory mediators, increases endothelium permeability (Medina-Leyte et al., 2021; Mundi et al., 2018; Özdoğu, 2007). ED initiates an irregular trans-endothelial flow which leads to abnormal accumulation of cells and other molecules in intima (Mundi et al., 2018).

- ***Endothelium as an Anti-coagulant Surface.***

Anti-coagulation feature is of vital place in the inner homeostasis and thrombosis mechanisms of the endothelium. Under physiological conditions, ECs block thrombosis by several anticoagulant and antithrombotic mechanisms (Medina-Leyte et al., 2021). In healthy status, ECs form an anticoagulant surface via several mechanisms e.g., inhibition of coagulant activation and platelet aggregation and fibrinolysis activity by secreting various mediator substances (e.g., tissue plasminogen activator, plasminogen activator inhibitor, prostaglandin 12, prostacyclin, NO, CD39, etc.). In the case of ED or inflammation, ECs behave like procoagulant by secreting thrombosis-triggering substances (e.g., thrombomodulin, von Willebrand factor, thromboxane A2, etc.) (Emre et al., 2004; Medina-Leyte et al., 2021; Özdoğu, 2007; Zoghi & Nalbantgil, 2002). In the last decade, signaling pathways and molecules playing a role in the inhibition of prothrombic incidents have been described (Kirsch et al., 2016; Medina-Leyte et al., 2021; Wu, Hu, Jiang, Wang, & Gong, 2019). Among these, the mitochondrial thioredoxin system blocks the ROS production in ECs. In

thioredoxin reductase 2 knock-out mice, the endothelium phenotype was demonstrated to be prothrombic which was considered of systemic origin (Kirsch et al., 2016). The inhibition of the Sirt1/FoxO1 signaling pathway was also reported to cause an increase in prothrombic von Willebrand factor secretion (Wu et al., 2019). Discovering the novel genes and pathways related to vascular endothelial function is of critical importance to uncover promising pharmacological approaches that are conceived to adjust endothelial activity for the treatment of thrombotic and cardiovascular disorders (Medina-Leyte et al., 2021).

- ***Endothelium in Regulation of Vascular Tonus.***

ECs regulate the vascular tonus by secreting endothelium-derived factors such as vasodilators e.g., prostaglandin, NO, and prostacyclin and vasoconstrictors e.g., endothelin 1 (ET-1) and platelet-activating factor (Godo & Shimokawa, 2017; Medina-Leyte et al., 2021; Sağatlı, 2009). Along with these factors, ECs can also be maintained by other vasoactive substances in circulation such as bradykinin, thrombin, ADP, and ATP.

- ***Endothelium Supporting Immune System.***

The endothelium is involved in the secretion of interleukin-1 while presenting antigens to immunocompetent cells (Emre et al., 2004; Godo & Shimokawa, 2017; Zoghi & Nalbantgil, 2002). ECs mediated immune response via activation of toll-like receptor signaling. ECs residing in organs play a critical role in response against bacteria during sepsis (Xia, Menden, Korfhagen, Kume, & Sampath, 2018).

- ***Endothelium in Assign of Lipoproteins' Functions.***

Endothelium assigns the function of lipoproteins, which transit to the subendothelial space, by involving in the metabolism of lipoproteins in circulation (Emre et al., 2004; Özdoğu, 2007; Zoghi & Nalbantgil, 2002). The mediators that are associated with the specific functions of endothelium are summarized in Table 1. As healthy endothelium plays vital a role in vascular homeostasis, it has been previously reported that ED is implicated in the pathogeneses of vasospasm, thrombus formation, and vascular proliferation-associated diseases (Gavriilaki et al., 2019; O'Riordan et al., 2005).

Table 1. Endothelium-specific functions and endothelial mediators.

Function	Specific Function	Mediators
Barrier function	Exchange of small and large molecules	Transition though cells and cytoplasm via diffusion, osmosis, vesicular transport; endocytosis, exocytosis
Homeostasis and inflammation	Thrombocyte adhesion and activation	Von Willebrand factor, P-selectin, E- selectin, thrombocyte activating factor
	Fibrinolysis	Plasminogen activator inhibitor-1, plasminogen activator, urokinase
	Coagulation	Thrombomodulin, heparan sulfate, tissue-type plasminogen activator
Regulation of vascular tonus	Smoot muscle relaxation	Endothelium-derived relaxing factor, prostacyclin, bradykinin, acetylcholine, nitric oxide, hyperpolarizing factors
	Smooth muscle contraction	Thromboxane A2, endothelin 1, angiotensin II.
Endocrine and remodeling	Chemotactic	Monocyte chemotactic protein, Interleukine-8
	Growth factors	Heparin-binding epidermal growth factor, M-colony stimulating factor, vascular endothelial growth factor, platelet derived growth factor, transforming growth factor
	Cellular adhesion	E-selectin, intracellular cell adhesion molecule, vascular cell adhesion molecule-1

3. Endothelial Dysfunction

ED is the perturbation of the balance between vasodilation and vasoconstriction (Park & Park, 2015). The term ED was first used to define insufficient endothelium-dependent vasodilation against certain stimulators namely acetylcholine and bradykinin (Endemann & Schiffrin, 2004).

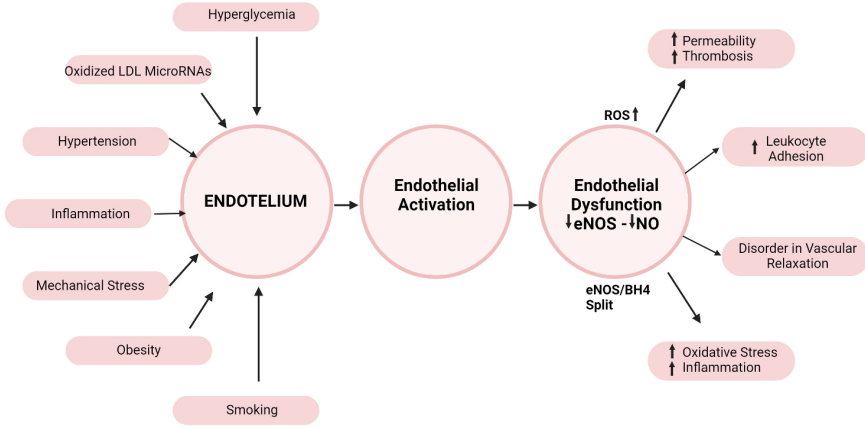

Fig. 4. Alterations in endothelium status in case of endothelial dysfunction. Modified from (Park & Park, 2015).

ED is a multifactorial disorder in which ROS production is included. Among molecular mechanisms involved in oxidative stress-induced ED, the inhibition of eNOS by both reactive oxygen and nitrogen species, up-regulation of ET-1 upon superoxide/hydrogen peroxide production, and angiotensin-II-mediated activation of NADPH oxidase (Nox1 or Nox2) are included (Medina-Leyte et al., 2021). In ED, the bioavailability of NO reduces due to decreased synthesis and elevated oxidative stress levels which results in an impaired arterial dilatation as a response to biological and mechanical stimuli (Fig. 4) (Gavriilaki et al., 2019; Park & Park, 2015). Nevertheless, a broader perspective is required for the definition of ED; insufficiency in vascular smooth muscle tonus, reduction in antithrombotic feature in addition to impaired barrier function, damaged synthesis function, decreased proliferation of neutrophils and monocytes (Mason, 2018; O'Riordan et al., 2005; Park & Park, 2015). The molecules with altered expression levels during ED are listed in Table 2.

Table 2. Altered expression of molecules during endothelial dysfunction. Modified from (Sena, Pereira, & Seica, 2013).

	Molecules	Alteration
Vasodilators	NO, eNOS, PGI2	↓
Vasoconstrictors	ET-1	↑
Adhesion molecules	P-selectin, VCAM-1, E-selectin, ICAM-1	↑
Thrombotic factors	vWF, PECAM-1, PAI-1	↑
	t-PA	↓
Endogeneous inhibitor of nitric oxide synthase	ADMA	↑

Pro-inflammatory molecules	CRP, IL6, TNF α	↑
Oxidative stress	ROS	↑

NO: Nitric oxide, eNOS: Endothelial nitric oxide synthase, PGI2: Prostaglandin I2, ET-1: Endothelin 1, ICAM-1: Intracellular cell adhesion molecule 1, VCAM-1: Vascular cell adhesion molecule 1, vWF: Von Willebrand factor, PECAM-1: platelet and endothelial cell adhesion molecule 1, PAI-1: Plasminogen activator inhibitor-1, t-PA: Tissue-type plasminogen activator, ADMA: Asymmetric dimethyl-arginine, CRP: C-reactive protein, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor α, ROS: Reactive oxygen species.

3.1. Endothelial Dysfunction-Related Diseases

As ED can be implicated in several diseases e.g., diabetes, atherosclerosis, hypertension, hypercholesterolemia, and cardiac insufficiency, it can also emerge as a result of these disorders (Sena et al., 2018). The distinctive characteristic of ED is endothelium-originated NO loss and impaired endothelium-dependent vasorelaxation characterized by augmented oxidative stress. Besides, ED is closely related to cardiovascular risk factors such as dyslipidemia, arterial hypertension, and metabolic syndrome (Lund, 2018; Sitia et al., 2010). The impaired insulin signaling in diabetes is also associated with ED by attenuating endothelial nitric oxide synthase activity and NO levels (Endemann & Schiffrin, 2004; Favero, Paganelli, Buffoli, Rodella, & Rezzani, 2014; Versari, Daghini, Virdis, Ghiadoni, & Taddei, 2009; Yang & Ming, 2006). ED plays a critical role in atherosclerosis onset and prognosis (Davignon & Ganz, 2004). Hence, the underlying mechanisms of diabetic ED-related atherosclerosis are critically significant (Dong et al., 2017).

3.1.1. Atherosclerosis

Atherosclerosis is an inflammatory/fibrotic disease of arterial intima that emerges in the presence of atheroma or focal plaque formed by the accumulation of lipid and fibrous elements in the inner layer of middle and large arteries (Etli, Yavuz, Kayan, & Sezer, 2012; Lusis, 2000). Atherosclerosis is one of the major factors in the physiopathology of cardiovascular diseases as the primary cause of death in developed countries (Drouet, 2002). The deformations in the endothelium result in both structural and functional impairments. Hence, the blood flow gets damaged, the permeability to macromolecules like LDL increases, and therefore fragile regions develop in favor of atherosclerotic lesion formation in vascular endothelium (Mizuno et al., 2011).

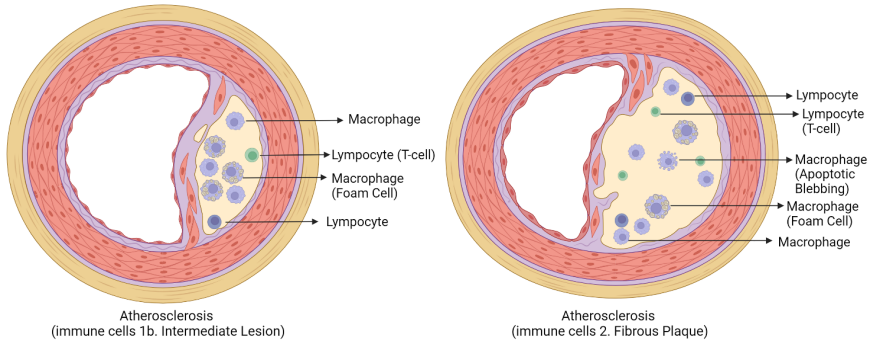

Fig. 5. Implication of immune system cells in different stages of atherosclerosis progression Modified from (Jebari-Benslaiman et al., 2022).

Atherosclerotic lesion progression increases with the risk factors namely hypertension, hypercholesterolemia, diabetes, and smoking. The accumulation of monocyte-originated macrophages and LDL in arterial intima produces a fatty streak (Etli et al., 2012).

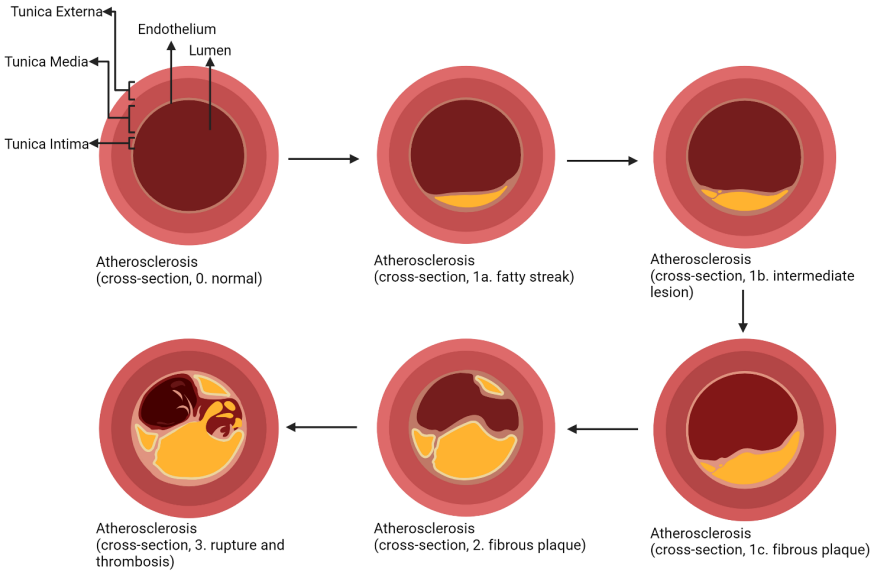

Fig. 6. Atherosclerosis progression in vascular system Modified from (Jebari-Benslaiman et al., 2022).

Within the first 10-year lifespan of a human being, these fatty streaks are observed in the aorta ensuing coronary and cerebral arteries. Early atherosclerotic lesions are composed of macrophages stacked with cholesterol under endothelium, which are called “foam cells” (Fig. 5). Then the lesion formation in endothelium occurs together with dynamic alterations in the blood flow. The growth factors and cytokines secreted by inflammatory cells lead to a layer formation that consists of smooth muscle cells, collagen content, and fat fibrils. This whole process takes place by the activation of inflammation and protease secre-

tion (Etli et al., 2012). Fatty streaks, which may not be clinically relevant, can be a prognostic indicator for more progressive lesions. The progressing lesions that might be as advanced as blocking blood flow can result in thrombosis and blood coagulant-inducing myocardial infarction and brain stroke (Fig. 6) (Jebari-Benslaiman et al., 2022; Lusis, 2000; Mizuno et al., 2011). Atherosclerosis together with hyperlipidemia lead to ED. In the case of hypercholesterolemia, the increased transition rates of LDL into intima from vascular lumina, owing to its high lipid content, causes lipid accumulation. Thus, the luminal lipid aggregation causes inflammation that leads to the formation of enzyme and oxygen radicals and eventually ROS which damages the endothelium integrity by bringing about cytotoxicity after the lipid peroxidation of the EC plasma membrane (Önder & Nalbantgil, 1997). EC damage can be considered as the first stage of atherosclerosis and cardiovascular diseases' onset (Fig. 7) (Park & Park, 2015). The migration of vascular smooth muscle cells into intima is a prerequisite for the progression of vascular disorders. The following stage is ED-induced atherosclerosis (Milutinovic, Suput, & Zorc-Pleskovic, 2020). Last but not least, the growth factors and endothelium-derived adhesion molecules are of critical importance in the formation and progression of atherosclerosis. The main ones of these molecules are fibroblastic growth factor, platelet-derived growth factor, vascular and intercellular adhesion molecules, and endothelial leukocyte adhesion molecule. These factors lead to collagen formation within the endothelial layer by inducing the proliferation and migration of smooth muscle cells and the chemotaxis of inflammatory cells (Etli et al., 2012).

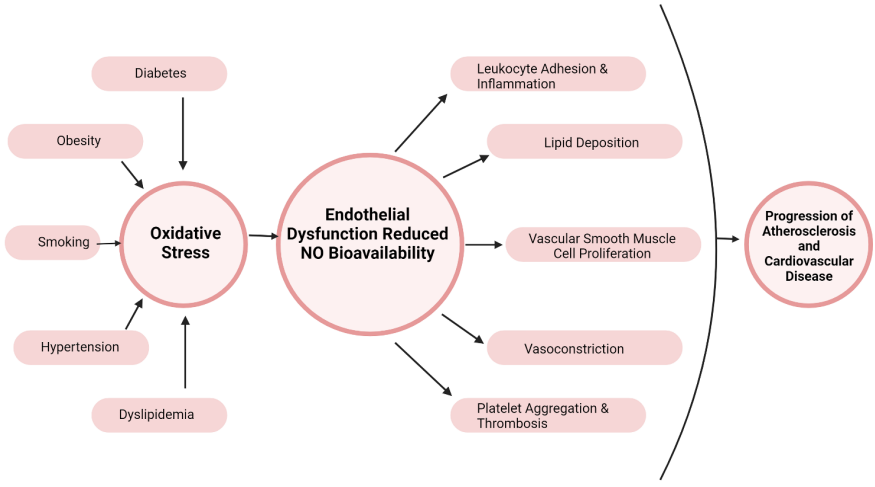

Fig. 7. The role of endothelial dysfunction in atherosclerosis-induced cardiovascular diseases. Modified from (Park & Park, 2015).

3.1.2. Hyperlipidemia

The increased levels of cholesterol and LDL cause morphological changes in the vascular wall by altering inflammation and proliferation

rates. These modulations increase the tendency towards atherosclerosis and eventually contribute to ED by decreasing endothelium-depended vasodilatation response. In the case of hypercholesterolemia, elevated circulating cholesterol levels reduce NO activity in ECs while superoxide radicals increase NO levels counteractingly which causes eventual increased LDL oxidation. Oxidized LDL exacerbates ED by augmenting the ROS production (Gürel, 2009). Besides, oxidized LDL mediates the foam cell formation in the atherosclerotic plaques after being taken up by macrophages. Macrophages phagocytose oxidized LDL and store it in the form of cholesterol esters that accumulate in the cell as fatty droplets. This process continues until the macrophage transforms into a lipid-loaded foam cell (Libby, 2000; Lusis, 2000; Zengin, 2012). Hypercholesterolemia impairs the NO-dependent vascular dilatation. NO is a fast-reacting locally active agent that is produced after catalysis by nitric oxide synthase (Ignarro, 1989). NO initiates vasodilation by activating guanylate cyclase within the smooth muscle cells which results in intracellular cGMP elevation. Eventually, this dephosphorylates weak myosin chains and leads to a reduction in intracellular Ca^{2+} levels and vasodilatation. Hypercholesterolemia reduces the NO levels as well as inactivates the NO via oxygen radicals produced due to the inflammation appearing during atherogenesis (Gültekin, Ersanlı, & Küçükateş, 1996).

3.1.3. Diabetes Mellitus

Diabetes is a metabolic disease characterized by high fasting and satiety plasma glucose levels (Zoghi & Nalbantgil, 2002) and it is among the capital risk factors for cardiovascular diseases (Keskin & Balcı, 2011). Hyperglycemic conditions cause oxidative stress and increase antioxidant enzymes in human ECs (Ceriello, dello Russo, Amstad, & Cerutti, 1996). Previous studies reported that hyperglycemic status directly leads to ROS formation (Giugliano, Ceriello, & Paolisso, 1996). Considering the fact that ROS is one of the factors involved in the NO bioavailability loss and thereof ED emergence, hyperglycemia can be accounted for as one of the ED risk factors (Harrison, 1997). ED plays a pivotal role in the onset and progression of atherosclerosis as the major mechanism of diabetic macrovascular complications (Jiang et al., 2015). The high glucose levels in diabetic patients often cause elevations in TNF-α, IL-6, and IL-1β and thereof boost inflammation (Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995; Spranger et al., 2003). The elevating levels of TNF-α, IL-1β, and NF-κB increase atherosclerosis together with cardiovascular complications (Ait-Oufella, Taleb, Mallat, & Tedgui, 2011; Kleemann, Zadelaar, & Kooistra, 2008; Popa, Netea, van Riel, van der Meer, & Stalenhoef, 2007; Ramji & Davies, 2015; Shanmugam, Reddy, Guha, & Natarajan, 2003; Tedgui & Mallat, 2006). The increasing levels of growth factors in

diabetes mellitus induce both ET-1 upregulation and smooth muscle cell proliferation and neovascularization. Insulin paves the way for inflammation by increasing ET-1 secretion while hyperglycemia-inducing glycation end products promote proinflammatory cytokine secretion by interacting with ECs (Coco et al., 2019; Gürel, 2009).

3.1.4. Hypertension

Endothelium under normal conditions keeps vascular homeostasis against mechanical and biochemical stimuli. The disturbance in endothelial vasodilatation-vasoconstriction balance lays the ground for hypertension (Gürel, 2009). The altered expression of endothelial mediators during ED increases peripheral vascular tonus. NO, secreted from the endothelium, supports the regulation of arterial blood pressure via relaxing the smooth muscle cells beneath the endothelial layer (Park & Park, 2015; Zoghi & Nalbantgil, 2002). The attenuated NO secretion and the weakened response of vascular smooth muscle cells to NO cause the dysregulation of endothelium-dependent vasodilation which is attributed to the essential hypertension. During hypertension, despite the normal levels of ET-1, as a strong vasoconstrictor, it has been asserted that ET-1-mediated vasoconstrictive activity can remain high (Gürel, 2009; Zoghi & Nalbantgil, 2002).

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