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Research & Reviews in Health Sciences

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gece kitaplığı

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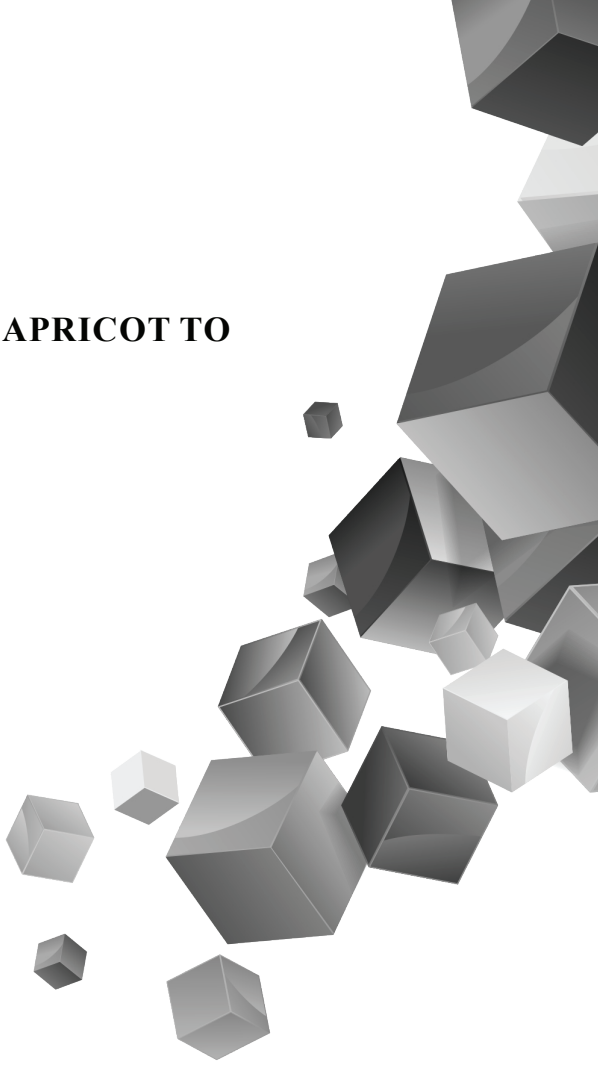
VARIOUS BENEFITS OF APRICOT TO OUR HEALTH



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Alternative medicine practices are increasingly being applied every day in order to protect healthy individuals from diseases, reduce the amount of chemicals exposed to modern drugs in sick individuals, or often support the existing treatment procedure. It has been seen that approximately 80% of the world population uses herbal medicines in the field of health services and the number of patients seeking alternative and herbal treatment continues to increase day by day. Several in vivo and in vitro studies have reported that the components of fruit / dried fruits have beneficial effects on several health disorders. Apricot (Prunus armeniaca L.) is a rich source of bioactive components such as thiamine, riboflavin, niacin, pantothenic acid, vitamin B6 and folic acid, vitamins such as vitamin C, minerals such as potassium, calcium, magnesium, carotenoids and polyphenols, which are important for its effects on health. In this study, it is presented the latest scientific evidence on apricot and apricot kernel bioactives and their benefits to health.

Alternative Medicine and Fruits

The increase in consumer demand for naturalness, the increase in the orientation to the bioactive and functional components of plant-based foods, fruits and vegetables and the increase in the incidence of chronic and incurable diseases such as diabetes, cancer, HIV / AIDS and arthritis, have sparked interest in alternative medicine studies in recent years. The use of Complementary and Alternative Medicine (CAM) is increasing rapidly in the treatment of many diseases (Farooqui et al, 2016:6).

While The National Center for Complementary and Alternative Medicine defines CAM as "a group of medical and health care systems, practices and products not currently considered as part of traditional medicine" and complementary medicine is used conjugated with conventional therapy, alternative medicine is used instead of traditional medicine (Ernst, 2000:321). It is seen that approximately 80% of the world population uses herbal medicines in the field of health services, and the number of patients seeking alternative and herbal treatment continues to increase day by day (Kamboj, 2000:78).

There are more than 100 different treatment types under the title of CAM treatments. However, five different clinical disciplines (acupuncture, chiropractic, herbal medicine, homeopathy and osteopathy) come to the fore as they are based on educational and professional standards. CAM treatments are recommended for chronic pain affecting the spine, joints and muscles, control of nausea, eczema and other skin complaints, asthma, cancer, and migraine etc. (Saks, 2001:49).

Several in vivo and in vitro studies have reported that the components of fruit / dried fruits have beneficial effects on lipid, modulation of carbohydrate metabolism, liver function, appetite control, cardiometabolic syndrome, and tumorigenesis (Alasalva, Salvadob, Ros, 2020:314)

Apricot (Prunus sp.):

Apricot is one of the temperate tree fruit species with a total production of approximately 2.6 million tons in the world; Among the main producers, the leading country is Turkey (370,000 tons), Iran (285,000 tons) and Italy (244,000 tons) are among the other producing countries (Gatti, Defilippi, Predieri, Infante, 2009:7). Important apricot varieties grown in Malatya province in Turkey, where 80-85% of the world's dried apricots are exported, are known as Hacıhaliloğlu, Kabaası, Hasanbey, Soğancı and Çataloğlu (Otlu, Kıran, Karabulut, Karabulut, 2019:25;Yıldırım, Tilkat, Onay, Ozen, 2007:2). All these ingredients, which increase the nutritional value, make apricots a medicinal golden nutrient. The development and nutraceutical properties of apricots vary according to their varieties, cultivation method, storage conditions, fruit and development stages (Bae et all,2014:87).

Apricot (Prunus sp.), which has an important place in the world, is a member of the Amygdaloideae (Prunoideae) subfamily, which includes the most common stone fruit genus from the Rosaceae family including about 100 genera and about 3.100 species (Hummer and Janick,2009:1;Shi, Li, Sun, Zhou,2013:55).

Apricot (Prunus armeniaca L.) is a rich source of bioactive components such as thiamine, riboflavin, niacin, pantothenic acid, vitamin B6 and folic acid, vitamins such as vitamin C, minerals such as potassium, calcium, magnesium, carotenoids and polyphenols, which is important for its effects on health (Voi, Impembo, Fasanro, Castaldo, 1995:8; Hegedus et all., 2010:75). Apricot contains mono-poly saccharides, proanthocyanidins, hydroxycinnamic acid derivatives, polyphenols containing both hydro and lipophilic components, and antioxidant types such as anthocyanins (Schmitzer et all., 2011:91; Ruiz, and Egea, 2008:163).

Use of Apricot in Health Sciences Research:

It is known that red, orange, yellow and dark green fruits and vegetables generally contain high amounts of carotenoids such as β-carotene, lutein and lycopene. Eating the recommended amount of fruits and vegetables every day meets the intake of β-carotene and other carotenoids as we need. β carotene has been shown to react with peroxyl (ROO), hydroxyl

(OH) and superoxide (O2 -) radicals (El-Agamey et all., 2004:430). It has also been reported that β-carotene can control transcription factors (Niles 2004:555; Donato and Noy, 2005:65).It is known that regular consumption of fruits and vegetables in sufficient quantities provides high protection in many cancers such as epithelial cells, lung, cervix, esophagus, stomach, colon and pancreas (Winston,1997: 97).

A natural pigment lycopene made by plants is an oil-soluble carotenoid. Besides protecting plants from stress, lycopene helps them utilize solar energy to make food. Lycopene is found in fruits and vegetables as well as apricots. Lycopene taken with fruits and vegetables has been reported to positively affect the antioxidant activity and communication between cells (Rao and Agarwal,1999:19).

It has been reported by Rakhmanov et al., that the natural protein-vegetable diet including apricot has positive effects on fat and carbohydrate metabolism and liver function. It has been reported to have an improvement in atherogenicity index, decrease in systolic arterial pressure and diastolic arterial pressure, and positive effects on cardiovascular system function in patients with hypertension (Rakhmanov, Istomin, Narutdinov, Kropachev, 2014:83)

In a study of 1358 H. pylori positive Japanese adults who were not aged, It was determined that the anti-H. pylori IgG antibody titres were significantly lower in the group in which apricots, rich in polyphenols and having significant antioxidant activity, were added to the diet. (Enomoto et all., 2010:64). In a case-control study on dietary habits in Portugal, it was reported that the group with high consumption of fruit and dairy products had a lower risk of stomach cancer (Bastos, Lunet, Peleteiro, Lopes, Barros, 2010:127). It is known that polyphenolic compounds and flavonoids detected in apricot fruit have antioxidant activity (Greger and Schieberle 2007:55; Ahmed, Rashid, Mansoor, Ansar, 2002:117). On the antiradical activity of apricot fruit studies by radical scavenging methods such as DPPH / copper ion reduction antioxidant capacity (CUPRAC) / 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) / hydroxyl / superoxide / Trolox-equivalent antioxidant capacity have also shown that it has high radical scavenging activity and has an oxidative damage healing, preventive and antibacterial effect (Ugras et all., 2010:30; Vardi, Parlakpinar, Ozturk, Ates, Gul, Al,2008:46; Leccese, Bartolini, Viti, 2008:37; Rashid, Ahmed, Bibi, Kazmi, Ansar, 2005:6).

Two new flavonoid group compounds in the form of 4′,5,7-trihydroxy flavone-7-O-[β-D-mannopyranosyl (1‴→2″)]-β-D-allopyranoside (1) and

3,4′,5,7-tetrahydroxy-3′,5′-di-methoxy flavone 3-O-[α-L-rhamnopyranosyl (1″→6″)]-β-D-galactopyranoside have been isolated. It has been determined that different extracts obtained from the fruit have antibacterial activity against Gram positive, Gram negative bacteria and fungi (Rashid, Ahmed, Mahmood, Ahmad, Bibi, Kazmi, 2007:30; Abtani, Ghazavi, Karimi, Mollaghasemi, Mosayebi, 2008:8; Abtani, Ghazavi, Karimi, Mollaghasemi, Mosayebi, 2008:8)

In vivo cardio protective activity of apricot feed with respect to antioxidant phenolic content in rats exposed to myocardial I / R (Parlakpinar, Olmez, Acet, Ozturk, Tasdemir, Ates, 2009:47). Prunus armeniaca fruit shows antitubercular activity (Sehgal, Siddheswaran, Kumar, Karthiyayini, 2010:2)

In their study with Wistar rats, they reported that feeding with apricot feed had beneficial effects on liver steatosis caused by CCl4 due to its antioxidant nutrient (β-carotene and vitamin) content and high radical scavenging capacity. They reported that dietary intake of apricots may reduce the risk of liver steatosis and damage caused by free radicals. (Ozturk, Gul, Ates, Ozturk, Cetin, Vardi, 2009:102)

Amygdalin and Amygdalin Metabolism

Amygdalin (D (-)-mandelonitrile-β-D-gentiobioside) is one of the cyanogenic di-glycosides found in high amounts in plants belonging to the Prunus genus of the Rosaceae family. It is usually found in the seeds of fruits such as apricots, almonds, cherries, apples, plums, pears and peaches. Kernels containing high amounts of amygdalin have a bitter taste (Çelik and Yıldırım,2017:6; Barakat, 2020:61).

Amygdalin consists of benzaldehyde, nitrile, and two molecules of glucose (Sireesha, Reddy, Reginald, Samatha, Kamal, 2019:23) while benzaldehyde may have analgesic and anti-cancer effects, the nitrile group may have anticancer properties (Chang et all., 2006:29). The reduction of amygdalin is mainly due to-galactosidase enzymes. Cyanogenic glycosides taken into the body by humans and animals can form hydrogen cyanide (HCN) with the effect of intestinal microorganisms or plant food-derived enzymes. Enterobacter aerogenes, Streptococcus fecalis, Clostridium perfringens and Bacteroides fragilis are bacteria that can form HCN from amygdalin in human intestine(Cresseya and Reeveb, 2019:125; Nyirenda, 2020). The destruction steps of amygdala are given in Figure 1. with the complete destruction of 1 g amygdaline, 54 (0.054 g) mg of HCN is released

(Kovacikova et all., 2019:103). There are several ways in which cyanide is metabolized in humans.

Approximately 80 % of the cyanide taken into the body is converted to thiosionate (SCN-) by sulfurizing in the presence of thiosulphate by the activity of the rhodanase (thiosulfate sulfur transferase) enzyme in the liver, and thiosionate is also excreted in the urine. In addition, 3-mercaptopyruvate sulfur transferase enzyme provides the conversion of cyanide to thiosionate. Although the concentration of enzymes that enable the conversion of cyanide to thiosionate varies according to tissues, it has been reported that the highest amount is observed in the liver and kidneys. Cyanide reacts with the amino acid cysteine to convert aminothiazoline carboxylic acid and iminothiazolidine carboxylic acid (Speijers,1993:30; Hea, Wua, Wang, Xie, Chena, Wang,2020:254; Liczbinski and Bukowska, 2018:40).

Amigdalin Amount Found in Apricot Kernels:

The amount of amygdalin found in apricot seeds is affected by factors such as the cultivated geography, the structure of the soil and the storage of the seeds. However, it is known that bitter apricot seeds contain more amygdalin than sweet seeds. Karsavuran et al. reported that the amount of amygdalin found in bitter apricot seeds belonging to different species in Malatya province was between 13 mg/g and 44 mg/g (average 26 mg/g) (Karsavuran, 2015:40). In another study conducted in Malatya province, it was reported that the average amount of amygdalin in bitter apricot seeds was 55.5 mg/g (Yildirim and Askin, 2010:9). In another study, they reported that the amount of amygdalin obtained from apricot seeds obtained from Rosaceae species was 14.4 mg/g (Bolarinwa, Orfila, Morgan, 2014:152).

Use of Apricot Seeds in Health Sciences Research:

It has been reported that amygdalin treatment in cancer cells results in a synergistic reaction of hydrogen cyanide and benzaldehyde, resulting in a toxic compound capable of destroying the cancer cell (Song and Xu, 2014:10). In another study, it was reported that intramuscular injection of amygdalin in rabbits caused less hepatic cyanide toxicity compared to oral administration, and intramuscular administration of 0.6-3 mg / kg amygdalin during 14-day follow-up did not have a significant side effect (Kovacikova, 2019:103). In the study conducted by Duracka et al, it was reported that 0.6-3 mg / kg amygdaline had an antioxidant effect by reducing reactive ROS production in rabbits (Duracka et all., 2016:23). The

core of P. armeniaca has been reported to show anti-inflammatory activity (Hwang, Kim, Kim, Lee, Shim, Yin, 2008:31).

In rats with liver necrosis with dimethylnitrosamine, 0.5mg/kg, 1 mg/kg and 1.5 mg/kg of ground apricot kernels were added to the feed, respectively, and it was aimed to observe the effectiveness of different doses of apricot kernel. According to the results of this study, a significant decrease in serum ALT, AST and MDA levels in all groups receiving ground apricot kernel; increased enzyme activities of antioxidant systems such as SOD, CAT and GSH-Px have occurred. As a result of the histopathological examination performed in the same study, it was stated that liver necrosis regressed in the groups consuming ground apricot kernels and the dose of 1.5 mg / kg was the most effective dose in the said regression (Abdel-Rahman, 2011:10).

In the in vitro study conducted by Cassiem and Kock, seed extracts obtained from apricots collected from different regions were treated with colon cancer cells at different doses and for different durations, and proliferation and cell cycle data of cancer cells were obtained. According to these results, it has been observed that apricot extracts slow down the proliferation of cancer cells, cause morphological changes and affect the cell cycle. However, it has been stated that these effects differ depending on the dose and duration of administration (Cassiem and Kock, 2019:19).

In a study investigating the effects of different doses of apricot extract on oral squamous cell cancer, it was reported that apricot extract slowed the proliferation of cancer cells and this effect was observed at the highest level at a dose of 100µg/ml. In the same study, IC50 value of apricot extract was calculated as 61 µg/ml (Sireesha, Reddy, Reginald, Samatha, Kamal, 2019:3). The effects of amygdalin on cell growth and oxidative stress in human breast cancer cell lines were investigated. The fast growing cell line MCF7 has proven to be more sensitive to amygdaline than the slow growing cell line T47D. Amygdaline has been reported to decrease glutathione reductase activity, and high reactive oxygen species (ROS) levels in T47D in MCF7 (Abboud, Al Awaida, Alkhateeb, Abu-Ayyad, 2018:1). It has been reported that amygdalin and a monoclonal antibody-glucosidase companion play an active role in the treatment of bladder cancer cell lines and the therapeutic effects of amygdaline are enhanced by the ADEPT method (Syrigos, Rowlinson-Busza, Epenetos, 1998:78)

In the study conducted by Kim et al. apricot seed extract was applied topically at doses of 0.5 mg/ml and 1 mg/ml to the eyes of rats in which keratoconjunctivitis was induced, and it was stated that dry eye due to

conjunctivitis decreased in rats as a result of this application. In the same study, it was observed that corneal epithelium damage was significantly reduced, corneal integrity was regained and mucin-4 layer was improved in the 1 mg / ml dose of apricot seed applied group. In addition, as a result of biochemical analyzes performed on corneal conjunctival cells, it has been reported that apricot seed extracts decrease the expression of inflammatory markers such as matrix metalloproteinase-9, TNF-α and IL-6 (Hyun et all., 2019:24).

In the study performed by Raj et al., apricot seed extract at doses of 200 mg / kg and 400 mg / kg was administered to rats with liver damage by giving nicotine. In rats receiving 400 mg / kg dose of apricot seed extract, SGOT, SGPT, CRT, GGTP and Total bile acids levels decreased, albumin and total protein levels increased compared to the group given only nicotine. Also, improvements were observed in plasma HDL, LDL, ALP, TC, TG, TBL and DBL levels for both doses of apricot seed extracts (Raj, Mishra, Mishra, Khan, 2019:9). There was an increase in liver function parameters induced by carbon tetrachloride injection in rats. After application of 70% and 99.9% ethanolic apricot seed extracts, serum AST, ALT, ALP total, direct bilirubin, albumin, total proteins, alpha-fetoprotein, malondialdehyde (MDA) and nitric oxide (NO) levels decreased, hepatic reduced glutathione (GSH) It was determined that the level increased, showed anticancer activity against hepatocellular carcinoma (Ramadan, Kamel, Awad, Shokry, Fayed, 2020:9)

Kovacikova et al., in a study they conducted on rabbits, gave pure amygdalin to a group of experimental animals intramuscularly, and apricot kernel orally to animals in another group, and analyzed biochemical and hematological data for a period of 14 days. With this study, it was stated that there were no significant differences in the biochemical and hematological measurements of the experimental animals and even if the amount of amygdalin was high, no health threatening situation was observed in 14 days (Kovacikova et all., 2019:103).

Yamshanov et al. transplanted two different types of tumors (lymphosarcoma and Ehrlich carcinoma) into experimental animals and observed the effects of eating apricot kernels with feed, and investigated that tumor sizes were reduced in both tumor types and the life span of rats was prolonged. In the same study, it was stated that treating apricots with 100-110 degrees heat did not cause a decrease in antitumor activity, but could reduce intoxication (Yamshanov, Kovan'ko, Pustovalov, 2016:160). In another study investigating the effect of apricot seed oil in rats with

immunosuppression with cyclophosphamide, researchers observed that serum IgA, Ig M and Ig G levels increased, and the release of cytokines such as IL-2, IL-12 and TNF-a increased in rats treated with apricot oil. In liver tissue examinations, they reported that oxidative stress decreased and degeneration caused by immunosupression in other organs was prevented (Tian et all., 2016:51).

Rafaat et al. reported that all of the seed extracts regulated insulin and blood sugar levels, but infrared-supported core extracts were less toxic, in their studies investigating the effects of apricot seed extracts obtained by different extraction methods on diabetic rats (Raafat, El-Darra, Saleh, Rajha, Maround, Louka, 2018:29).

In a study where pure amygdaline was applied on cervical cancer cell lines (HeLa), decreased viability of amygdalin-treated cells, decreased Bcl-2 expression, increased Bax and Caspase 3 proteins, and increased apoptosis rate of cancer cells. In the same study, it was reported that the growth of tumor tissue was inhibited as a result of the application of amygdalin in HeLa cancer tissue transplanted to experimental animals (Chen et all., 2013:35). In the studies of our group, it has been found that massaging with apricot seed oil after heavy exercise reduces oxidative stress and the fatigue period after exercise lasts shorter (Kafkas, Karabulut, Kafkas, Otlu, 2013:172). In another study where we investigated the effect of adding bitter apricot seeds to the diet on liver damage caused by carbon tetra chloride, we determined that there were significant changes in serum and liver oxidative stress parameters in the groups eating apricot kernel and this effect was also observed histo-pathologically (Karabulut et all., 2014:2).

Toxicity Cases Related to Apricot Seed Consumption:

As a result of the search made in PubMed with the words “cyanide toxicity apricot”, it was observed that there are four case reports of individuals who experienced poisoning as a result of apricot seed consumption in the last 50 years (1969-2021).

In two of the four reports in question, individuals experiencing toxicity consumed more apricot seeds (8-30 apricot seeds) than the recommended daily amount (2-3 seeds/day) (Akıl, 2013:44; Konstantatos, Kumar, Burrell, Smith, 2017). In one of them, it was reported that toxicity occurred due to a food supplement that the manufacturer provided for consumption without determining the amount of cyanide (Sivakumaran, Lajevardi, Wright, Shaw, Halder, 2015). In the last case report, it was reported that a 67-year-

old male patient who consumed apricot seed extract every day for 5 years without the recommendation of a specialist, showed a hypoxic picture during general anesthesia and the patient's data returned to normal when the use of the extract was discontinued (Suchard, Wallace, Gerkin, 1998).

A 58-year-old male patient who received palliative chemotherapy for metastatic colon carcinoma used 70 apricot kernels in small pieces a day for 45 consecutive days until one week before his outpatient clinic visit. Liver tests were found to be abnormal in the peripheral blood analysis of the patient. After the last apricot seed intake, thiocyanate concentration of 71 mg/l per week was measured, considering the first order kinetics and the elimination half-life calculated as 9.5 days, it was predicted that the thiocyanate concentration on the last intake day could be 118 mg/l toxic (Seghers, Veen, Salome, Hamberg, 2013:).

45-day-old 16 male rabbits were divided into four groups (control group without apricot kernels) and fed with crushed apricot kernels at doses of 60, 300 and 420 mg / kg b.w mixed with commercial feed. Morphometric evaluation of rabbit livers after the application of apricot seed showed that the binuclear cells increased in the vena central region at the highest dose compared to the control and in the peripheral region at all doses. 300 and 420 mg / kg b.w. Significant inhibition of the number of binuclear cells in the central zone at doses, in the peripheral zone at all doses used has been observed (Kolesárová et all., 2020:7). In another study, amygdalin cytotoxicity was determined in liver (Huh-7) and colorectal (HT-29) cancer cell lines as an IC50 of 100 μM and 30 μM, respectively (Badr, Wahdan, AbdelFattah, 2020:7). In another case report, a 3-year-old girl had respiratory distress and coma following tonic-clonic convulsions after eating 3 apricot seeds. The presence of severe metabolic acidosis (pH 6.91, bicarbonate [$HCO3^-$] 5.6 mEq/L, base excess−26.0 mEq/L), blood cyanide level was reported to be 3.15 mg/L 3 hours after ingestion. Since he was brought from a medical center every 4 hours, hydroxocobalamin could not be administered immediately, and clinical improvement was reported after a 3-hour hemodialysis session (Dalkıran, Kandur, Ozaslan, Acıpayam, Olgar, 2020:36).

Conclusion:

Alternative medicine practices are increasingly being applied every day in order to protect healthy individuals from diseases, reduce the amount of chemicals exposed to modern drugs in sick individuals, or often support the existing treatment procedure. Thus, we present the latest scientific evidence on apricot and apricot kernel bioactives and

their benefits to health. Apricot fruit has a complementary nutritional profile and bioactives and can be included in a healthy diet as a snack, as a supplement to daily fruit consumption, or in recipes such as dried fruit salads, cakes, rice.

The sugar part in the chemical structure of amygdaline ensures that it has appropriate biological properties that lead to increased pharmaceutical effects against various diseases such as cancer. Enzymatic digestion leads to the release of hydrogen cyanide, which can have anti-tumor effects. Hydrogen cyanide is known to have negative effects on healthy cells. The benefit of consuming apricot kernel or seed extracts due to the amygdalin it contains has been demonstrated by scientific studies. For this reason, apricot seed-based products are used as a supportive product in many countries. However, it should be kept in mind that cyanide toxicity may occur as a result of unconscious use and high consumption. For this reason, we would like to underline that, just like modern drugs, foods related to alternative treatment should be used on expert advice and at doses and times determined by the expert. Similarly, we recommend that children should not consume bitter apricot kernels but if they do, it must be under the supervision of their parents. Monoclonal antibodies cross-linked with the-glyco-sidase enzyme increase the degradation of amygdalin by binding to the specific antigen on cancer cells, and cancer cell death can be targeted by the final release of cyanide at the tumor site. By encapsulating amygdaline with nanoformulation, its effect can be enhanced by gradual release of amygdalin by transporting it to the target cancer site.

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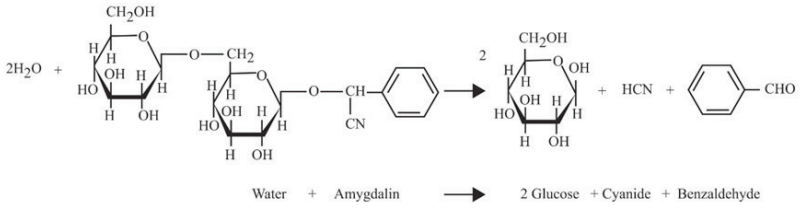
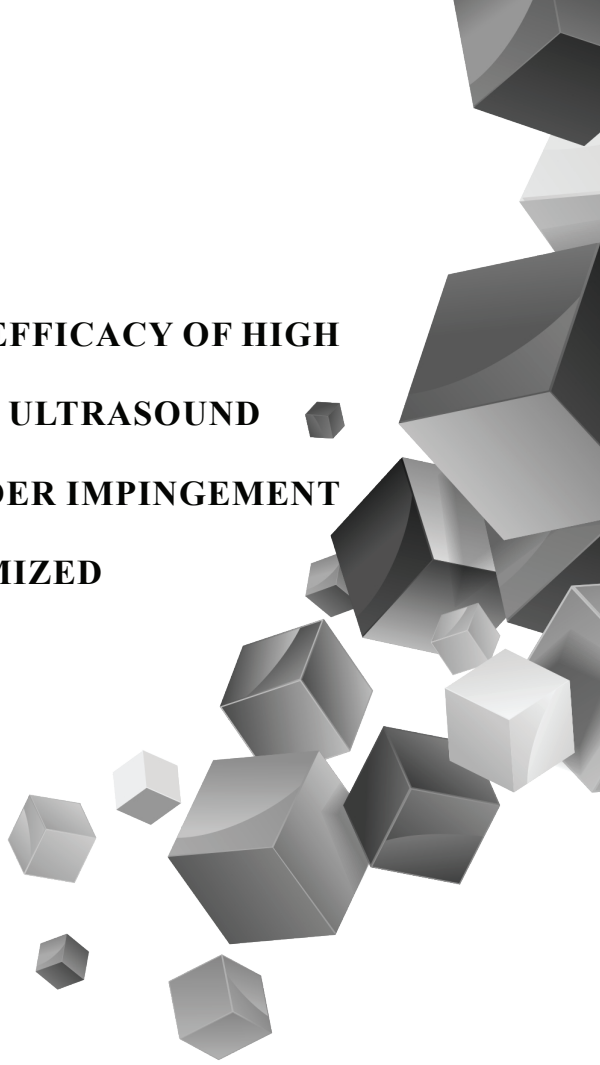
Figure 1: The destruction steps of amygdalin.



Chapter 2

COMPARISON OF THE EFFICACY OF HIGH INTENSITY LASER AND ULTRASOUND THERAPIES IN SHOULDER IMPINGEMENT SYNDROME: A RANDOMIZED CLINICAL TRIAL



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INTRODUCTION

Subacromial Impingement Syndrome (SIS) commonly causes shoulder pain and is an impingement of the supraspinatus tendon, subacromial bursa and bicipital tendon between the coracoacromial arch and caput humeri (Calıs&Berberoglu 2011:47). SIS was first described by Neer. Neer stated that SIS was responsible for more than 95% of rotator cuff ruptures and classified the SIS into 3 stages. The 1st stage is characterized by hemorrhage and edema in the rotator cuff and subacromial bursa. Stage 2 includes irreversible rotator cuff alterations including tendinopathy and fibrosis. Stage 3 involves chronic changes including partial or total rotator cuff rupture (Thortnton&McCarty 2013:22, Bal&Eksioglu 2009:27).

Often, SIS causes discomfort and pain of the lateral and anteroposterior localization of the shoulder spreads to the deltoid and biceps region. Pain increases with forced internal rotation, especially during night and abduction (Yavuz&Duman 2014:27). There are many accepted standard methods for the treatment of SIS such as nonsteroidal anti-inflammatory drugs, corticosteroid injections and physiotherapy applications. There has been little evidence to prove or refute the effectiveness of these treatments. A large part of conservative treatment includes physiotherapy applications and these applications are usually the first step of treatment (Abrisham&Kermani 2011:30).

Therapeutic ultrasound (US), bipolar interferential current, laser therapy, extracorporeal shock wave therapy (ESWT), transcutaneous electrical nerve stimulation (TENS) and pulse electromagnetic field therapy are used in physiotherapy applications (Santamato, Solfrizzi, Panza, Giovanna, Vincenza, Brian 2009:89). Different results have been reported in the literature regarding the efficacy of physiotherapy applications.

Ultrasound is a commonly used treatment in physical therapy (Webster, Harvey 1980:89). Although contradictory results have been reported about the efficacy of US, the Philadelphia panel included US therapy as an acceptable physical therapy in SIS in their recommendations (Philadelphia 2001:81).

Laser treatment is based on the belief that light can change the function of the cell and tissue depending on wavelength and/or coherence of the laser beam.[6] The main mechanisms of laser treatment are believed to be induction of cell proliferation, stimulation of protein and collagen

synthesis, acceleration of tissue repair and wound healing and relief of pain. All of these biological effects are secondary to the direct effects of photonic radiation rather than the thermal effects of laser beams (Yavuz&Duman 2014:27).

Low-level laser therapy (LLLT) has been used in multiple rheumatologic and musculoskeletal diseases. LLLT has been documented for its analgesic, anti-inflammatory and biostimulant effects. Different results have been reported regarding the efficacy of LLLT in the treatment of SIS. There are studies stating that LLLT is an effective treatment in reducing functional loss or disability in patients with SIS, but studies indicating that it is not superior to placebo (Yavuz&Duman 2014:27, Yeldan&Cetin 2009:31, Bigliani&Morrison 1986:10).

High-intensity laser therapy (HILT) has been recently used in treatments. The rays are obtained by small and slow absorption of light through chromophores. This absorption is obtained not by concentrating the light but by stimulating the tissues due to increased mitochondrial oxidative reaction and production of adenosine triphosphate, RNA or DNA as a result of diffuse propagation of light in all directions (photobiological effects). There have been few studies in the literature evaluating the effectiveness of HILT in shoulder tendinopathies (Santamato, Solfrizzi, Panza, Tondi, Frisardi, Leggin 2009:89).

The purpose of this study was to evaluate the efficiency of HILT and US therapy in the treatment of SIS and also to compare the effectiveness of these two treatments to determine whether they have superiority to each other.

METHODS

This prospective and randomized clinical study was performed on patients with diagnosis of SIS who presented to the Physical Medicine and Rehabilitation Outpatient Clinic of Adıyaman University Faculty of Medicine. Ethical approval was obtained from the local ethics committee of the university before the study (Adıyaman University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee). This study was conducted following the principles of the Declaration of Helsinki. All participants were informed about the study and written informed consent was obtained from the participants before they were enrolled in the study. Patients with chronic pain were included in the study. The diagnosis of Subacromial impingement syndrome was established by the same physiatrist in the outpatient clinic. For the diagnosis of SIS,

Neer test, Hawkins test, presence of pain following active elevation of the shoulder and presence of pain following isometric resistive abduction were evaluated. Those with at least three positive results from these tests were included in the study. The clinical diagnosis was established with shoulder magnetic resonance imaging (MRI) evaluations performed by the same radiologist. After imaging, a total of 70 patients between the ages of 20 and 65 with stage 1 or stage 2 impingement were included.

Exclusion criteria included: a history of direct trauma to the shoulder, rotator cuff rupture on MRI, inflammatory rheumatic disease, neurological disease, active infection, pregnancy, malignant disease, a history of extrinsic disease reflected on the shoulder such as cervical spondylitis, a history of any physical therapy within 6 months prior to the study, a history of previous intra-articular or subacromial steroid injection or the diagnosis of adhesive capsulitis.

Participants were randomized into two groups. Group 1 (n = 35) was the HILT group and Group 2 (n = 35) was the US therapy group. The treatment was performed over 10 total sessions for 2 consecutive weeks (5 times a week) in both groups by the same physiotherapist.

An infrared laser [gallium-aluminum-arsenide (GaAlAs)] (Chattanooga, TN, USA), 850 nm wavelength, 100 mV output, continuous wave and a 0.07 cm^2 spot area was used. HILT was applied with a dosage of 3 joule/cm^2 (15 joules total), according to the World Association of Laser Therapy (WALT) standard for the treatment of shoulder disorders, at a maximum of five painful points for one minute at each point over the subacromial region of the shoulder. HILT was applied for each patient for five minutes. During the laser treatment, the patient and physiotherapist used protective glasses for safety.

A Pagani DT-20 US device (Electronica Pagani S.R.L., Italy) was used for the US therapy. US therapy was administered to the area over the subacromial region of the shoulder with slow circular movements, at a frequency of 1 MHz and an intensity of 2 W/cm^2, in continuous mode. US therapy was applied for each patient for five minutes.

All participants were asked not to use any analgesic or anti-inflammatory drugs and not to engage in any activity that would force the shoulder in their daily living activities during the study.

Outcome parameters

The outcome measures were severity of shoulder pain and the Shoulder Pain and Disability Index (SPADI). Pain severity was determined by a visual

analogue scale (VAS), on which the patients could indicate their assessment along a 100 mm line ranging from 0 ("no pain at all") to 100 ("worst pain ever"). The functional status was evaluated using the SPADI, which is a self-administered shoulder-specific questionnaire including pain and disability subscales (Roach&Budiman-Mak 1991:4). The pain subscale consists of five items and the disability subscale consists of eight items. Subjects were asked to answer each item using a 0-100 mm VAS. The SPADI score ranged from 0 to 100, with high scores indicating high disability.

In order to evaluate supraspinatus local sensitivity score (SLSS) objectively, pain measurement was performed through scapular notch using Algometry before and after treatment in all patients. The pressure algometer used in algometric measurement is a metal piston with a round rubber disc of 1 cm at the end connected to a dial where the pressure is measured in kilograms. The dial is arranged to show up to 17 kilograms in 200 gram sections. During the application, it was applied perpendicularly to the skin and by increasing the pressure at a constant speed. The values obtained were pressed 3 times with the algometer to the limit of pain and averaged.

All measurements were conducted and recorded by the same physiatrist at the beginning of treatment, at the completion of treatment and one month after treatment.

Statistical analysis

The number of patients to be included in the study was calculated using the G-power software package for power analysis (ver.3.1.9; Franz Faul, Kiel University, Kiel, Germany). Since two groups and three measurements will be made, comparing the averages within the group and analyzing the interactions between the groups_according to the 'Repeated measures ANOVA' test, the total sample number of 0.95 power and 0.05 significance level was determined as at least 66 with a 0.20 effect size.

SPSS (ver.15, SPSS Inc, Chicago III, USA) statistics program was used for statistical analysis. Categorical variables were shown as percentages, continuous variables as mean ± SD and median (minimum-maximum). T test was used for independent variables in comparing the means of two independent groups. Repeated measures ANOVA test was used to analyze the pre-treatment, post-treatment and 1-month post-treatment measurements of the groups. The significance level was accepted as $p<0.05$ in all statistical analyzes.

RESULTS

There was no statistically significant difference between the groups in terms of demographic and clinical data (p> 0.05) (Table 1).

Table 1: *Demographic and clinical features*

	Group I	Group II	p^1
Number of patients (M/F)	35 (14 / 21)	35 (12 / 23)	
Age (years) (mean±SD)	46.5 ± 9.6	47.7 ± 7.8	0.607
Pain duration (weeks) (mean±SD)	40.0 ± 96.7	37.0 ± 57.7	0.887

M: male; F: female; [1]: t-test for equality of means

The decreases in VAS and SPADI scores at the end and one month after treatment were statistically significant in both groups (p <0.001, p <0.001, respectively). The mean SLSS score was decreased in both groups but it was not statistically significant (p> 0.05) (Table 2).

Comparing groups, the improvements in VAS and SPADI scores of the group 2 (US therapy group) were significantly higher than those of the group 1 (HILT group). This significance was more prominent in the first month. When the measurements at the end of treatment were taken into consideration, no difference was found between the groups in terms of improvements in VAS scores (Table 3).

Table 2: *Comparisons of before treatment vs. after treatment (repeated measures) scores*

	Before treatment	After treatment		Repeated measures ANOVA	
		Immediate	1 month	***p***	***p***
	(t_0)	(t_1)	(t_2)	**(t_0 vs. t_1)**	**(t_0 vs. t_2)**
Group I					
VAS	6.4 ± 1.1	4.6 ± 2.0	4.9 ± 1.3	**<0.001**	**<0.001**
SPADI	64.4 ± 15.2	49.1 ± 16.9	49.9 ± 13.6	**<0.001**	**<0.001**
Mean SLSS	61.4 ± 22.2	59.1 ± 19.0	56.8 ± 9.9	0.516	0.257
Group II					

VAS	6.3 ± 1.1	4.0 ± 1.6	3.7 ± 1.6	**<0.001**	**<0.001**
SPADI	64.5 ± 13.5	40.0 ± 17.0	38.6 ± 15.4	<0.001	**<0.001**
Mean SLSS	65.1 ± 19.7	60.6 ± 12.3	58.0 ± 6.9	0.255	0.077

VAS: visual analog scale; SPADI: shoulder pain and disability index; SLSS: supraspinatus local sensitivity score; t_{0-2}: time periods of measurements.

Table 3: *Group comparisons and group-time interactions*

Parameter	Repeated measures *(interactions between time and groups)*		ANOVA
	p^1	p^2	p^3
VAS	**0.031**	0.244	**0.005**
SPADI	**0.026**	**0.049**	**0.025**
Mean SLSS	0.489	0.672	0.647

VAS: visual analog scale; SPADI: shoulder pain and disability index; SLSS: supraspinatus local sensitivity score; 1:p values summarized from "Tests of Between-Subjects Effects" table; 2,3: p values summarized from "Tests of Within-Subjects Contrasts" table [2: before treatment vs. immediate after treatment; 3:before treatment vs. 1st month after treatment]

DISCUSSION

In this study, both US therapy and HILT were shown to be effective in reducing shoulder pain and improving shoulder functions. However, reduction in shoulder pain and improvement in shoulder functions were increased in the US group when compared to the HILT group. This increase was more prominent in the first month.

The efficacy of US therapy on SIS has been shown in many studies (Downing&Weinstein 1986:66, Akman&Demirhan 1993:27). It has also been reported that therapeutic US therapy for shoulder soft tissue disorders is effective in reducing pain and improving quality of life and daily living activities (Philedelhia 2001:81, Robertson&Baker 2001:81). Akgün et al. reported a substantial reduction of pain and increased joint range of motion in patients undergoing US therapy (Akgün&Karamehmetoğlu 1997:22). Calis et al. reported a substantial reduction for pain during both rest and movement in the group treated with US (Calis&Berberoglu 2011:47). In our study, we also observed a substantial reduction for pain for the US group compared to the HILT

group, and we found that this effect was more pronounced in the first month.

The laser stimulates cellular metabolism through biostimulation and increases blood flow in the capillaries through arterial vasodilation (Yavuz&Duman 2014:27). The number of studies evaluating the efficacy of laser treatment in SIS patients is limited. LLLT has been performed for treatment of multiple musculoskeletal syndromes such as fibromyalgia, myofascial pain syndrome, lateral epicondylitis, low back pain, and tendinitis (Bal&Eksioglu 2009:27).

Some systemic reviews and randomized clinical studies have reported beneficial effects of LLLT in the treatment of shoulder pathologies (Blair&Rokito 1996:78, Neer 1972:54, Mao&Jaw 1997:78). However, there have been studies reporting that LLLT is not different from placebo in shoulder pathologies. Bal et al. reported no significant difference and no additional benefit of LLLT to exercise in their study comparing the results of the home exercise group and the LLLT group in addition to home exercises (Bal&Eksioglu 2009:27). Vecchio et al. reported no substantial differences for pain, function, range of motion (ROM) and muscle strength between the LLLT and ROM exercises group and the placebo LLLT and ROM exercises group in the treatment of SIS (Vecchio&Kavanagh 1995:34). The differences might be due to differences in subjects or different laser types and/or doses used.

Higher intensity laser beams obtained by slow absorption of light through chromophores have been recently used in the treatment of various musculoskeletal pathologies (Kujawa&Zavodnik 2004:22). HILT rapidly reduces inflammation and pain. It performs these effects very rapidly by increasing blood flow and vascular permeability as well as photochemical/photothermic effects in tissues (Zati&Degli 1997:7). HILT has been shown to palliatively affect nerve endings, however, there has been no evidence of the reduction in inflammation (Tsuchiya&Kawatani 1994:7, Nicolau&Martinez 2004:35).

The only study in the literature comparing the efficacy US therapy and HILT in treatment of SIS was conducted by Santamato et al.. In contrast to our results, the HILT group was reported to have decreased pain, increased joint movement, improved function and increased muscle strength compared to the US therapy group. They reported that HILT may be a new therapeutic approach in shoulder pathologies (Santamato et al. 2009:89). However, the results of the study covered only the 2-week period of treatment. In our study, we found that improvements in VAS

and SPADI scores were higher in the US therapy group compared to the HILT group. We also observed that the difference was more pronounced at the first month control visit.

Limitations of this study include the absence of a control group, limited follow-up periods and results, relatively small number of patients and a single-center study.

CONCLUSION

More studies are necessary to validate the efficacy of HILT for SIS. Our study showed that HILT is comparable to US therapy in the affected shoulder for reducing pain and increasing joint movement. However, it is suggested to conduct long-term and placebo-controlled studies with more patients.

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Conflict of interest: The authors declare that there is no conflict of interest.

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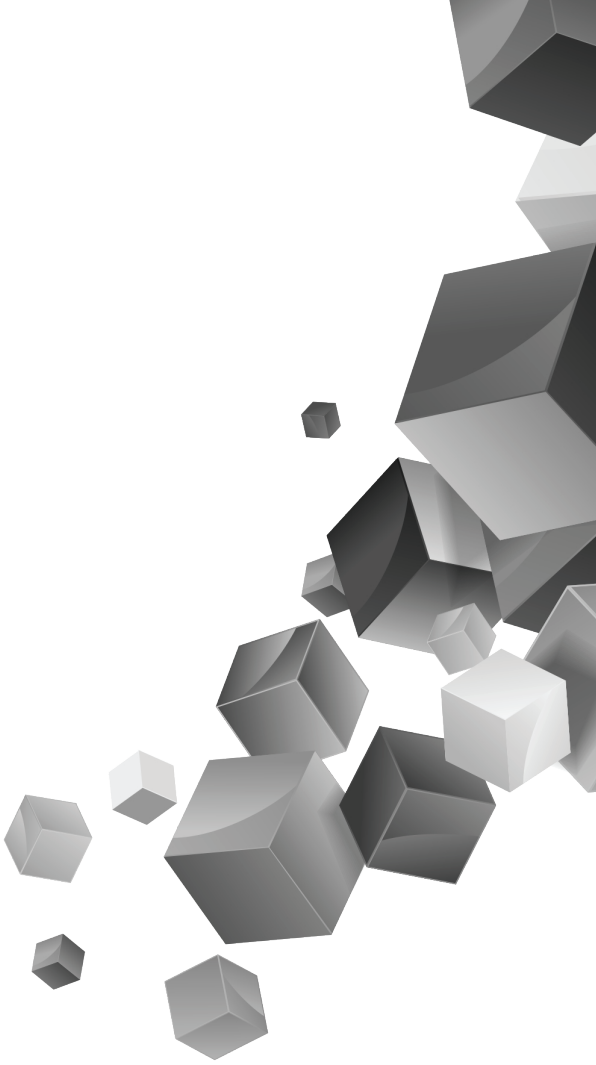
Chapter 3

ENZYME INHIBITION



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Enzymes

Enzymes known as biological catalysts accelerate reactions in living organisms, at the same time they provide 100% product yield without creating any by-products. Except for a small group of catalytic RNA molecules (ribozymes), all enzymes have protein structure. They constitute the largest and most specialized group of proteins.

The majority of research in the history of biochemistry has been studies on enzymes. The first important trials on catalysis were made on digestion in the stomach between 1760-1825. The first study on a particular enzyme was carried out by S. S. Berzelius, a Swedish chemist, in 1835 and showed that diastase hydrolyzes starch in vivo at higher efficiency than sulfuric acid. L. Pasteur proved by experiments that the fermentation process was carried out by enzymes in 1860. Approximately 2000 enzymes have been identified until today. Many of them were obtained in pure form, their kinetics were examined, and more than 200 were crystallized. However, genetic studies show the existence of many enzymes that have not yet been detected.

Inhibition

The reduction or even destruction of the activities of enzymes both in vivo and in vitro by some compounds is called inhibition. Compounds that cause inhibition are also called inhibitors. Inhibitors are generally small molecular weight compounds or ions. Although these substances can bind to enzymes, they do not act like substrates and form products. Thus, the enzyme cannot fulfill its catalytic task. Enzyme inhibition is highly important as the inhibition of enzymatic activity is a checkpoint for biological systems. Most toxic compounds and drugs act on enzyme activity. Because of this effect, inhibitors have an important role in elucidating both enzyme action mechanisms and metabolic pathways.

Enzyme inhibition is examined in two main groups as irreversible and reversible.

Irreversible Inhibition

In irreversible inhibition, the enzyme is irreversibly inactivated by the inhibitor. In addition, the inhibitor binding with the active site of the enzyme is tight, or the inhibitor is located near the active site by covalent bonds.

$$E + I \rightarrow EI$$

$$(EI: Inactive\ Enzyme\ Complex)$$

Examples of irreversible inhibitors of enzymes;

- Alkylating agents such as iodoacetate inhibit sulfhydryl group-containing enzymes in their active sites. In addition, iodoacetate causes alkylation of the imidazole ring of histidine residues in the enzyme's structure. Ribonuclease treated with iodoacetate at pH 5.5 is inactivated by alkylation of the two histidine residues it contains.

- In enzymes with serine residues in their active sites, the enzyme is inhibited by binding organic phosphate compounds to the hydroxyl group of the serine (Fig. 1).

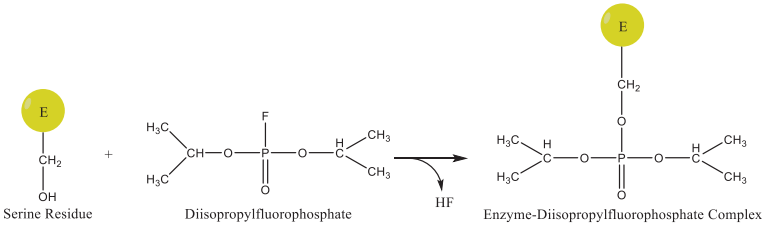

Figure 1. Inhibition with organic phosphate compounds as an example of irreversible inhibition

The acetylcholinesterase enzyme, which has a highly significant role in the transmission of nerve impulses, is irreversibly inhibited by nerve gas poisons. Of these gases, diisopropylfluorophosphate (DIPF) reacts with the serine amino acid in the active site of the enzyme and the inactive diisopropylfluorophosphate-enzyme is formed. Acetylcholine increases the permeability of the membrane to Na^+ and causes its depolarization.

$$Acetylcholine \rightarrow Acetat + Choline$$

In poisoning with organic phosphate compounds, acetylcholine cannot be broken down and continuous depolarization of the muscles occurs. Poisoning with organophosphates used as insecticide in agriculture results in death. Organic phosphate-linked inactive acetyl choline esterase (AChE) can be released spontaneously. However, this spontaneous hydrolysis occurs quite slowly. Therefore, nucleophilic agents are used in the treatment, which provide a much faster reactivation of the enzyme. Developed by the Germans during the Second World War, the nerve gases tabun, sarin and soman are organic phosphate compounds that inhibit acetylcholine esterase. Inhaling these gases, which are liquid at normal temperature and pressure, results in death due to respiratory ability.

Reversible Inhibition

In reversible inhibition, when the inhibitory effect is removed, the enzyme becomes active again. There are three types of reversible inhibition defined according to the effects of inhibitors on the reaction kinetics of the enzyme:

i. Competitive
ii. Noncompetitive
iii. Uncompetitive

In reversible inhibition, the interaction of inhibitor and enzyme is in the form of an equilibrium reaction.

$$E + I \leftrightarrow EI$$

K_i is the dissociation equilibrium constant of the enzyme-inhibitor complex.

$$K_i = \frac{[E]\,[I]}{[EI]}$$

K_M is defined as the enzyme's affinity for the substrate, while K_i is defined as the enzyme's affinity for the inhibitor.

i. Competitive Inhibition

In this type of inhibition, the inhibitor wants to bind to the site where the substrate binds to the enzyme. This type of inhibitor is called competitive inhibitor and the inhibition that occurs is called competitive inhibition. In competitive inhibition, enzyme and inhibitor compete in binding to the active site of the enzyme. This race is due to the structure of the inhibitor similar to that of the substrate. Since the inhibitor is structurally similar to the substrate, it easily binds to the active site and prevents the substrate from binding. Thus, no product is formed. Because the inhibitor occupies the active site, the substrate cannot bind (Fig. 2).

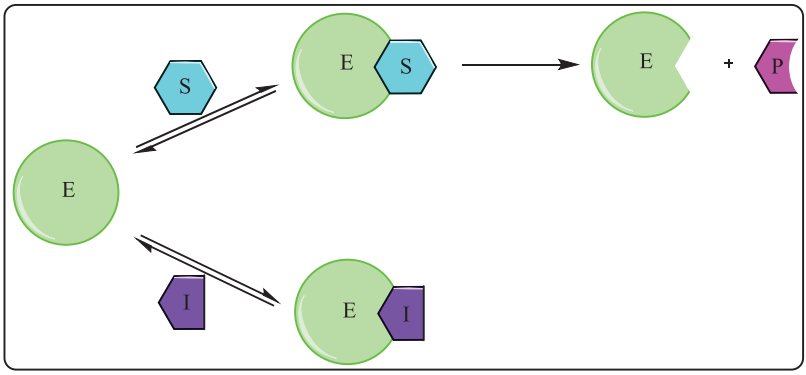

Figure 2. Binding patterns of substrate and inhibitor in competitive inhibition

Based on this situation, the Michael-Menten equation is expressed as follows;

$$\vartheta = \frac{V_{max}\,[S]}{[S] + K_M\,(1 + \frac{[I]}{K_i})}$$

Competitive inhibition can be easily determined experimentally. When the substrate concentration of the medium is increased at a constant inhibitor concentration, the reaction reaches maximum speed. In competitive inhibition, the known K_M for the substrate of the enzyme increases. This means the increase in substrate concentration required to reach maximum speed. The newly formed K_M value is always greater than the actual K_M value of the enzyme (Fig. 3). As seen in Figure 1, while the competitive inhibitor does not affect the V_{max} value, the K_M value increases.

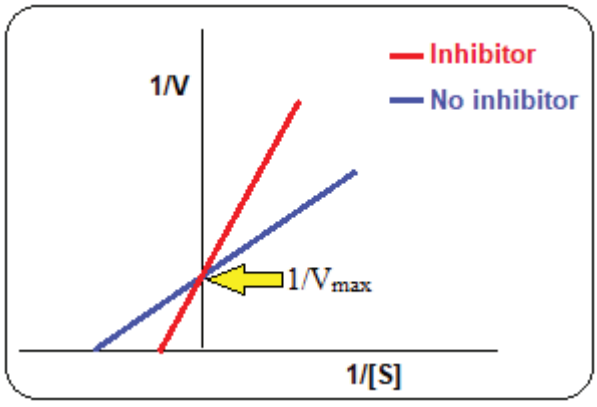

Figure 3. Lineweaver-Burk plot for competitive inhibition

The effect of malonate on succinate dehydrogenase is one of the classic examples of this type of inhibition. The task of succinate dehydrogenase is to catalyze the formation of fumarate by removing two hydrogen atoms from the succinate molecule. Malonate is structurally similar to succinate, but, unlike succinate, it contains a missing methylene group. Malonate binds to the enzyme with the two carboxyl groups it contains, but remains unchanged since it does not have two hydrogen atoms it can give (Fig. 4).

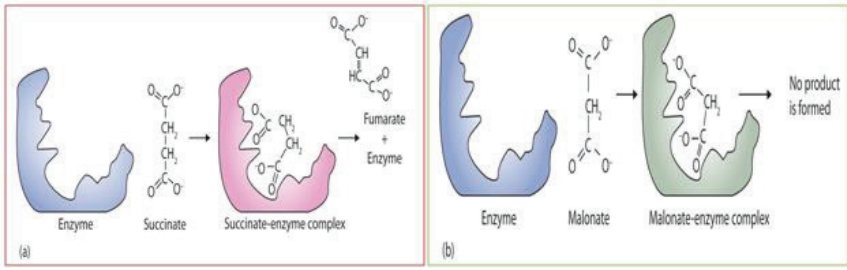

Figure 4. The binding of succinate (a) and malonate (b) to succinate dehydrogenase

The end product formed in purine catabolism in humans is uric acid. Xanthine oxidase catalyzes the formation of uric acid by providing the oxidation of hypoxanthine and xanthine, respectively. Allopurinol, a hypoxanthine analog, is both a competitive inhibitor and a substrate of xanthine oxidase. While allopurinol inhibits the formation of xanthine and uric acid, it transforms itself into alloxanthine.

In enzymes with two substrates, higher concentrations of the second substrate compete with the first substrate for binding to the enzyme. For example, both aspartate and a-ketoglutarate are substrates of the enzyme in the transamination reaction catalyzed by aspartate aminotransferase. High amounts of a-ketoglutarate inhibit the enzyme and the inhibition is competitive compared to aspartate.

In addition, competitive inhibition may occur with product accumulation as a result of reaction without inhibitor. The resulting product acts as an inhibitor. Alkaline phosphatase, which enables organic phosphate esters to break down into alcohol and inorganic phosphates, is inhibited by the inorganic phosphate formed. Alkaline phosphatase breaks down organic phosphate esters into alcohol and inorganic phosphates, and alkaline phosphatase is inhibited by the inorganic phosphate formed.

There are cases where competitive inhibition is used in medicine for therapeutic purposes. For example, in methanol poisoning; methanol is converted to formaldehyde by alcohol dehydrogenase enzyme.

Formaldehyde causes damage to many tissues, especially the eye. When ethanol, which is the main substrate of alcohol dehydrogenase, is administered, competition begins between ethanol and methanol for binding to the enzyme. When the ethanol concentration is increased further, the conversion of methanol to toxic formaldehyde is prevented. Methanol is also excreted in the urine.

ii. Noncompetitive Inhibition

In noncompetitive inhibition, the inhibitor is not structurally similar to the substrate. While the substrate binds to the active site of the enzyme, the inhibitor binds to a site other than the enzyme's active site. Therefore, the substrate and the inhibitor are not in a race with each other to bind to the enzyme (Fig. 5)

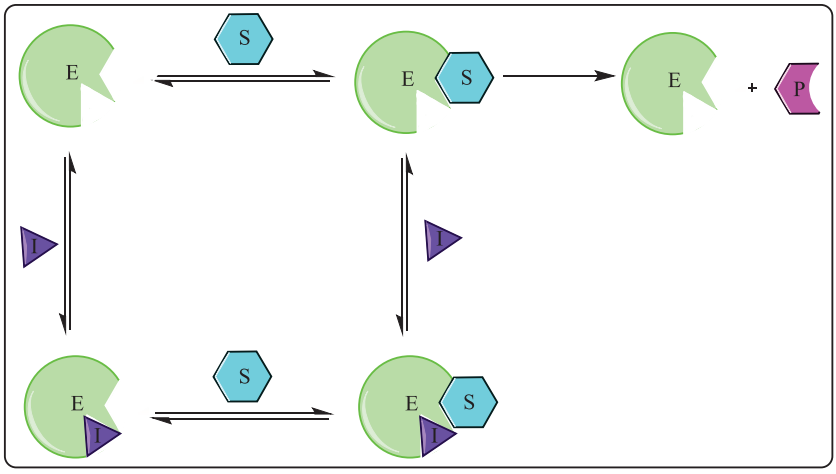

Figure 5. Binding patterns of substrate and inhibitor in noncompetitive inhibition

Noncompetitive inhibition depends only on inhibitor concentration. Inhibition cannot be eliminated by increasing the substrate concentration. The inhibitor binds to free enzyme or enzyme-substrate complex. The complexes formed in both cases, both EI and ESI, are catalytically inactive. Since the affinity of the substrate to the enzyme does not change, the K_M value does not change, but the V_{max} value decreases (Fig. 6).

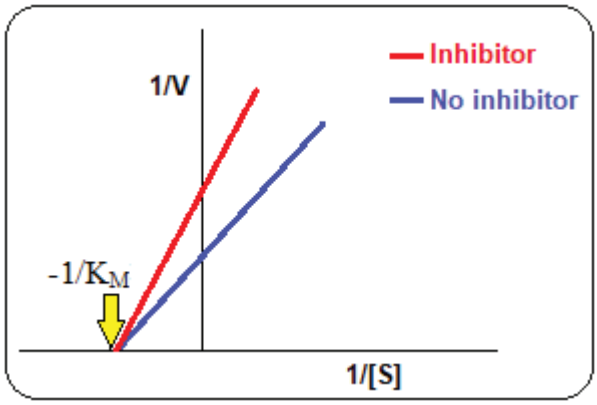

Figure 6. Lineweaver-Burk plot for noncompetitive inhibition

The activities of enzymes that need metal ions in catalysis can be inhibited noncompetition as a result of the alternate binding of some compounds to metal ions. Enzymes with -SH groups, which play a role in protecting the tertiary structure of molecules, are inhibited by heavy metals such as Ag, Hg and Pb. Therefore, anemia as a result of lead poisoning is explained by non-competitive inhibition. The porphobilinogen synthase and ferrokelatase enzymes, which are in the first step of the heme group synthesis, contain sulfhydryl groups and are inhibited by Pb. Eggs are also effective as an antidote in heavy metal poisoning. Ovalbumin, which has excess sulfhydryl groups, binds free metal ions and prevents its absorption from the intestine.

iii. Uncompetitive inhibition

In this type of inhibition, the inhibitor only binds to the enzyme-substrate (ES) complex (Fig. 6).

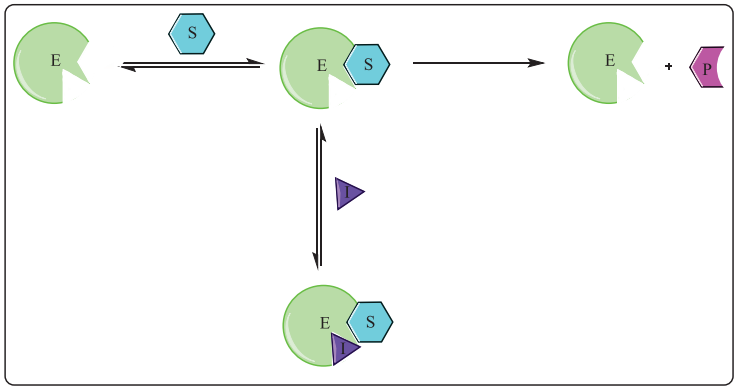

Figure 7. Binding patterns of substrate and inhibitor in uncompetitive inhibition

Uncompetitive inhibition is rare in reactions with a substrate. However, it is more common in reactions with two substrates. Inhibition increases by increasing the substrate concentration in the presence of a uncompetitive inhibitor. In the presence of a uncompetitive inhibitor, the K_M decreases as the ES complex is constantly removed from the reaction. At the same time, V_{max} decreases as there will be a continuous ESI complex (Fig. 8).

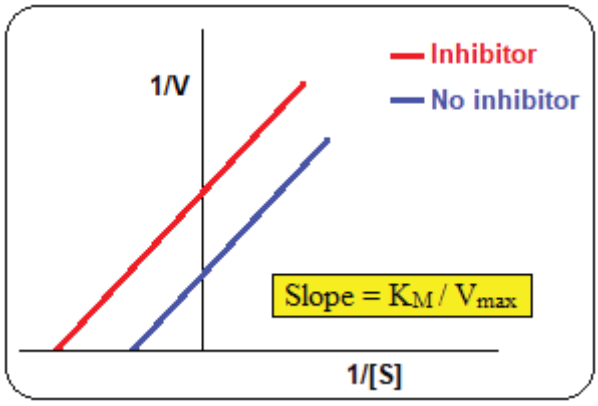

Figure 8. Lineweaver-Burk plot for uncompetitive inhibition

Table 1. V_{max} and K_M values in competitive and noncompetitive inhibition

Inhibition Type	V_{max}	K_M
Competitive	$V_{max}^{*} = V_{max}$	$K_M^{*} = K_M\,(1 + \frac{[I]}{K_i})$
Noncompetitive	$V_{max}^{*} = \frac{V_{max}}{(1 + \frac{[I]}{K_i})}$	$K_M^{*} = K_M$
Uncompetitive	$V_{max}^{*} = \frac{V_{max}}{(1 + \frac{[I]}{K_i})}$	$K_M^{*} = K_M\,(1 + \frac{[I]}{K_i})$

In vitro Inhibition Studies

Before examining the inhibitor's *in vitro* inhibition effect on an enzyme, the first thing to do is to obtain the enzyme pure. Thus, inhibitory effects of an inhibitor can be investigated on pure enzyme. Firstly, the activity of the enzyme at different inhibitor concentrations is measured. Without inhibitor, the control activity is assumed to be 100%. The percent activity

vs inhibitor concentration graph is drawn for each inhibitor. Next, a Lineweaver-Burk plot is plotted by using three different inhibitor concentrations and five different substrate concentrations. With the help of the graphic obtained in the next step, the K_i value is calculated and the inhibition type is determined.

Enzyme Inhibitions in Drug Therapy

About half of the most commonly used drugs show their effects by inhibiting specific enzymes. A group of drugs used in the treatment of hypertension by inhibiting the angiotensin converting enzyme (ACE) that catalyzes the synthesis of the vasoconstrictor angiotensin II.

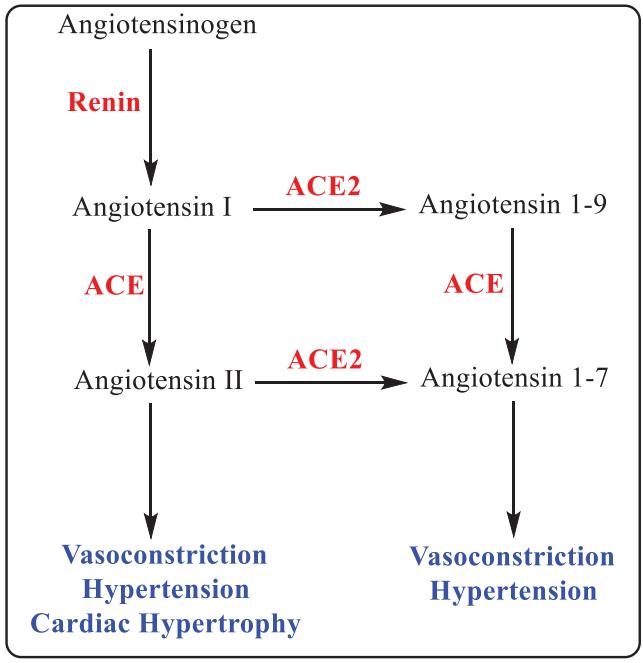

Figure 9. Reaction catalyzed by angiotensin converting enzyme (ACE)

In 'myasthenia gravis', which is an autoimmune muscle disease, the contraction function is impaired due to the presence of antibody blocking the acetylcholine receptor. Acetylcholinesterase inhibitors are used in treatment to increase the effectiveness of acetylcholine. Drugs used in the treatment of Alzheimer's disease are generally specific inhibitors of the AChE enzyme. In some studies, it has been found that AChE enzyme inhibitors also have an inhibition effect on CA isoenzymes. Rivastigmine and galantamine drugs significantly inhibit CA I and II isoenzymes.

Rivastigmine; It inhibits the AChE enzyme weakly, selectively and reversibly. It is used in the treatment of mild to moderate Alzheimer's disease. Galantamine; It is an alkaloid isolated from the Galanthus woronowii plant. It is used in the treatment of moderate Alzheimer's disease. It inhibits the AChE enzyme strongly, selectively and reversibly. It shows its inhibition effect competitively.

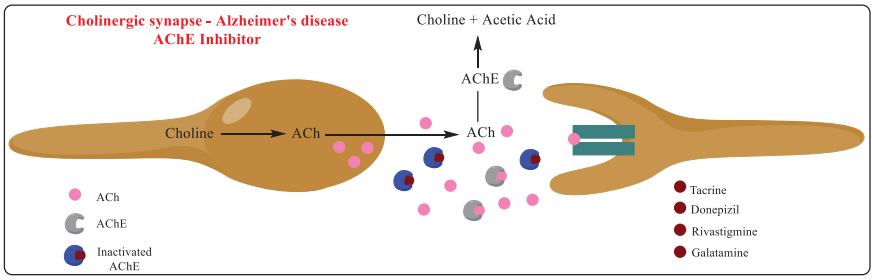

Figure 10. Binding status of inhibitors of the AChE enzyme

The mechanism of antabus therapy in alcoholism is also associated with enzyme inhibition. Ethanol is metabolized in the body in two steps. Acetaldehyde formed by alcohol dehydrogenase is then oxidized to acetic acid by aldehyde dehydrogenase. Disulfiram (Antabus) used in alcohol treatment inhibits the enzyme by covalently binding to aldehyde dehydrogenase. Drinking alcohol causes severe headache and vomiting due to acetaldehyde accumulation.

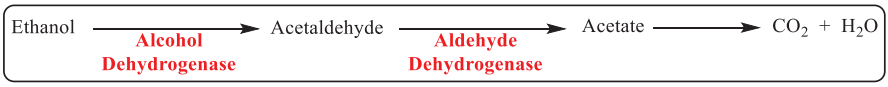

Figure 11. Ethyl alcohol metabolism

The cyclooxygenase enzyme, which initiates prostaglandin and thromboxane synthesis from arachidonic acid, is inhibited by anti-inflammatory drugs such as indomethacin and ibuprofen. These drugs compete with arachidonic acid and inhibit the synthesis of proinflammatory biomolecules. Aspirin (acetylsalicylic acid) suppresses inflammation by irreversibly inhibiting the enzyme by acetylating the serine residue in the active site of the cyclooxygenase enzyme.

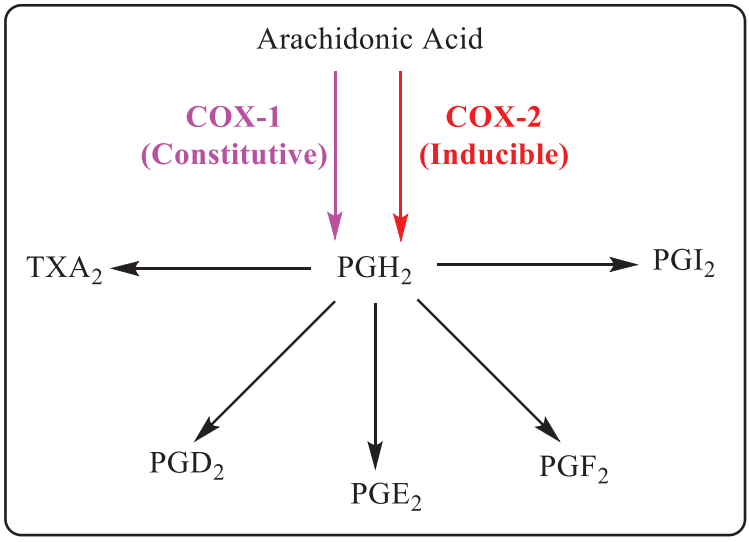

Figure 12. Catalysis mechanisms of COX enzymes

Drugs that inhibit enzymes specific to microorganisms are used in the treatment of bacterial infections. Beta-lactam antibiotics show their effects by inhibiting the enzymes involved in the synthesis of the bacterial cell wall. Penicillin inhibits transpeptidase involved in the synthesis of bacterial cell wall. The lactam ring of penicillin, which binds to the active site of the enzyme, opens and forms a covalent bond with the serine residue in the active site.

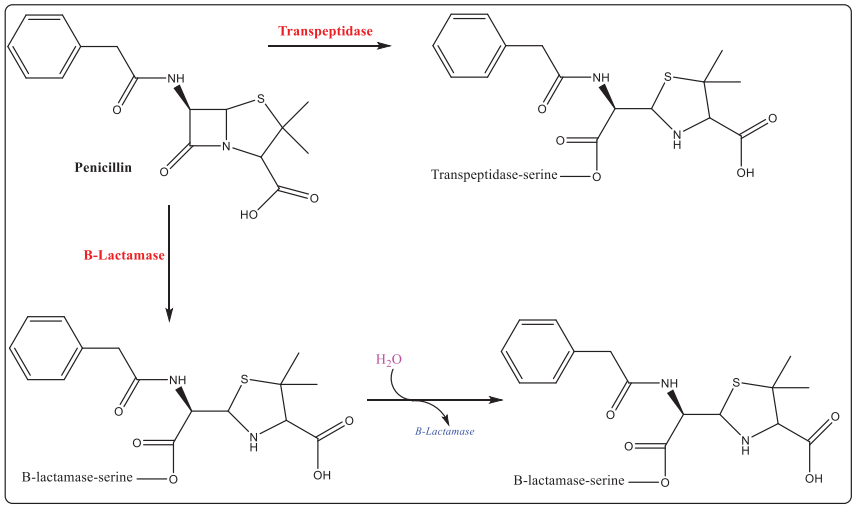

Figure 13. The inhibition mechanism of penicilin

Monoamine oxidases (MAO) are enzymes that inactivate biological amines such as dopamine and serotonin, which are important neurotransmitters of the organism, by deamination. N,N-dimethylpropargilamine is an MAO inhibitor. The compound formed by oxidation of the inhibitor by MAO inhibits the same enzyme by covalent binding. N, N-dimethyl propargilamine is used in the treatment of Parkinson's disease in which dopamine levels are low.

Statins used in the treatment of hypercholesterolemia are competitive inhibitors of the enzyme (HMG-CoA reductase) involved in cholesterol biosynthesis.

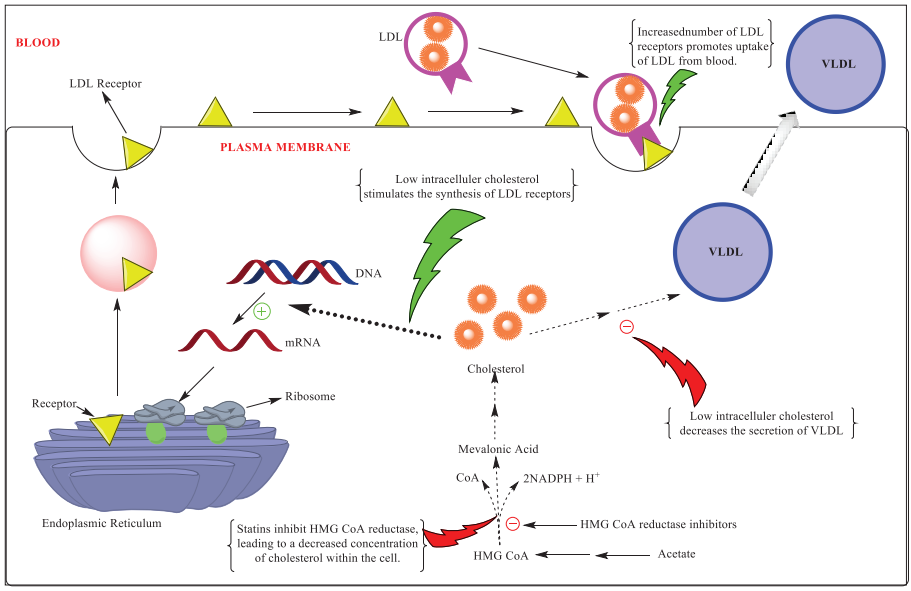

Figure 14. Inhibition mechanism of cholesterol biosynthesis

Nucleoside analogs (acyclovir and gancyclovir) are used in the treatment of herpes simplex virus (HSV) and cytomegalovirus (CMV) infections. These analogs are activated when phosphorylated. The thymidine kinase of the virus cell with low substrate selectivity readily phosphorylates its nucleoside analogs. These activated agents inhibit viral DNA polymerase with competitive inhibition and prevent virus replication.

Some inhibitors are specific for certain isoenzymes of enzymes. For example, sildenafil is used in the treatment of erectile dysfunction by inhibiting a specific isozyme of phosphodiesterase (PDE_5). PDE_5 functions by breaking down cGMP, a vasodilator molecule. In case of PDE_5 inhibition, cGMP shows a longer vasodilator effect.

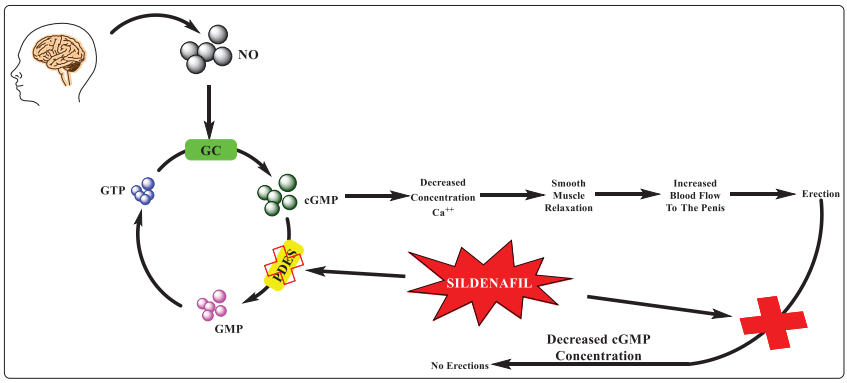

Figure 15. Use of sildenafil in treatment

Used in cancer treatment, methotrexate is a competitive inhibitor of the dihydrofolate reductase enzyme. Tetrahydrofolate (FH_4), the coenzyme in DNA (thymidylate) synthesis, is oxidized to dihydrofolate (FH_2) during the reaction and reduced back to tetrahydrofolate by dihydrofolate reductase. Cells with high division rate are damaged when thymidylate synthesis is halted due to methotrexate (leukemia, cancer cells).

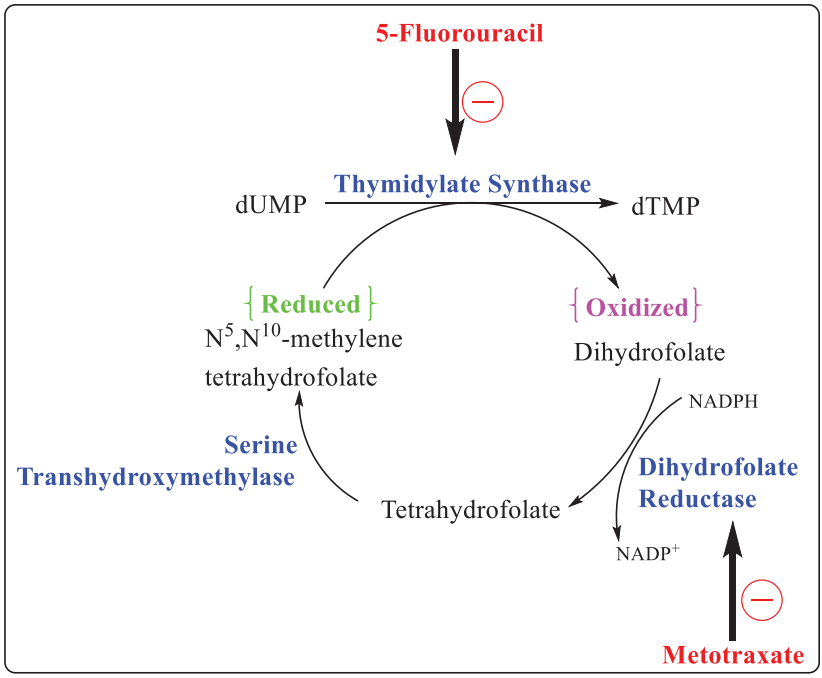

Figure 16. The mechanism of action of the inhibitor used in some cancer treatments

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Chapter 4

ERYTHROCYTE DEFORMABILITY

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INTRODUCTION

Blood is a tissue that is vital for the continuation of life in multicellular organisms. Blood is a tissue in which blood cells are dispersed in suspension in liquid medium called plasma, filling the vascular system and circulating the whole body in this system thanks to the pumping power of the heart. The blood constitutes a system that brings the nutrients to be used by the cells along with oxygen to the interstitial fluid and also takes the metabolism residues and carbon dioxide formed by the cells from there. It fulfills its regulatory role by contributing to keeping the pH and temperature of the indoor environment constant and by conveying the messages that will ensure the mutual communication between the hormones it carries and the organs. However, the movement of blood tissue within the vascular system depends primarily on its own properties and fluidity (Charm & Kurland, 1974; Errill, 1969). The composition and physical properties of blood reflect the internal environment and changes in the internal environment.

Blood tissue physically consists of a suspension of cellular elements in plasma. About 40-50% of blood in volume consists mainly of erythrocytes (Chien, 1987; Wintrobe, 1981), which are biconcave discoid cells with an average diameter of 8 μm, a small portion of other blood cells, and the remaining 50-55% of plasma. The fluidity of the blood tissue also changes depending on the erythrocyte mass in the first plane, the properties of the plasma and the relationship between these two phases (Chien & Sung, 1990; Mohandas, 1992; Mohandas & Chasis, 1993; Mohandas & Evans, 1994). However, the physical properties of erythrocytes play a decisive role in this system. The erythrocyte membrane plays an important role in determining the cell's ability to change shape and maintaining the cell shape in the biconcave disc structure by ensuring its structural integrity.

Erythrocyte deformability can be defined as the deformation of the erythrocyte in response to the forces applied to it during blood flow. Erythrocyte deformability, besides its decisive role on massive blood flow in large vessels, also contributes to the maintenance of the capillary circulation in the most appropriate way to the needs of the tissues. At this level of the circulatory system, erythrocytes must pass through capillary vessels smaller than their size (Mohandas, 1992). During this transition, the tight relationship between the erythrocytes and the capillary wall creates an ideal environment for the exchange of respiratory gases (Mohandas & Chasis, 1993; Wintrobe, 1981). It is possible for cells with a diameter of about 8 μm to pass through capillary

vessels with a diameter of 3–5 μm only if these cells change their shape. The ability of erythrocytes to fulfill their functions in microcirculation depends on these abilities. Erythrocyte deformability, which is extremely important for both mass blood flow in the large vessels and circulation in the capillary vascular bed, plays a determining role on the rheological properties of the circulatory system as a whole.

During some pathological and physiological processes, erythrocytes may move away from these structural features (Dormandy, Boyd, & Ernst, 1981; Hasegawa et al., 1995; McMillan, Utterback, & Mitchell, 1983; Mohandas & Shohet, 1981; Noji, Taniguchi, & Kon, 1991; Saldanha et al., 1999; Sheetz, 1983; Weed, 1970). As a result, erythrocyte deformability is also affected. Depending on the degree of deformability change, the changes that will occur in capillary circulation and in the exchange of respiratory gases establish the physiopathological relationship between the basic processes affecting the erythrocyte structure and the pathological changes at the tissue level.

1. MASS BLOOD FLOW

In vessels large enough to allow the blood tissue to behave as a whole, the blood is exactly a biphasic suspension (Errill, 1969). Under these conditions, flow can be seen in laminar or turbulent character depending on the geometric properties of the vascular system, the physical properties of the blood and the flow rate. Laminar flow is a regular flow that occurs in the form of sliding layers of liquid on each other and has low hydraulic resistance (Lowe, 1988). Under physiological conditions, the blood flow in most of the vascular system is laminar in character. Due to local changes in vessel geometry and sudden increases in blood flow velocity, blood flow may turn into turbulent. Under these conditions, the current resistance also increases.

1.1. Capillary Blood Flow (Microcirculation)

Capillary vessels in the circulatory system are 3–8 μm in diameter. Under these conditions, it is impossible to consider blood as a whole two-phase fluid system. Instead, the behavior of the blood cellular elements and plasma in the microcirculation must be evaluated separately. The flow velocity in these vessels, which may have a smaller diameter than the size of blood cells, is largely related to the deformability of blood cells (Chien, 1987).

2. ERYTHROCYTES

Erythrocytes are highly specialized cells whose main function is to transport respiratory gases (Wintrobe, 1981). Erythrocytes with an average volume of approximately 90 femtoliter and an average surface area of 140 μm^2 are produced in the bone marrow and lose their nuclei before entering the peripheral circulation (Figure 1) (Chien, 1987; Wintrobe, 1981). Other organelles also disappear from circulation within a few days. Erythrocytes that do not have cytoplasmic organelles such as nuclei, mitochondria and ribosomes cannot synthesize proteins, perform oxidative reactions associated with mitochondria, and undergo mitosis (Wintrobe, 1981). Thus, erythrocytes can be defined as consisting of a simple membrane surrounding proteins and electrolytes. Hemoglobin (Hb) constitutes more than 95% of cytoplasmic proteins. The biconcave disc shape of erythrocytes is very suitable for them to maintain their functions. Thanks to this special shape, the ratio of cell surface to volume reaches the highest possible value, thus facilitating gas transfer. In addition, the fact that the biconcave disc structure is more capable of changing shape than the sphere contributes to the optimal movement of erythrocytes in microcirculation (Mohandas & Chasis, 1993; Wintrobe, 1981). When erythrocyte movements are observed in small vessels, it is seen that the biconcave disc moves orientatively in the direction of flow, similar to a parachute when viewed from the side. Thus, deformable erythrocytes can pass easily through vessels with a maximum diameter of 4 μm (Wintrobe, 1981). Factors involved in maintaining and maintaining the normal erythrocyte biconcave disc shape are: 1) elastic forces in the membrane 2) surface tension 3) electrical potential at the membrane surface 4) osmotic or hydrostatic pressures and 5) surface / area volume relationship. In addition, the properties of the environment in which erythrocytes are located are of great importance in maintaining cell shape (Wintrobe, 1981).

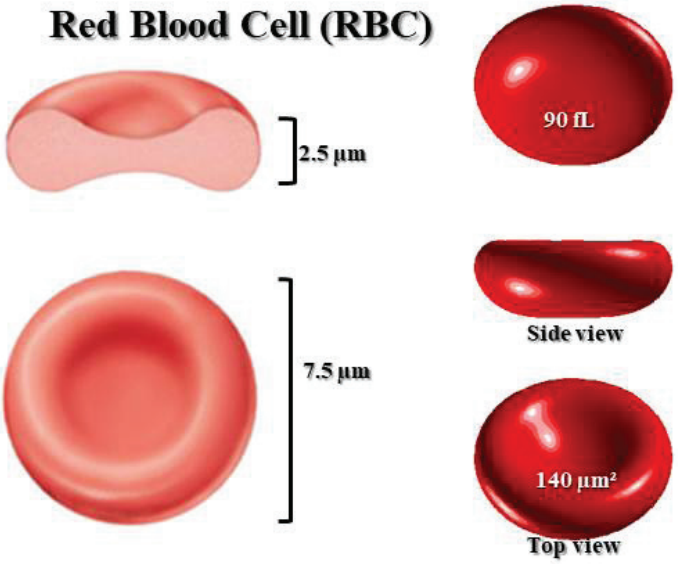

Figure 1: *Morphological structure of red blood cell*

2.1. Erythrocyte Deformability

The term deformability generally refers to the deformation of any structure under the effect of a force (Chien, 1987; Noji et al., 1991). When the forces that cause the elastic bodies to change shape disappear, the objects return to their former shape, that is, the event is reversible (Hochmuth & Waugh, 1987). The amount and shape of the deformation depends on the speed, shape, size and direction of the applied force (Chien, 1987). Their ability to change shape is essential for erythrocytes to perform their circulatory functions.

2.2. Factors Determining Erythrocyte Deformability

There are three main factors that regulate the erythrocyte's ability to change shape. These can be listed as: 1) Erythrocyte geometry (surface area-volume relationship) 2) Cytoplasmic viscosity 3) Rheological (visco-elastic) properties of the erythrocyte membrane (Chien, 1987; Mohandas & Chasis, 1993; Mohandas, Chasis, & Shohet, 1983; Mohandas, Clark, Jacobs, & Shohet, 1980; Mohandas & Shohet, 1981; Sheetz, 1983; Weed, 1970).

2.2.1. Erythrocyte Geometry (Surface area-volume relationship)

The biconcave disk shape of the normal erythrocyte provides an advantageous surface area-volume relationship in order to keep the erythrocyte surface area constant and change its shape (Mohandas, 1992; Mohandas & Chasis, 1993; Mohandas et al., 1983; Weed, 1970). The surface area of human erythrocytes with a volume of 90 fL is approximately 140 μm^2. This figure is significantly higher than the surface area of the sphere of the same volume, 97 μm^2 (Wintrobe, 1981). This excess in the surface area caused by the erythrocyte structure being in the form of a biconcave disc allows the cell to change shape without any change in the surface area (Chien, 1987; Wintrobe, 1981). Thanks to these features, erythrocytes can show linear elongation up to 250% of their original size, but even a 3-4% increase in surface area causes the cell to break down. The loss of membrane causing a decrease in the surface area or the increase in erythrocyte fluid content, which causes an increase in cell volume, makes the erythrocyte shape more spherical. In this spherical structure, the surface area to volume ratio has decreased. According to Euclid's laws, in order for a sphere to change shape, it must increase its surface area. Since the force required to increase the surface area is four times the force required to change shape in the fixed area, more force must be applied to change the shape of a spherical cell compared to a biconcave disk structure (Mohandas & Chasis, 1993; Weed, 1970).

2.2.2. Cytoplasmic Viscosity

Cytoplasmic viscosity determined by intracellular Hb concentration is another determinant of erythrocyte deformability (Chien, 1987; Mohandas & Chasis, 1993; Mohandas et al., 1983; Mohandas & Evans, 1994; Sheetz, 1983). Hb concentrations of erythrocytes obtained from normal individuals are between 27–37 g/dL, and the cytoplasmic viscosity in this range is up to 5–15 centi poise (cp) (Mohandas & Chasis, 1993; Mohandas et al., 1983). In cells with normal hemoglobin concentration, the effect of cytoplasmic viscosity on erythrocyte deformability is negligible, and the viscous distribution of forces applied to the cell membrane is the main factor determining erythrocyte deformability (Alonso, Pries, & Gaehtgens, 1993; Lowe, 1988; Mohandas et al., 1983; Schmid-Schonbein, Wells, & Goldstone, 1971; Wells & Schmid-Schonbein, 1969). Cross bridges between Hb and membrane proteins may occur as a result of the increase in the mean erythrocyte hemoglobin concentration (MCHC) due to cellular dehydration (Mohandas, 1992;

Mohandas & Chasis, 1993; Wintrobe, 1981). This situation adversely affects the erythrocyte deformability by restricting the movement of spectrin molecules (Mohandas, 1992; Mohandas & Chasis, 1993; Wintrobe, 1981).

2.2.3. Rheological Viscoelastic Properties of Erythrocyte Membrane

The forces that cause the erythrocytes to change shape during blood flow almost always act from outside the cell. The part of the erythrocyte that is directly exposed to these forces is its membrane. Thanks to the flexible structure of the erythrocyte membrane, it transfers the forces acting from the outside of the cell to the cytoplasm, allowing the erythrocyte cytoplasm, which is almost exclusively composed of Hb suspension, to participate in the current. Thanks to this feature of the membrane, erythrocytes can adapt geometrically to the flow conditions in the best way (Chien, 1987; Hochmuth & Waugh, 1987; Wintrobe, 1981). The behavior of the erythrocyte membrane in the presence of any stress is quite complex and depends not only on the size of the force applied to it, but also on the duration of its application (Mohandas & Chasis, 1993; Mohandas et al., 1983). When short-term (<100 sec) and 10–6 dyne forces are applied, it shows large elastic shape changes in response, but returns to its former shape when the force disappears. When these forces are applied for a longer period (5-10 minutes), the membrane behaves like a semi-solid and even when the force is removed, the cell cannot fully return to its former biconcave disc shape. When large forces (>10–6 dyne) are applied, the erythrocyte membrane is permanently deformed. Thus, the same membrane can respond in three different ways depending on the magnitude of the force applied to it and the duration of its application. This feature of the membrane is due to its elastic structure, and the interaction between different membrane components is responsible for these different responses (Chien, 1987; Mohandas & Chasis, 1993; Mohandas et al., 1983).

2.3. Physiological and Pathological Factors That Change Erythrocyte Deformability

Erythrocyte deformability can be changed experimentally by the effect of various factors, and deviations from normal can be seen in erythrocyte deformability in various disease tables. The increase in the osmotic pressure of the environment in which the erythrocytes are located causes both a decrease in the erythrocyte volume and an increase in the RBCs, provided that the cell surface area is constant

(Mohandas & Shohet, 1981). At high osmolarity, there may also be a decrease in erythrocyte deformability by interacting between membrane components. A decrease in osmolarity causes an increase in erythrocyte volume and affects deformability in the opposite direction (Lowe, 1988; Mohandas & Chasis, 1993; Mohandas et al., 1983; Schmid-Schonbein et al., 1971; Wells & Schmid-Schonbein, 1969).

However, these effects are only valid for a certain range of osmolarity changes. In general, very large changes in the direction of both increase and decrease in osmolarity reduce erythrocyte deformability (Chien, 1987). An increase in osmolarity causes a decrease in cell volume and an increase in Hb concentration. Although such an erythrocyte passes through a narrow channel more easily than the diameter of a normal erythrocyte, its deformability ability is reduced in the mass flow. On the contrary, in a hypoosmolar environment, the mass flow is facilitated by a decrease in hemoglobin concentration and thus in cytoplasmic viscosity, while the flow in a narrow channel becomes difficult as the volume will increase in a certain surface area. However, this dilemma only applies to osmolarity changes in a certain range. Osmolarity change negatively affects erythrocyte deformability at both ends under all conditions.

It is known that increases in intracellular Ca^{++} concentration cause a decrease in erythrocyte deformability (Delaunay, 1977; Sheetz, 1983; Weed, 1970). When the intracellular Ca^{++} concentration is increased using the Ca^{++} ionophore A23187, a series of complex biochemical reactions occur, involving the activation of Ca^{++}-ATPase in the erythrocyte with the aid of Ca^{++} binding calmodulin, changes in the composition and metabolism of membrane lipids and the outflow of K^{+} ions and water (Gardos effect) (Mohandas & Shohet, 1981; Noji et al., 1991). In the experiments in which erythrocytes were placed in an environment containing high K^{+} ions to prevent K^{+} outflow and related dehydration, no decrease in erythrocyte deformability was found. Therefore, it has been suggested that the reason for the decrease in deformability caused by the increase in intracellular Ca^{++} concentration in the erythrocyte is dehydration due to Gardos effect (Noji et al., 1991).

Increased intracellular Ca^{++} concentration causes K^{+} ions to exit the cell, water molecules also follow K^{+} ions, intracellular viscosity and Hb concentration increase. This situation results in a decrease in erythrocyte deformability (Mohandas & Chasis, 1993; Mohandas & Shohet, 1981; Noji et al., 1991; Wintrobe, 1981). ATP deprivation in the erythrocyte membrane is particularly important in terms of pump activities. ATPase

enzymes in the membrane are largely involved in maintaining the size, shape and deformability of the cell by regulating the cation and water content of the erythrocyte.

It is known that erythrocytes turn into echinocytes when they are deprived of ATP, and also deformities occur, resulting in the loss of some membrane material by breaking their membranes. Thus, lack of ATP results in a decrease in erythrocyte deformability (Chien, 1987; Sheetz, 1983; Weed, 1970). Erythrocyte deformability is generally changed in hematological diseases, especially in erythrocyte deformities (Chien & Sung, 1990; Mohandas, 1992). In patients with sickle cell anemia who have erythrocytes containing hemoglobin S, erythrocyte deformability is severely impaired mainly due to Hb polymerization. In diseases such as hereditary spherocytosis and hydrocytosis, where ion transport mechanisms of erythrocytes are impaired, erythrocyte deformability is affected by changes in cell surface-volume ratio and cytoplasmic viscosity (Clark, Shohet, & Gottfried, 1993; Mohandas & Chasis, 1993). In hereditary spherocytosis, which is one of the most common hemolytic anemias characterized by erythrocyte membrane defect, the ability to change erythrocyte deformation is also reduced (Iolascon et al., 1998).

In addition to hematological diseases, changes in erythrocyte deformability have been detected in various clinical pictures, especially pathologies involving the circulatory system. It has been shown that the ability of erythrocytes to change shape decreases and blood viscosity increases in patients with acute myocardial infarction (Dormandy et al., 1981; Saldanha et al., 1999). It is known that the ability to change erythrocyte shape is reduced in diabetes mellitus (Kunt et al., 1999; McMillan et al., 1983). It has been reported that blood viscosity increases and erythrocyte deformation ability decreases in hypertension (Baskurt, Gelmont, & Meiselman, 1998; Hacioglu, Yalcin, Bor-Kucukatay, Ozkaya, & Baskurt, 2002). It is known that the ability of erythrocyte deformation is impaired in experimental sepsis and ischemia-reperfusion injury models (Kayar, Mat, Meiselman, & Baskurt, 2001).

3. CONCLUCSION

The erythrocyte membrane, like other cell membranes, is a selectively permeable structure with special pumps, channels and ion gates. Again similar to other cell membranes, it consists of a two-lined lipid matrix and several proteins located in or on its cytoplasmic face. The erythrocyte membrane, which is suitable for the liquid-mosaic model, is very flexible and can quickly respond to the forces applied to it, making

large shape changes without deteriorating its structural integrity. While the highly flexible lipid layer of the erythrocyte membrane (together with transmembrane proteins) is more responsible for the isolation of the cell from the external environment, the rigid skeletal protein network contributes greatly to the erythrocyte membrane's ability to change shape by providing stability. The erythrocyte membrane plays an important role in determining the cell's ability to change shape and maintaining the cell shape in the biconcave disc structure by ensuring its structural integrity.

Changes in the structural and functional properties of erythrocytes are important for all tissues in the organism, as they closely concern the fluidity of the blood tissue, especially the behavior of blood in the microcirculation. The ability of erythrocytes to fulfill their functions in microcirculation depends on these abilities. Erythrocyte deformability, which is extremely important for both mass blood flow in the large vessels and circulation in the capillary vascular bed, plays a determining role on the rheological properties of the circulatory system as a whole.

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Chapter 5

VEGETERIAN DIETS:

EVERYTHING ABOUT HISTORY,

HEALTH AND SPORT PERFORMANCE

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INTRODUCTION

Vegetarian and vegan words which mean healthy and strong and derived from Latin word vegatus (Bedin et. al., 2018), refer to nutritional approaches that have attracted attention in recent years and are increasingly known today (Çakıcı et al., 2020). Vegetarian/vegan diets are becoming increasingly common in the world (Leitzmann, 2014), people don't consume and use animal-based products for various reasons such as ecological, environmental sustainability, compassion for animals, better health and religion (Constatin, 2019). Elaborating on these, these reasons are divided into two groups: nature-centered (ecocentric) and human centered (anthropocentric) (Dilek, 2018). In the anthropocentric approach; there are reasons such as person's health problems, weight control, dislike and rejection of animal-based foods, economic problems, social environmental impact (being affected by vegetarian/vegan people) and religious belief (Shani and DiPietro, 2007). In the ecocentric approach, there are factors that emphasize the natural environment and animal ethics, such as moral/ethical reasons, becoming aware of the damages caused by industrial animal husbandry, and considering that vegetarianism or veganism is important in combating global famine (Rivera and Shani, 2013). To sum up, if a person puts himself/herself in the center and s/he chooses a vegetarian/vegan diet, the situation refers to the anthropocentric approach; if a person believes that s/he is a part of the nature, the situation expresses the ecocentric approach (Dilek, 2018).

According to the universal definition of International Vegetarian Union (IVU), "Veganism means not consuming any animal products, not using products derived from animals, not engaging in any activities or intermediaries that restrict animal freedom." (Sünnetçioğlu et al., 2017). According to the definition of veganism that is made by The Vegan Society in 1979; it is the philosophy and life style that avoids all forms of exploitation and cruelty to which animals are subjected for food, clothing or other purposes and promotes the development and use of non-animal use alternatives for the benefit of humans, animals and the environment (Tunçay Son and Bulut, 2016). Respect for animal rights is generally seen in veganism and environmental factors such as health, culture and religion are in the forefront in vegetarianism (Vatan and Türkbaş, 2018). In the world, these nutritional approaches are mostly seen in India (20-40%), Mexico (20%), Brazil (14%), Switzerland (14%), Taiwan (13-14%), Israel (13%), Austria, Germany, Jamaica, Sweden and Vietnam (10%) (Vikipedia, 2020a). India is an important example that

the reason of vegetarian dietary preference can only be religious/cultural (Tunçay Son and Bulut, 2016).

The vegetarian/vegan diets that are widespread today are frequently discussed by healthcare professionals and a clear approach can't be showed to this issue. There are contradictory statements about the subject in both written sources and media news. When the web pages, acknowledged first research source by society, are examined; there are reports that a vegan family receive imprisonment for causing growth and development retardation by feeding their babies vegan (Anonymous, 2019a); in a similar situation, taking custody of children from the family (Anonymous, 2016); striking news that the risk of bone fracture is 40% higher in vegans (Anonymous, 2020a). Contrary to these news, a vegan diet from identical twins has a decrease in fat mass and an increase in brain functions (Anonymous, 2020b) and there are also reports that many diseases can be prevented with vegetarian diets (Anonymous 2019b). Therefore, in this article, it is aimed to give a comprehensive information about vegetarian/vegan nutrition and to examine this type of nutrition in terms of health and especially athletes. According to the results of the literature review, it was aimed to eliminate the aforementioned contradictions and to make concrete suggestions.

History of Vegetarian Diets

The first traces of vegetarian nutrition in the history are found in the groups that adopted the orphism philosophy of the Ancient Greek society living in the 500s BC. It is known that those who adopt orphism don't consume meat (Vikipedia, 2020b). The Greek philosopher and mathematician Pythagoras (570-495 BC), who is regarded as the basis of vegetarianism, described meat consumption as an indicator of violence (Tunçay Son and Bulut, 2016), Empedocles (494-434 BC), who also lived in the same period, stated that there was a connection between vegetarianism and reincarnation. (Vikipedia, 2020b). Especially with the influence of Pythagoras, the vegetarian/vegan diet spread rapidly among the Greek society and it influenced the Roman society. So much so that even the gladiators known as sports heroes, fighting machines and presumably expected to consume a diet rich in high protein and animal foods were found to be vegetarian/vegan (Akıncı and Türkay, 2020). The fact that the historian Plinius (AD 25-79) called the gladiators as barley eaters (hordearii) and the strontium element, whose level increased with plant-based nutrition, was higher in their bones which are thought to

belong to gladiators as a result of excavations seems to support this situation (Akıncı and Türkay, 2020; Anonim, 2014).

After the Greek and Ancient Roman times, there isn't enough information about vegetarianism until the 16th century. It is known that many scientists such as Leonardo da Vinci in the Renaissance and Tryon, Rousseau, Voltaire in the enlightenment period followed a vegetarian/vegan diet. However, it is estimated that the number of vegetarians was a few during this period (Leitzmann, 2014; Türkmen, 2015). After the 1800s, Alexander Haig, a London scientist, used vegetarianism for the first time as the term of health, focused on the adverse effects of red meat and its products on the human body, asserted that red meat triggers uric acid in the human body and also, he rejected meat consumption in his own diet. In addition, who was one of the first scientists to mention about the health benefits of vegetarian nutrition in the field of medicine, referred this subject in his book, "Uric Acid as a Factor in the Causation of Disease", written in 1892 (Vatan and Türkbaş, 2018).

Analyzing the recent process of vegetarianism, after the first vegetarian association (Vegetarian Society) that was established in England in 1847, the American Vegetarian Society was established in 1860 and the German Vegetarian Society was established in 1867. Afterwards, vegetarian associations all over the world gathered in 1908 and merge under the name of International Vegetarian Union (IVU), The Vegan Society and the associations in European countries gathered in 1944 and merge under the name of European Vegetarian Union in 1985 (Tunçay Son and Bulut, 2016; Kuz, 2018; Vatan and Türkbaş, 2018). In 1978, the International Vegetarian Union approved the 1st of October as the "World Vegetarian Day" and the 1st of November as the "World Vegan Day". Every year, the first week of October is celebrated as the International Vegetarian Week and the entire month of October is celebrated as a vegetarian awareness month (Vikipedia, 2020c).

Vegetarian Diet Types and Effects on Health

There are some differences between vegetarian and vegan diets. The main difference between the two diets is that vegan individuals completely refuse animal products and vegetarian individuals can consume various animal products. According to the type of consumption of animal products, vegetarian nutrition is divided into classes. In addition, veganism is considered as a subtype of vegetarian diet (Gökçen et al., 2019). Information about vegetarian diet types is given in Table 1 (Çakıcı et al., 2020; Gökçen et al., 2019; Genç, 2020).

Table 1. *Types of Vegetarian Diets*

Types	Description
Cognitive Vegetarianism	• These people consume all kinds of animal foods but rarely have a vegetarian diet.
Ethical Vegetarianism	• These individuals chose to be vegetarians in order to reduce death and persecution.
Semi-Vegetarianism* (Flexitaryen)	• People who are on a diet of transition to vegetarianism consume milk, dairy products, eggs, a certain amount of white meat or fish per week and don't consume only red meat. Some of them consume organic red meat.
Pesko-Vegetarian *	• They are people who consume milk, dairy products and do not consume all other animal food.
Polo-Vegetarian *	• These people only consume poultry.
Lacto Vegetarian	• These people only consume milk and dairy products.
Lacto-ovo Vegetarian	• Only secondary products (such as eggs, milk, honey) that was produced from animals are eaten. It is the most popular type of vegetarian diet.
Suprotarian Vegetarianism	• This diet usually includes rice, sprouted grains, and legumes.
Veganism	• It is a vegetarian type that these people don't consume meat and meat products, honey, milk, eggs, yogurt, kefir that was obtained from animals, and also don't use clothes that was made of animal products such as wool, silk and leather.
Ravists	• These individuals argue that the food should not be cooked. They believe that the nutritional value of the food will decrease if it is cooked.
Fruvitarians or Fruitists	• 75% of the foods consumed are raw fruits or fruit-like vegetables (tomatoes, etc.), grains and oil seeds. At the same time, vegetables and fruits that aren't completely plucked while harvesting are consumed.
Zenmacrobiotic Diet	• While the diet of people following this diet consists of vegetables, fruits, grains and legumes, some people only eat cereal products.

** This group isn't considered as vegetarian by International Vegetarian Union because it consumes meat.*

Vegetarian diets have advantages over western diets. Obesity, hypertension, cardiovascular diseases, Type 2 diabetes, and mortality risk in all cancers are reduced in individuals who follow a vegetarian diet (Meline et al., 2016). In Adventist Health Research-2; it was found that the overall mortality rates of vegan, lacto-ovo vegetarian and pesco-vegetarians were significantly lower when compared with non-vegetarians (Orlich et al., 2013). There are studies showing that obesity (Braithwaite

et al., 2003; Le and Sabaté, 2014), Type 2 diabetes (Tonstad et al., 2009; Pilid et al., 2014; Yokoyama et al., 2014), cardiovascular diseases (Wang et al., 2015; Dinu et al., 2017) and carcinogenesis (Tantamango-Bartley et al., 2013; Chang et al., 2017) are less in vegetarians. This is explained by the fact that individuals consume high amounts of nutrient-rich food (vegetables, fruits, legumes, oilseeds) from protective nutrients (antioxidants, vitamins and minerals, polyphenols, fiber) against diseases and they don't consume or they less consume individuals consume high amounts of nutrient food (industrial and animal origin foods) from nutrients (simple sugar, saturated fatty acids, trans fatty acids, sodium) that increase the risk of chronic disease (Dewell et al., 2008).

Nutritional Content of Vegetarian Diets and Possible Problems

When the nutrient content of vegetarian diets is compared to normal diets, vegetarian diets appear to be rich in complex carbohydrates, carotenoids, fiber, vitamin C, vitamin E, folic acid and omega-6 fatty acids and magnesium (Balcı, 2018). In a study involving approximately thirty-four thousand people (twenty-one thousand omnivores, ten thousand vegetarians), it was found that individuals following a vegetarian diet received more fiber, thiamine, vitamin C, vitamin E, magnesium, folic acid and iron (Maziars ve ark., 2020). However, some vegetarian diets (especially veganism) contain low amounts of nutrients such as vitamin B12, vitamin D, iodine, iron, calcium, zinc and omega-3 fatty acids. In this case, various health problems can be seen depending on the lack of nutrients (Aksoy Kendilci, 2020).

Among these health problems, there are conflicting results about osteoporosis which is the most discussed. It is stated that vegetarian diets, which can lead to insufficient protein and calcium intake and low body weight, negatively affect bone health; however, at the same time, these diets show alkaline properties. Alkaline environment has positive effects on bone mineral density (Kuz, 2018; Ayaz, 2018). In addition to this, high protein intake (especially animal protein) causes an increase in calcium excretion in the urine. Postmenopausal women who consume large amounts of animal protein and lower amounts of vegetable protein have a high rate of bone resorption and the risk of hip fracture increases (Kuz, 2018). Although there are studies showing that bone mineral density is low in vegan people (Fontana et al., 2005; Knurick et al., 2015), there are some studies that vegetarian diets don't have a significant effect on bone mineral density (Wang et al., 2008) and vegan diets don't clinically result in an increase in fracture risk (Ho-Pham et al., 2009).

Protein deficiency is considered to be the greatest deficiency of vegetarian diets, while lacto-ovo, ovo and lacto are rarely seen in vegatarians, they are more common in vegans. However, the protein requirement for all ages can be obtained with a properly planned vegetarian diet, even for athletes. Vegetable proteins are known to be poor in lysine, methionine, isoleucine, threonine and tryptophan, in addition to being less absorbed than animal proteins. For this reason, in addition to legumes rich in lysine amino acids, whole grains, nuts and seeds rich in tryptophan and methionine can be consumed in larger amounts and the protein and essential amino acid needs of a vegetarian diet can be met (Ongan and Ersoy, 2011; Demir and Seran, 2017; Rogerson, 2017; Ayaz, 2018). When it is examined in terms of calcium, vegetarians can get calcium with the recommended amount of diet, while vegans can be seen insufficient intake. Although vegetable sources contain calcium, calcium absorption is very low in vegetables such as spinach rich in oxalate, beet greens and chard. Therefore, vegans should consume low-oxalate vegetables such as broccoli, soy milk, soy yogurt, and use a variety of calcium supplements (Ongan and Ersoy, 2011; Özcan and Baysal, 2016; Demir and Seran, 2017).

Cited as another noteworthy problem, vitamin B_{12} deficiency is common in those who follow a vegetarian diet (Karabudak and ark., 2008; Pawlak, 2015). It has been stated that B_{12} deficiency varies between 0-86.5% in vegetarians and that deficiency is more common in vegans (Ayaz, 2018). For this reason, lacto-ovo vegetarians should obtain vitamin B_{12} from dairy products, eggs and reliable vitamin B_{12} sources (enriched foods and supplements), vegans should get enriched soy and rice drinks and some breakfast cereals. Otherwise, vitamin B_{12} supplements are required (Kuz, 2018). In vitamin B_{12} deficiency, pernicious anemia, growth retardation, depression, arteriosclerosis due to increased homocysteine and heart diseases can be seen. Moreover, it has been stated that there is a relationship between low serum B_{12} levels and low bone density in children (Özcan and Baysal, 2016).

Other nutrients to be considered in vegetarian diets can be shown as omega-3 fatty acids, iodine, iron and zinc. Eicosapentaenoic acid (EPA) and docosahexaenic acid (DHA), known as fatty acids that are produced from omega-3 in the body, have been reported to be lower in vegetarians (Agnoli et al., 2017). These fatty acids are the leading fatty acids of the 3rd series prostanoids and thromoboxanes (TXA 3, PGE 3, PGI 3) and the 5th series leukotrienes (LTB 5, LTC 5, LTE 5) with anti-inflammatory properties. It is reported that they have positive effects in the prevention

and treatment of diseases such as various cancer types, cardiovascular diseases, hypertension, rheumatoid arthritis, osteoporosis, diabetes, asthma, Alzheimer's, depression and schizophrenia (Çelebi et al., 2017). Vegetarians can increase omega-3 fatty acid levels by preventing dietary factors (high omega-6 intake, low protein, biotin, pyridoxine, calcium magnesium, copper and zinc intake) that restrict the conversion of omega-3 to EPA and DHA (Agnoli et al., 2017). For vegetarians, food such as walnut, canola oil, soy, flax seeds, sea vegetables, seaweed and derivatives are recommended as sources of omega-3 fatty acids (Özcan and Baysal, 2016). Flaxseed oil contains high amounts of omega-3 fatty acids. In order to get this effect fully, the seeds must be crushed and consumed in powder form (Demir and Seran, 2017).

Vegans and some vegetarians (non-consuming seafood or milk and) face an iodine deficiency. For this reason, iodine deficiency should be prevented by using seaweed, iodized salt or iodine supplements. Also, it should be kept in mind that vegetables such as potatoes, soy, broccoli and brussels sprouts, which vegans consume in high amounts, reduce iodine absorption due to their goitrogen content (Özcan and Baysal, 2016). Iodine deficiency is the main cause of hypothyroidism, and this condition is characterized by fatigue, sensitivity to cold, constipation, dry skin, muscle aches and voice changes (Bakır and Şahin, 2019). While iron deficiency anemia cases in vegetarians are similar to non-vegetarians; it is stated that mainly vegetarian diets have lower zinc bioavailability due to their high phytic acid content (Kuz, 2018). Adequate intake of both nutrients is important for maintaining health.

Vegetarian Diets in Athletes

In the last half of the 19th century, while it was believed that meat consumption is necessary for athletic performance, it was known that vegetarian athletes strive to prove the opposite. The facts that diet without meat consumption are applicable for athletes and that champion athletes prefer vegetarian diet have made this diet popular among athletes (Duncan, 2015). In written and visual media sources, it is seen that important names such as Myke Tyson, Serana Williams, Kyrie Irving, Barny du Plessis, Lewis Hamilton, Tia Blanco, Jahina Malik, Fiona Oakes among the champion athletes prefer a vegetarian diet. Although there is no clear information about the prevalence of vegetarian athletes today, based on the data about general population 8% of the them is lacto-ovo vegetarian, 3% of them is vegetarian (meat-chicken-fish non-consuming) and 1% of them is vegan and these rates are expected to

be similar in athletes (Duncan, 2015). In some studies, it is observed that the prevalence of vegetarian athletes varies between 2-5% (Mullinix et al., 2011; Kiertscher and DiMarco, 2013), but this rate increases in female athletes. 30-50% of female athletes follow a semi-vegetarian diet and consume low amounts of meat (Nieman, 1999) and in another study conducted on Indian national female athletes, it was concluded that 22.2% of the athletes were lacto-vegetarian and 15.9% of them is lacto-ovo vegetarian (Khanna et al., 2006).

Advantages of vegetarian diets for athletes are stated that vegetarian diets include complex carbohydrate sources (cereals, legumes, root vegetables) that provide energy for a longer period of time during training or competition (Constatin, 2019), alkaline food (fruits, vegetables, legumes) that is likely to reduce the slowing effect of the acid environment formed in the muscles during training or competition (Barnard et al., 2005) and vitamin C, vitamin E, beta carotene and other antioxidants that minimize the damage caused by increased oxidative stress as a result of sport activities (Kahleova et al., 2011). However, there is a study stating that long-term strict vegan diets have no effect on preventing oxidative stress and chronic diseases (Vanacore et al., 2018).

The disadvantages of vegetarian diets for athletes are that these diets are plant-based and contain anti-nutritional factors such as phytic acid, oxalates, trypsin inhibitors that limit the absorption of some micronutrients and are less bioavailable and less digestible. Additionally, vegetarian diets can lead to negative energy balance in athletes by easily saturating even with low energy intake due to their high fiber content (Constatin, 2019). It is stated that oligomenorrhea and amenorrhea, which is seen especially in female athletes because of insufficient energy intake, are more common in vegetarians (Borrione et al., 2009). Insufficient energy intake causes estrogen deficiency by changing the release of Gonadotropin Releasing Hormone (GnRH) and indirectly causes irregular menstrual cycle and decrease in bone mineral density (Zergeroğlu, 2017). Moreover, vegetarian female athletes' lower circulating estrogen levels have been associated with higher fiber and lower fat intake (Borrione et al., 2009).

As mentioned in the previous section, athletes who follow a vegetarian diet are at risk of deficiencies in energy, protein, essential fatty acids, iron, zinc, iodine, calcium, vitamin D and B_{12} (Melina et al., 2016; Rogerson, 2017). It is defended that insufficient protein intake and some amino acid deficiencies (especially carnitine, carnosine and

creatine) will negatively affect athlete's performance (Nebl et al., 2019). Proper nutrition provides athletes with an opportunity to have optimum health, increase in lean body mass and low-fat percentage, long-term endurance in training and competitions and faster recovery afterwards (Canbolat et al., 2018). For this reason, a nutritional guideline containing recommendations for daily intake is presented in Table 2, taking into account the nutrients with a high risk of deficiency in vegetarian athletes.

Table 2. *Spesific Nutrition Guide for Athletes and Vegeterian Athletes*

	Athletes Specific Considerations	**Vegetarian Athletes Specific Considerations**
Energy [1]	25-35 kcal/kg/day [a] 40-70 kcal/kg/day [b,c,d]	
Carbohydrates [2]	6-7 g/kg/day	3-10 g/kg/day
Proteins [2]	1.5-2.5 g/kg/day	1.8-2.7 g/kg/day
Lipids [2]	1.2-1.7 g/kg/day	0.5-1.5 g/kg/day or 30% of daily caloric intake
Omega-3 Fatty Acids [3]	1-2 g/day (EPA/DHA ratio: 2/1)	
Creatin [4]	1 g/day	2 g/day
Vitamin B_{12} [5]	2.4-2.5 mcg/day	
Vitamin D [5]	15 mcg/day	
Iron [5]	15-18 mg/day	
Zinc [5]	11-15 mg/day	
Calcium [5]	1300-1500 mg/day	
Iodine [5]	120-150 mcg/day	

1. *Kerkscik et al. (2018)* ***2****. Constantin et al. (2019).* ***3****. Rogerson (2017).* ***4****. Borrione et al. (2009).* ***5.*** *Benardot (2012).*

a. *General physical activity 30-40 minutes/day, 3 times a week*

b. *Moderate levels of intense training 2-3 hours/day, 5-6 times a week*

c. *High-volume intense training 3-6 hours/day, 1-2 sessions/day, 5-6 times a week*

d. *Moderate levels of intense training use lower level of range, high-volume intense training uses upper level of range*

The Effect of Vegetarian Diet on Athlete Performance

There is no clear thought about the positive or negative effects of vegetarian diets on performance if athletes eat the recommended amount of carbohydrates. The proper diet, which provides sufficient energy and nutrients, allows the athlete to maintain their health and performance (Borrione et al., 2009). A well-planned vegetarian diet is sufficient in terms of all nutrients and energy (Kuz, 2018). Despite this information, contradictory results are observed in studies examining the effect of vegetarian diets on athlete performance.

When the studies on this subject were examined, aerobic capacities of vegetarian athletes were found to be higher than omnivorous athletes in a study conducted on endurance athletes, and at the end of the study, it was stated that vegetarian diets were applicable to athletes (Lynch et al., 2016). In a study conducted on Indian female athletes, it was found that the endurance and recovery times of omnivorous athletes were better than lacto-ovo and lacto vegetarians (Khanna et al., 2006). Contrary to these studies, in some cross-sectional studies, no difference was found between the aerobic and anaerobic performances of omnivorous athletes and vegetarian athletes (Hanne et al.,1986; Nebl et al., 2019).

When the intervention studies were examined, no difference was found between the performances of the athletes who were given western-style diet and lacto-ovo vegetarian diet during 1000 km marathon running in 20 days (Eisinger et al., 1994). In another study, aerobic performance and isometric contraction strength of athletes that follow western-style diet and lacto-ovo vegetarian diet for 6 weeks were found to be similar. Also, it has been determined that the serum testosterone concentrations of athletes who follow a lacto-ovo vegetarian diet decreased. This situation was explained by the binding of fibers to steroid hormones with increasing fiber consumption. Taking into account that testosterone accelerates muscle growth, not observing a decrease in the performance of lacto-ovo vegetarian athletes was associated with the duration of the study (Raben et al., 1992).

CONCLUSIONS

The American Nutrition Association states that vegetarian diets, which are planned with experts, are healthy, nutrient-sufficient, and may benefit the prevention and treatment of some diseases. In addition, it is stated that well-planned vegetarian diets are suitable for individuals and

athletes at all stages of the life cycle, including pregnancy, breastfeeding, infancy, childhood and adolescence (Craig and Mangels, 2009). However, there are institutions (German Nutrition Association) that oppose the implementation of these diets for children (Ayaz, 2018).

When the athletes are discussed, it is seen that vegetarian diets are becoming popular among athletes, some of professional athletes prefer these nutritional approaches and these diets have positive effects on health. However, as a result of the studies, it couldn't be revealed that vegetarian diets have a clear advantage over normal diets in terms of athlete performance. While performance has been found to be improved in vegetarian athletes who are only loaded with creatine (Borione et al., 2009), this increase is thought to be due to the generally low creatine level in vegetarians.

In conclusion, if normal individuals and athletes meet their energy and nutrient needs, it doesn't matter whether their diets include vegetarian or omnivorous features. For this reason, especially the guide given in Table 2 is important for athletes to maintain their performance and health. In addition of these, the lack of sufficient studies on the effect of vegetarian diets on athletes makes this field still interesting today.

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Chapter 6

METABOLIC RESPONSES TO ENERGY DRINKS

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Introduction

Physical activity can be defined as bodily movements that cause energy consumption above the basal level with a body movement created by skeletal muscles. It is thought that exercise positively affects human health in many ways, and regular exercise reduces the risks of some diseases and is an effective method to prevent these diseases (Russel et al. 1995). In some cases, appropriate exercise programs are effective in reducing deaths caused by cardiovascular system diseases. It also provides benefits such as improvement in hematological profiles, hypertension and lipid metabolism (Van Camp et al. 1994), physical fitness and psychological health (De Moor et al. 2006).

During physical activity or exercise, some energy systems come into play in our body and provide the formation of different metabolic activities in the body depending on factors such as the movement style of the exercise, the basic characteristics of the sport, its duration and intensity. There are four different energy sources, consisting of muscle and liver glycogen, glucose in the blood and fats that can provide the energy required for the body during exercise. With aerobic and anaerobic energy metabolism, the required energy is generated by the production of ATP, which is stored in muscles. When necessary, when one of the ATP phosphate bonds is broken down, ATP is transformed into adenosine diphosphate and energy is supplied with a phosphate molecule. Three metabolic systems are important for physical activities. These systems; phosphogen system, anaerobic glycolysis lactic acid and aerobic systems. The purpose of these metabolic processes is to reproduce ATP existing in the muscle (Günay, Tamer, Cicioğlu, 2010).

Although the importance of exercise and physical activity is better understood day by day, individuals choose a sedentary life by being negatively affected by environmental factors such as eating habits, intensity of work life and stress. Stress factors, negative eating habits, lack of regular and adequate nutrition make the human body vulnerable to diseases. It has to struggle with many diseases such as cardiovascular diseases and metabolic disorders. Studies in the literature have proven that exercise has many positive physiological, physical, psychological and dynamic effects (Boşnak-Güçlü et al. 2008). The positive effects of exercise on biochemical parameters have been demonstrated in many studies (Turğut and Sarikaya, 2020; Turğut et al. 2018; Cınar et al. 2017; Pancar et a. 2018; Pancar, Özdal, Vural, 2018). Regular exercise causes more oxygen consumption in working muscles. Depending on

this consumption, reactive oxygen species are released in the body and oxidative stress can increase and damage many tissues and organs (Biçer, 2008). These radicals increase during exercise and return to their previous levels after exercise. With the increase in the functions of skeletal muscle cells by contraction, metabolism is affected, causing an increase in cytokines. Due to the excess production of various anti-inflammatory cytokines with exercise, the acute phase response is developed by the body (Kaspais and Thompson, 2005).

The consumption of energy drinks is increasing, without knowing the effects and mechanisms of the ingredients. People in line with different goals and demands; They consume energy drinks with the thought of having fun, increasing performance and increasing concentration. As a result, individuals encounter negative physical, physiological and psychological problems (Görgülü et al. 2014). It is thought that the consumption of energy drinks arbitrarily by individuals who display a sedentary lifestyle not only gives energy, but also causes the person to be metabolically distressed and causes some organs and tissues to fat in the body.

Exercise and Energy Drinks

Definition of energy drinks; the Turkish Food Codex regulation states in the Energy Drinks Communiqué (Communiqué no 2006/47) that "it refers to beverages that provide energy to the human body due to the usable carbohydrate content in its composition and can contain functional substances, vitamins and minerals, whose limits are determined in the product properties" (Pancar, 2020). Energy drinks first entered the world agenda in the 1960s in Asia and Europe, then became popular first in Europe and then in North America (Görgülü et al. 2014). The United States of America is ranked first in the world consumption of energy drinks. As of 2007, it ranks first with 3.8 qt units per person, approximately 290 million gallons of energy drink consumption. In addition, the age of consumption is gradually decreasing and the consumption rate is increasing (Dikici, Aydın, Kutlucan, 2012). Turkey is also in Turkey has entered the market in 1998. Although the consumption of energy drinks is increasing gradually, the total annual consumption in the Turkish energy drink market is approximately 50 million boxes. About 40 domestic and foreign companies are active in the energy drink market (Iyadurai, Chung, 2007).

Energy drink consumption in western countries compared with Turkey is more. The reason for this is that beverage companies have

entered the market of these countries and these communities have met energy drinks long before (Gözler, 2016). Energy drinks are products that are mostly consumed by young people for many different purposes and are considered to increase mental, physical and psychological performance (Varım et al. 2015). However, the negative effects of these drinks also cause psychological and physical consequences, and noticeable psychological damages (Görgülü et al. 2014). Although there are many commercial forms of energy drinks, the ingredients they contain are basically in different proportions; It contains ingredients such as caffeine, taurine, glucuronolactone, inositol, ginseng, B vitamin complexes, guarana and carnitine (Bahadırlı et al. 2015). Interest in energy drinks is increasing day by day and many young people and athletes consume these drinks as sports drinks because they believe that they increase energy and performance and do not know the difference between them. The amount of caffeine in energy drinks should be well adjusted and the purpose of use should be determined exactly. The caffeine dose should not exceed 150 mg / L (Sipahi et al. 2014). The increase in cognitive performance is due to the stimulating effect of caffeine. However, the stimulating effects of many substances found in energy drinks with each other and their interactions need to be investigated (Babu, Church, Lewander, 2008). As a matter of fact, many warnings such as not recommended for individuals under the age of 18 on the product labels within the scope of energy drinks, the elderly such as the elderly, diabetics, individuals with high blood pressure, pregnant and breastfeeding women, those with metabolic diseases, individuals with kidney failure and individuals who may be sensitive to caffeine (Pancar, 2020).

Ingredients of Energy Drinks

Energy drinks contain substances that affect many functions. These contain different doses of caffeine, taurine, inositol, glucuronolactone, carnitine, guarana, B vitamin complexes and ginseng substances. Caffeine is found naturally in the fruits, seeds and leaves of many plants, and the most common sources are tea leaves, coffee and cocoa beans, and cola seeds. The first information about the consumption of caffeine is known as a tea consumed by boiling its leaves in China in 2700 BC. However, the serious introduction of caffeine into our lives began with the discovery of the coffee plant by the Ethiopian shepherd Kaldi in the middle of the 8th century (Garipağaoğlu and Kuyrukçu, 2009). Caffeine is rapidly absorbed from the gastrointestinal tract shortly after oral administration, peaking in the blood within a period of 15-120 minutes.

Caffeine is metabolized in the liver and many metabolites such as paraxanthin (80%), theobromine (15%) and theophylline (4%) emerge as a result of its absorption (Harland, 2000; Kendrick and Day, 2007). It is transported by simple diffusion and 80% of caffeine, which is carried in plasma due to albumin, is found in the brain, and since there is no obstacle during its passage to cells, it rapidly spreads to all cells and tissues in the body, including the brain, testis and fetus (Rakıcıoğlu, 1993). Caffeine provides a moderate stimulating effect in the central nervous system, depending on the rate of consumption. This effect is achieved through adenosine, which is a neurotransmitter and is found in many regions of the brain (Lieberman, 2003). In addition, it has been stated that it negatively affects sleep, delays the process of falling asleep, causes an increase in heart rate and blood pressure, and increases stomach acid. It is also stated that energy drinks containing caffeine reduce the reaction time, increase oxygen and non-oxygen capacity, have an ergogenic effect, and reduce insomnia in motor vehicle users (Curry and Stasio, 2009). Studies have shown that when caffeine and glucose are consumed together, there is an increase in cognitive performance (Kennedy and Scholey, 2004) and effects on improvement in individual fatigue (Scholey and Kennedy, 2004). While moderate consumption of caffeine does not cause adverse effects in healthy individuals, excessive consumption (> 400 mg / day) can cause psychological changes in mood; irritability, tension causes adverse changes such as sleep disorders, arrhythmia, abdominal pain, and palpitations (Arpacı, 2011).

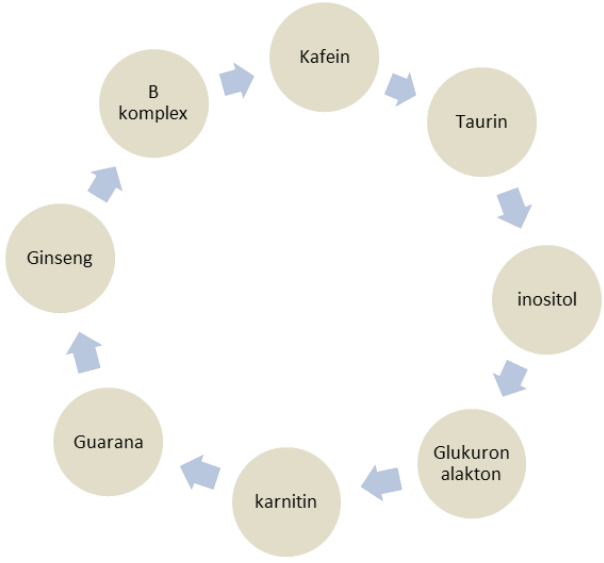

Figure 1. Substances in energy drinks

It is an intracellular amino acid that contains thiol and is the most abundant in humans and is a normal component of the nutritional diet in humans (Gaull, 1989). Taurine is found in different concentrations in central nervous system cells, and it is the most abundant free amino acid in skeletal and cardiac muscle cells. There are studies that modulate skeletal muscle function and show an increase in exercise capacity. In addition, it is emphasized that there may be improvements in DNA damage that may occur with exercise (Ballard et al. 2010). There are studies showing that it decreases anxiety level by increasing gamma amino butyric acid level, positively affects locomotor activity by increasing dopamine level and decreases oxidative stress at cellular level (Tribel et al. 2007). Taurine has also been reported to have numerous biological and physiological functions, including preventing bile acid conjugation and cholestasis, antiarrhythmic, inotropic and chronotropic effects, central nervous system neuro-modulation, retinal development and function, antioxidant and anti-inflammatory properties (Lourenco and Camilo, 2002; Sato and Kurasaki, 2003). There has not been enough scientific studies and related evidence to show how much taurine should be in the content of the energy drink. However, as the days pass, increasing levels of energy drinks contain high levels of taurine (Whirley et al. 2008).

It is a B complex vitamin that the body needs in low amounts to stay healthy. However, since this substance, which is a product of glucose in the body, is synthesized by intestinal bacteria, it is not considered a vitamin. Although inositol is present in all body tissues; Its density is found in the lens layer of the brain, heart and eye. In the brain, serotonin increases sensitivity to the hormone and the level of arousal, and there are studies showing that when a decrease in inositol level occurs, it triggers anxiety, depression, and panic attack disorders (Lewis et al. 2013).

It is a glucose metabolism product synthesized in the liver. However, energy drinks contain synthetic glucuronolactone and contain much more than its human value (Wolk et al. 2012). Since there are not enough studies regarding the positive and negative effects of this substance on humans, there is no evidence-based information about its impact area.

Carnitine is one of the amino acid components that play a role in the oxidation of fats that the body produces naturally. It consists of the synthesis of carnitine, lysine and methionine, which are stored in the brain, muscle tissues and heart. Although it is sufficient in daily nutrition, it is also produced in the liver. It has been suggested that energy drinks

manufacturers are required to help burn fat and improve performance (Babu et al. 2008).

This plant, whose botanical name is Paullinia Cupana, is a species of shrub originating from South America, grown in the Amazon and its fruits are used in the medical world. Guarana seeds are known as the richest caffeine source, as they contain three times more caffeine than coffee beans. It contains theobromine, theophylline and tannin along with caffeine. This ingredient shows the effects of caffeine. It has been emphasized in studies that it has effects such as increased attention and performance, reducing fatigue and stimulating the central nervous system. In addition, there is information that it has a strong diuretic effect, increases in fat breakdown and reduces broncho-spasm (Babu et al. 2008).

The ginseng plant, whose botanical name is Panax, is a hardy and perennial herb. It is a plant that can grow spontaneously, especially in the Far East and the American continent. The main active components of this plant, which is preferred because of its physical performance enhancing and mental benefits, as it is used for the treatment and prevention of diseases in many areas, are glycosidal saponins (glycosylate steroids) known as ginsenosides. It strengthens the brain with its effects on the central nervous system, generally protects homeostasis and protects the body against all kinds of negative effects of physical, chemical and biological factors, helps to eliminate disorders such as depression and anxiety, reduces stress, strengthens memory and increases motivation. It is recognized as a tonic and adaptogen (Nocerino et al. 200; Seo et al. 2008; O'Hara et al. 1998).

Because B vitamins are a group of vitamins, vitamins in this group are also called B complex vitamins. Vitamins in this group are named this because they are present together at the same time. Vitamins in this group: Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Nicotinamide, Niacin (Vitamin B3), Cholin (Vitamin B4), Pantothenic acid (Vitamin B5), Pyridoxine (Vitamin B6), Biotin (Vitamin B7), Folic acid (Vitamin B9), Cobalamin (Vitamin B12). These vitamins act as coenzymes to other enzymes (Tulum, 2007). B vitamins are said to play a key role in uncovering all the energy secreted by simple sugars and are used in energy drinks for this (Gözler, 2016).

Intensive use of energy drinks has become widespread due to the positive effect on cognitive and psychomotor functions, minimizing fatigue and increasing performance and endurance (Mets et al. 2011).

Metabolic responses to exercise and energy drink supplementation

The results of studies related to exercise have shown that exercise is a source of stress by increasing the production of free oxygen radicals, creating oxidative stress and on the other hand, it develops defense against oxidative stress by affecting antioxidant enzyme activity (Aguilo et al. 2003). While the proportions of oxidants and antioxidants that occur during exercise vary depending on the intensity of the exercise, more oxidants are formed in vigorous and intense exercises, while regular and short-term non-violent exercises stimulate the antioxidant systems more (Radak et al. 200; Atalay et al. 2004; Banerjee et al. 2003). Studies have shown that exercise can scavenge harmful radicals, work as an antioxidant mechanism, and increase antioxidant enzymes as a result of exercise (Timothy et al. 2003).

Exercise is a factor that has positive effects on many systems and maintains order endocrinologically. It has been reported that regular physical activity or exercise reduces coronary heart diseases, ischemic necrosis and related mortality rates in humans (Lakka et al. 2005). It has been reported that exercise reduces triglyceride levels from blood lipids, increases HDL cholesterol levels, lowers blood pressure, regulates endothelial function and hemostatic factors, minimizes the risk of metabolic syndrome and the development of Type 2 Diabetes (Laaksonen et al. 2005).

Due to the high content of energy drinks, some substances (components such as taurine and glucuronolactone) in the body, their high content in energy drinks constitutes the source of important effects and side effects for the human body (Şen et al. 2015). There have been studies suggesting that obesity may also be a risk factor due to its high sugar content (9). In this case, it is thought that when the calories taken from the energy drink are not consumed as energy, they will accumulate as excess sugar and fat in the body and damage the tissues and blood circulation metabolism.

In a study, apelin, irisin, ghrelin, and leptin levels of basketball exercises applied five days a week for eight weeks were examined. At the end of the study, apelin and ghrelin levels increased after exercise, but a decrease in leptin, ghrelin, and irisin levels after exercise was observed when compared to other groups (Dündar et al. 2019). The increase in iris release due to exercise triggers the release of UCP1 in the subcutaneous adipose tissue. Betatrophin hormone, which plays an active role in glucose metabolism, also increases depending on the stimulation of

UCP1 (Sanchis-Gomar, Perez-Quilis, 2014).

In a recent study in animal experiments, an increase in blood fat and body weight was observed in rats supplemented with energy drinks and immobilized. Increase in blood lipids can lead to heart disease and hormonal disruptions. Controlled consumption of energy drinks is recommended. Individuals who lead a sedentary life should not be burdened with an extra calorie load as they cannot release their energy.

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Chapter 7

DEEP LEARNING STRUCTURES USED IN PULMONARY CANCER DIAGNOSIS

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This chapter presents an overview of deep learning (DL) structures in pulmonary cancer diagnosis. It is divided into three parts. In this first section, an introduction to pulmonary cancer diagnosis has given along with an overview of related research in this field. Hereafter, DL algorithms used in the diagnosis of pulmonary cancer is presented in section 2. In section 3, commercial software programs serving radiologists in the diagnosis of pulmonary cancer are outlined.

The chapter of the book has been prepared especially for software developers who want to work on pulmonary cancer diagnosis.

1.Introduction

Pulmonary cancer is considered as the deadliest cancer worldwide. It has been predicted to be one of the greatest single source of mortality among the European population in 2019 [1]. Bestow to GLOBOCAN statistics, about 18.1 million new cancer cases arrived in 2018 that sourced 9.6 million cancer deaths [2]. For this reason, many countries are developing strategies for the early diagnosis of pulmonary cancer [3].

What are the Cancer and Pulmonary Cancer?

Cancer is the unchecked growth of abnormal cells in the body [2]. Normal healthy cells are born, perform a certain function and later on die and are replaced by other cells. This is not the case for cancerous cells, cell death does not occur for these cells and they continue to multiply to form a large cell mass. These large masses of cells are known as tumours. Tumours can be broadly divided into two types; benign and malignant [4]. Benign tumours are not cancerous, they are harmless. It does not infect other tissues or spread. Malignant tumours and cancerous and infect other tissues as well as spread around the body.

Types of Pulmonary Cancer

There are two major types of pulmonary cancer, non-small cell cancer (NSCC) and small cell pulmonary cancer (SCC). NSCC accounts for about 85% of pulmonary cancers as presented in Fig.1.

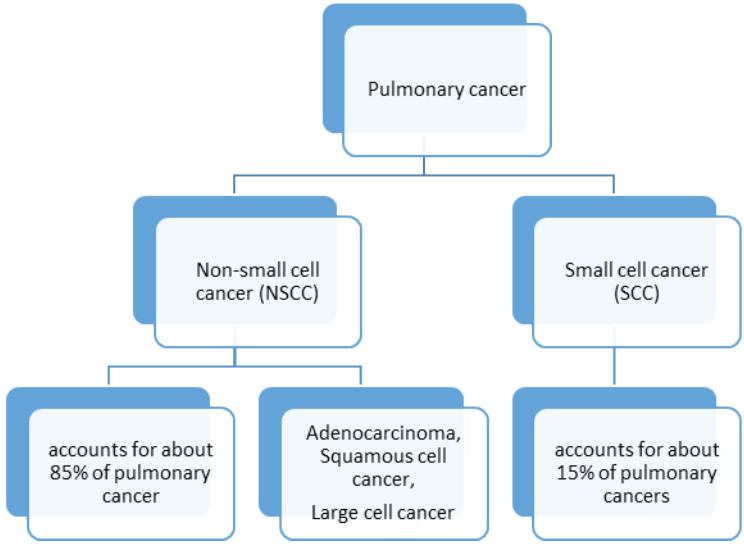


Fig.1 Types of pulmonary cancer

Laps of Pulmonary Cancer

For pulmonary cancer, staging is done by doctors to identify the rate at which the cancer is expanding and if it will in fact spread to other parts of the body. According to [5], the progression of pulmonary cancer disease can be branched into 4 laps. Fig. 2 shows laps of NSCC. SCC is more sensible to chemotherapy than NSCC.

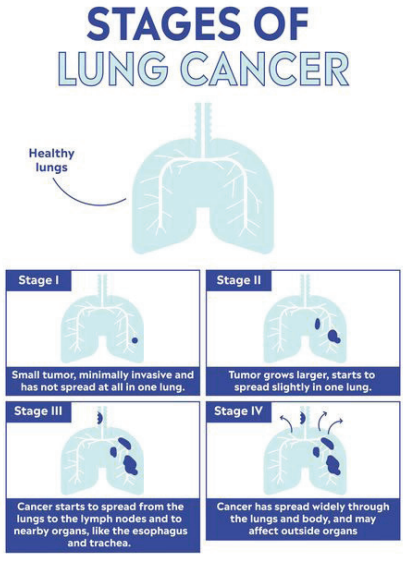


Fig.2 Laps of NSCC [6].

Lap 1: These are known as low grade cells and are similar in appearance to healthy cells where they do not spread to any lymph nodes.

Lap 2: Their appearance is more a typical to the common cell. It has a higher chance of spreading to different parts of the body.

Lap 3: They are known as high grade cells and have a very high chance of spreading to other cells. These cells look and behave nothing like normal cells.

Lap 4: This is the most advanced lap of pulmonary cancer, and is also described as advanced disease.

Types of Medicinal Imaging

Medicinal imaging shows a critical role in founding the diagnoses for numerous conditions and it is used generally in nearly every branch of medicine. The following will explain the types of medicinal imaging.

CT/PET Scan: A CT scan is equal to an x-ray, except that a CT scan takes many x-ray images from various angles. All these images are then united using a computer to prepare a very detailed image of the area being tested. With Positron emission tomography (PET) scan, a radioactive substance is insert the body. This substance is picked up by the PET scan to offer how certain organs are working inside the body. The PET scan not only offers images but also a live video supply of the radioactive substance traveling through the areas being tested [7, 8]. PET scan are often combined with CT imaging (Fig. 3).

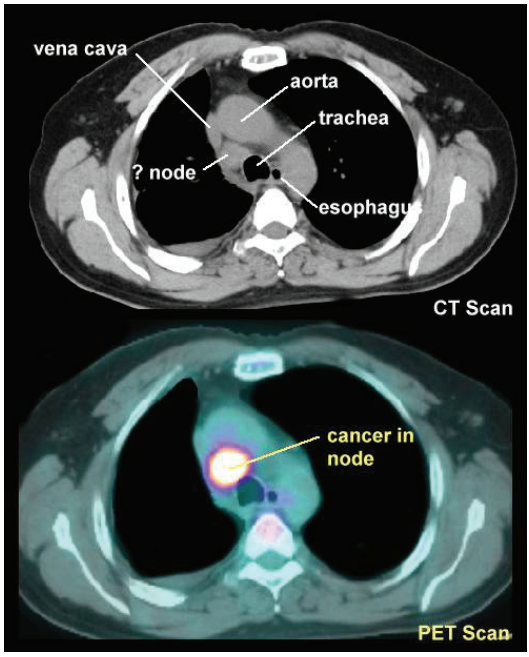


Fig.3 Comparison showing PET superior to CT [9].

MRI Scan: It is another medicinal imaging technique used to create images of organs in the body using strong magnetic fields and radio waves (see Fig.4). However, a disadvantage of taking magnetic resonance imaging (MRI) scans is that it does not achieve well on moving parts. Thus the expansion and contraction of the pulmonary during breathing would lead to a problem. As a result, it is rarely taken in pulmonary related conditions, but is used to check whether cancer has increase to other parts of the body [4].

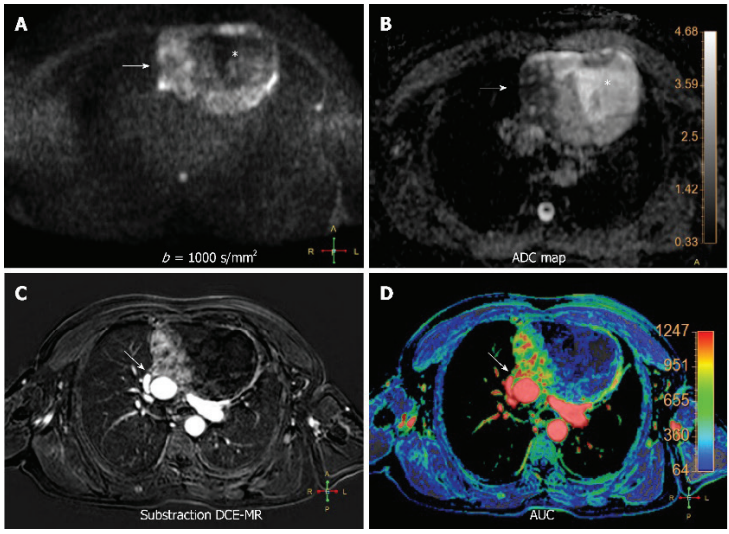


Fig.4 MRI scan [10].

X-rays: X-rays are used to detect problems with a person's musculoskeletal system; This means that x-rays are used to identify problems with our bones, muscles, ligaments, and tendons. In addition, the chest x-ray examines the lungs and bony thoracic cavity.

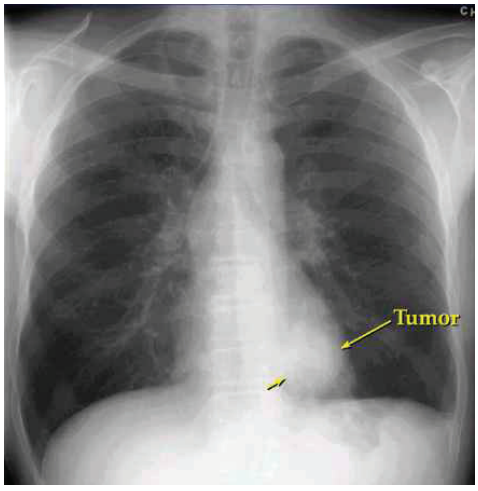


Fig.5 small tumor hidden behind the heart in the left lower lobe [11].

Ultrasound: Ultrasound is a very common procedure that can be used to detect the functions of certain organs. It is best known for testing and monitoring during pregnancy. The ultrasound tool sends tiny vibrations through the area being tested. When those vibrations hit the affected area,

they travel back to the ultrasound tool which then creates an image. This image outlines the area being looked at.

Images obtained with PET/CT can be very useful in various ways in the evaluation of suspected or pathologically proven cases of pulmonary cancer [12]. CT scan methods are regularly used to determine and recognize cancer, circulatory system diseases and conditions, provocative diseases, and head and domestic organ injuries. Many people suffer from medicinal negligence every day. So, adopting an automated computer system as a remedy would be very useful in diagnosing a patient's health from test results.

2.Deep Learning Structures used in Pulmonary Cancer

Low-dose pulmonary CT scanning provides an effective means of early detection, which can drastically reduce the pulmonary cancer mortality rate. Current pulmonary CT analysis research mainly includes nodule detection [13,14], and nodule classification [15,16,17,18].

2.1. Area of research in Pulmonary cancer

2.1.1 Pulmonary nodule detection

Detecting objects of interest is an essential part of analysis and is one of the most labor-intensive works for doctors. Various CNN architectures have been popularized to recognize several complain rapidly and exactly. R-CNN [19,20] associates place motions with CNN and has then been enhanced to Fast R-CNN [20] and Faster R-CNN [21] with superior achievement. There are several methods that computationally send the image detection problem using various regression; 2 of the most popular CNNs, you only look once (YOLO) [22] and the single-shot multi-box detector (SSD) [23] strongly predicts bounding boxes and classification feasibility.

Pulmonary nodule detection is another up-and-coming area for the application of DL. Pulmonary cancer is the outstanding source of cancer-related deaths worldwide, and chest radiography is the most universally used screening and imaging tool to detect pulmonary cancer. Unfortunately, however, due to the amazing reactions of anatomical complexity on chest radiographs, lung cancer screening using flat chest radiographs has allowed unsatisfactory results, with reports of unnoticed nodules being up to 40% [24, 25]. CAD models have been state-of-the-art to help doctors to detect pulmonary nodules.

2.1.2. Classification

One of the main tasks of radiologists is to constitute a useful differential diagnosis for medicinal images. This business can be detailed analytically as a classification work using input from medicinal images and any available clinical information [26].

2.1.3. Segmentation

Segmentation is mostly the dissolution of a region of interest (ROI) from the environment of the medicinal image. ROI is the part of the medicinal image that we wish to use. In the case of cancerous medicinal images, we use the lesion part to selection the features from the diseased part. The innovations of object classification have now removed to semantic segmentation. This is a familiar work for both natural and medicinal image analysis, whereby each voxel is classified in an image to adjust the boundary conditions that conclude a specific object [27].

2.2. Image Processing

The image processing method eliminates some problems in the image, allowing us to get more information than we would get in the normal image. One of the important usage areas of image processing techniques is the medicinal sector.

When using image processing techniques of medicinal images, some problems such as low resolution, noise, and the appearance of imaging structures are encountered. The reasons for these problems are the technological devices used and the negative factors encountered during image acquisition.

2.3. Deep Learning

Signal processing with convolutional neural network, video analysis, image analysis and detection, classification, medicinal image processing, etc. It has done important work. Some steps are taken while using this neural network. These are defined as pre-treatment, feature extraction and classification-detection. In each stage special approaches are exhibited and efforts are made to increase accuracy.

Many different approaches are presented especially for the feature extraction process. It is tried to reveal the specific points of the event desired to be detected by feature extraction. In the next process, neural networks are used to determine the class of properties determined using artificial neural networks [28-33].

2.4. Convolutional Neural Network Architectures

Convolutional neural network (CNN), which is a multi-layer feed forward neural network, is used especially for image analysis. CNN is the most extensively used deep learning model in medicinal image analysis. It is one of the artificial neural networks (ANN) that can be described by a convolutional layer that is other from different neural networks (see Fig. 6). A typical CNN consists of a convolutional layer, a pool layer, and a fully connected layer. The convolutional layer is the focus of a CNN. When used in a CNN, convolution means that a kernel is covered to the input data to give a feature map (see Fig. 7). It is thought that the cells in the center of the visual are branched into sub-regions to sheet the whole image, simple cells with edge-like features, and complex cells with larger receptors, focusing on the whole image.

The CNN algorithm, which is a forward neural network, was created by taking inspiration from the visual center of animals. The mathematical convolution process here is based on stimuli from a neuron's own field of stimulation can be thought of as the answer given. CNN consists of one or more fully connected layers, like one or more convolutional layers, a subsampling layer followed by a standard multi-layer neural network. One of the foreign items of CNN is an activation function. In spite of sigmoid or hyperbolic tangent functions were used previously, the most common nonlinear activation function is the rectified linear unit (ReLU) function [32,33,34]. CNN is the most widely used DL model in medicinal image analysis.

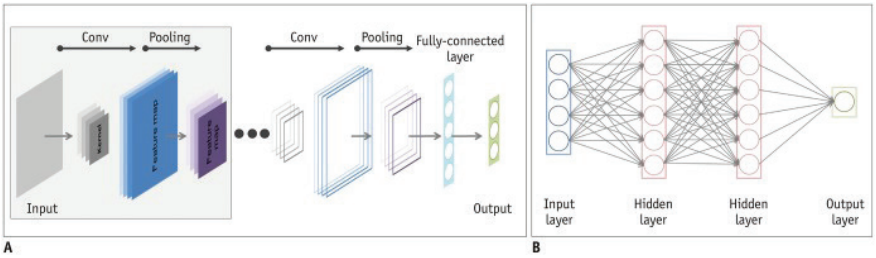
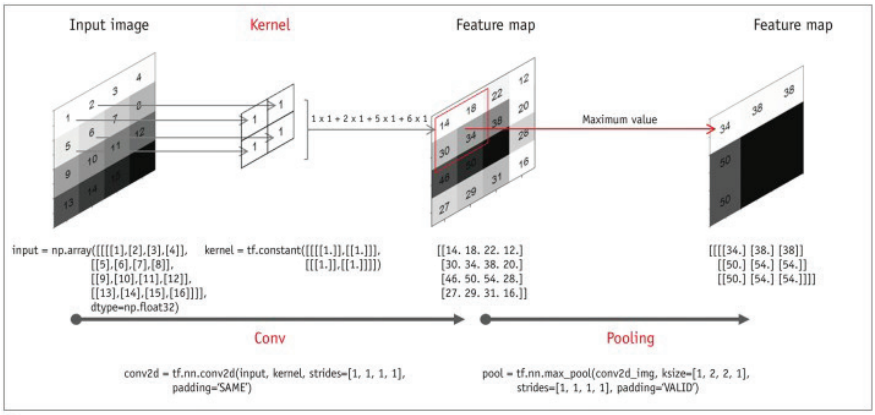


Fig. 6. Diagram of CNN models [32].



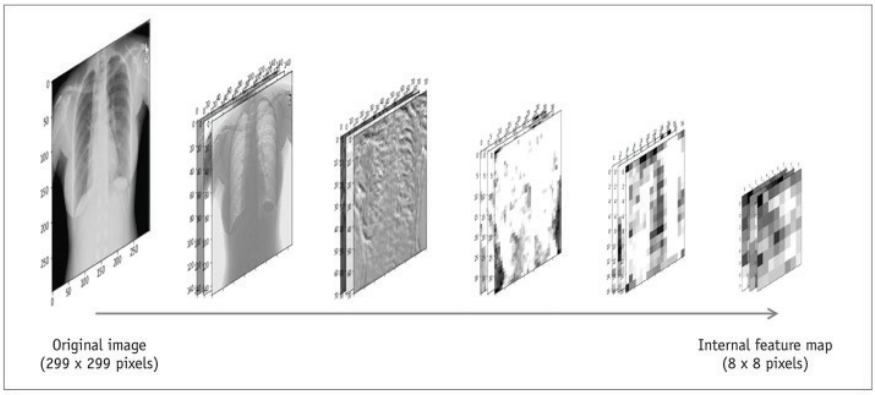


Fig. 7. Convolution and pooling [32].

2.5. Related Work

A lot of the papers examined here studied the nodule detection and nodule classification of pulmonary nodules from the medicinal images. The end-to-end papers covered in these reviews can help to build an effective CAD model to aid the radiologist. [52, 58] seem to work better than the other works reviewed here since its performance screens both the detection and classification tasks (see Table 1).

Table 1. *Summary of selected papers on pulmonary cancer detection and classification.*

Reference	Year	Methods	Tasks	Data set name	Sensitivity/ Specificity/ Accuracy (%)
35	2018	M- CNN	Nodule classification Malignancy estimation	LIDC-IDRI	89.40/93.18/95.61
36	2018	M-CNN	Automatic Nodule detection	LIDC-IDRI	96.64/71.43/82.51
37	2018	LeNet and AlexNet	Nodule classification	LIDC	82.23
38	2018	CNN Deep Fully CNN	Cancer detection and lap classification	LIDC-IDRI, RIDER, SPIE challenge, LUNA 16, LungCT-Diagnosis Shangai Hospital	83.91/89.32/86.02 86.54/74.58/80.64 81.22/82.97/84.87 73.14/81.95/80.12 82.54/93.60/89.52 83.67/96.17/86.32
39	2018	Deep reinforcement learning model	Nodule detection	LIDC-IDRI LUNA	58.9/55.3/64.4
40	2018	3D Faster R-CNN	Nodule detection and classification	LIDC-IDRI LUNA 16	95.8/90.44/ 81.41
41	2018	3D Multi-Output DenseNet	Diagnostic classification	LIDC-IDRI	ACC: 86.84 AUC:0,9010
42	2018	2D and 3D Fast Capsule networks	Nodule detection	General electric and siemens scanners	Precision/recall/ error rate 89.71/87.41/11.45 91.84/89.11/9.52
43	2018	SVM	detection	LIDC	82.5/50/92
44	2018	Probabilistic neural network (PNN)	classification	LIDC-IDRI Sincan Nafiz Koen Hospital	97.42/94.24/95.91
45	2018	CNN+SVM	classification	TCIA	ACC:91.9
46	2019	2D-CNN	Nodule detection	LUNA16	SENS:86.42
47	2019	GoogleNet CNN	detection	LIDC	100/-/99
48	2019	RGBPCANNet	classification	LIDC-IDRI	93.12/91.37/93.25
49	2019	MR CNN	classification	LIDC-IDRI	81/95/90
50	2019	2D CNN	classification	LIDC-IDRI	96/97.3/97.2
51	2019	2D Fully CNN, 3D Filtration		LIDC-IDRI	SENS:97.8
52	2019	GoogleNet and AlexNet	Nodule detection	LIDC-IDRI	ACC:99.05 ACC:99.91
53	2020	Logit-Boost	Nodule detection	LIDC-IDRI	96.88/100/99.23

54	2020	2D DCNN	Nodule detection and classification	Picture Archiving and Communication systems	ACC:99.42
55	2020	3D CNN SVM	classification	LIDC-IDRI LUNA2016 University Medical center Utrecht	ACC:91.22
56	2020	m-RPN, 3D-DeepCNN	Detection and diagnosis	SPH6 ANODE09 LIDC-IDRI LUNA2016	Sens:98.4 FROC:0.946
57	2020	Modified AlexNet	Pulmonary abnormality	LIDC-IDRI	Acc: 97,27
58	2020	2D-CNN	Cancer recognition	LIDC-IDRI SPIE-AAPM	Acc: 98.83 Acc:99.97
59	2020	CNN	classification	LIDC-IDRI	79.4/83.8/82.3
60	2020	V-Net	detection and diagnosis	LUNA16	Auc:0.98 Sens:96.5
61	2020	ResNet	Segmentation classification	LUNA16	Accuracy:88
62	2020	FPSOCNN	classification	LIDC	97.93/96.32/95.62
63	2020	ResNet	Nodule detection	LIDC	Accuracy:95.24
64	2020	CAD, U-Net	Nodule detection	TCIA LIDC-IDRI	AUC: 0.754
65	2020	VGG16	Nodule detection	LIDC-IDRI	MAP:82.7
66	2020	LTCoP LBP SVM	Nodule detection	LIDC-IDRI	Acc:91.5
67	2020	I3DR-Net RetinaNet	Nodule detection	LIDC Moskow Private Dataset	94.12/ mAP:49.61/81.84 65.90/ mAP:49.61/70.36
68	2020	a deep-learning-based model observer	Nodule detection	SOMATOM Force, Siemens Healthineers	Correlation coefficient: 0.98
69	2020	ACM SVM	Nodule detection and classification	LIDC and LUNA16	88.2
70	2020	Multi -scale dence CNN	Nodule detection	LIDC-IDRI	FROC:95
71	2020	3D CNN	Nodule detection	Fleischner Society Guidelines	SENS:93.09
72	2020	CNN RNN	Nodule detection	LUNA16 LIDC NDSB	AUC:0.78 AUC:0.86
73	2020	RetinaNet	Nodule detection	JSRT	0.97

74	2020	Multi-step cascaded Networks	Nodule detection	LUNA16 TianChi Dataset	89.92/94.85/93.96
75	2020	LoG algorithm	Nodule detection	TCIA	97

2.7. Programming languages for DL technologies

Python Programming Language

With more than 8.2 million developers worldwide using Python for coding, Python classes first with 100 points in the current annual ranking of popular programming languages by IEEE Spectrum [76].

R Programming Language

The framework provides code for supervised machine learning (SML) methods like classification, regression and survival analysis, as well as unsupervised methods like clustering.

Java and JavaScript

While Python and R continue to be favorited of ML enthusiasts, Java is gaining popularity among machine learning engineers from Java development backgrounds as they do not need to learn a new programming language such as Python or R to implement ML.

Julia

Julia is a high-performance, general-purpose dynamic programming language that has many dominant features for ML only, emerging as a potential competitor for Python and R.

Tensorflow: Google's generally used machine-learning framework with APIs for a wide change of languages.

List Processing

LISP is considered to be the most efficient and flexible ML language for solving specific problems, as it adapts to the solution a programmer encodes.

3. Some Commercial Sites for Pulmonary Cancer Diagnosis

In this section, information is provided about some commercial sites for pulmonary cancer diagnosis.

Blackford

Blackford delivers a dedicated platform and service for the effective selection, deployment, orchestration, and use of best-in-class medicinal imaging applications and AI [77]. Fast and easy to use, automated image

registration increases radiologist efficiency by 10-20%, and up to 50% for more challenging exams. It allows radiologists to quickly locate and compare an increased number of findings and model new measurements based on past exams, resulting in better reporting.

PixelShine

PixelShine permits medicinal imaging centers using legacy scanners to offer high-quality CT scans by taking them at a substantially reduced radiation dose. It can also improve the quality of blurry and waxy CT scans processed by IR software. Blurry CT scans can make the professional interpretation and diagnosis unclear – jeopardizing patient outcomes, but PixelShine can improve the quality of the scans resulting in better outcomes [78].

Zebra Medicinal Vision

Zebra-Med's Chest Solutions is a radiological package software that can provide multiple capabilities, including triage, visualization of puzzles, and automatic separation between normal runs [79]. Certain insights are more valuable when visualized because they are more difficult to find. Visualization also helps the radiologist to better understand the "why" behind an algorithm's decision. For these reasons, Zebra provides images of direct conversation to visualize suspicious pages in crawling (see Figure 8).

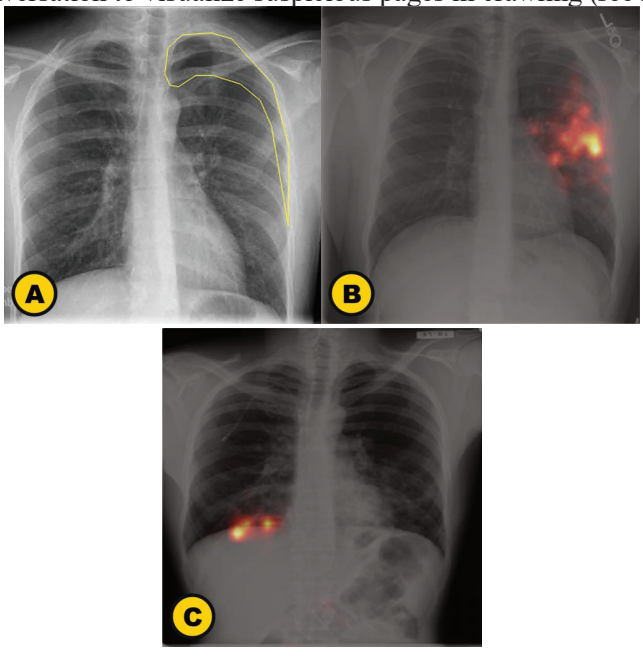


Fig.8 Pneumothorax (A), Consolidation (B), Pneumoperitoneum/free air (C) [79].

Aidoc

Aidoc develops advanced healthcare grade AI-based decision support software. This technology helps radiologists prioritize life-threatening cases and accelerate patient care by analyzing medicinal imaging to provide one of the most comprehensive solutions for marking acute abnormalities in the body [80]. Triage and notification software specified for use in analysis of 510 (k) CTPA images; It marks and transmits Pulmonary Embolism (PE). Triage and notification software specified for use in analysis of 510 (k) CT images; Marks and transmits incidental PE in GE and Siemens scanners.

aetherAI

With the development of full slide imaging technology, digital pathology has become a next generation milestone in the digitization of medical images. Offering a complete solution in digital pathology in addition to digital slide management and reading compilation, aetherAI integrates image disclosure as well as extraction and training of the deep neural network for research in the development and development of the AI module [81]. Thanks to the artificial intelligence platform due to AetherAI, different modules are developed for different genres; This lung cancer detection quantitative module can display cancer cell areas in digital pathological images and provides objective cancer area measurement to help biotech companies standardize and automate test development; This not only greatly increases production capacity, but also reliably replicate the control and operating systems of biotech companies as they expand rapidly and into inter-Regional branches.

Arterys

Arterys is the medical imaging AI platform that allows you to make leading AI clinical applications a native file that you currently do by reporting directly to your existing PACS or EHR focused workflow. AI assistants for longitudinal tracking and volumetric segmentation and analysis of pulmonary nodules. Standardizes the reporting of scan exams using the integrated Lung-RADS workflow [82].

Lunit

The AI generates the location information of detected lesions in the form of heat maps and outlines (see Fig.9). The AI generates an abnormality score which reflects the AI's calculation of the actual presence of the detected lesion. The AI provides a "case report" that summarizes the overall analysis result, narrowed down to each finding [83, 84].

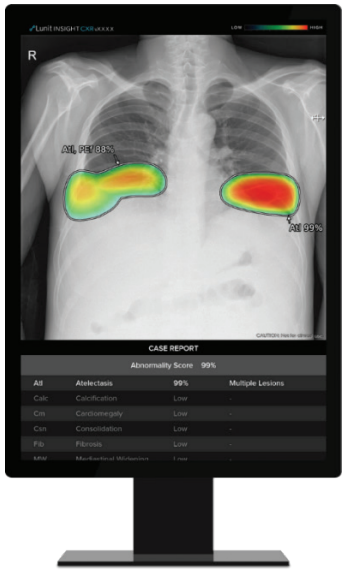


Fig.9 Screenshot of multiple lesions [83].

Aidence

Veye Chest supports the improvement of patient outcomes by delivering fast results, lowering the risk of misdiagnosis, and by detecting and segmenting possible early-lap pulmonary cancer [85]. Pulmonary nodule detection, quantification and growth assessment, fully integrated into the radiology workflow (see Fig. 10).

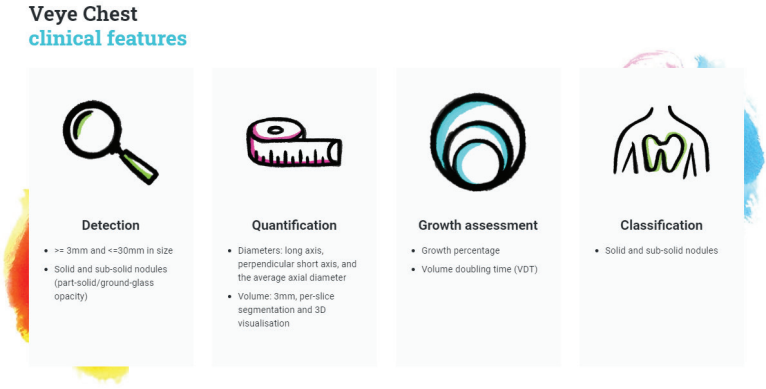


Fig.10 Veye Chest features [85].

OXIPIT

Oxipit ChestEye is the first CE-marked AI chest x-ray radiology package. The package can support the 75 most common radiological findings covering 90% of diagnoses encountered on a daily basis in a medicinal institution. The platform produces preliminary reports for healthy patients who adhere to the healthcare reporting practice.

ChestEye CAD is a fully automated CAD chest x-ray solution. It detects chest X-ray images and produces preliminary reports without any abnormalities. Preliminary healthy patient reports are generated only when the platform is quite confident of the results. ChestEye increases radiologist productivity by minimizing the radiologist input required for healthy patient chest X-ray reporting, and allows medicinal professionals to focus their attention on abnormal cases [86].

Abbreviations

AI, artificial intelligence

DL, deep learning

GLOBOCAN, global cancer observatory

NSCC, non-small cell cancer

SCC, small cell lung cancer

CT, computed tomography

PET, positron emission tomography

MRI, magnetic resonance imaging

CNNs, convolutional neural networks

R-CNN, region based convolution neural network

YOLO, you only look once

SSD, single-shot multi-box detector

CAD, computer aided diagnosis

ROI, region of interest

MAP, multiple abnormal pattern

ReLU, rectified linear unit

ILSVRC, ImageNet Large Scale Visual Recognition Competition

DANs, deep auto-encoders

RBM, Boltzmann's machine

SAE, stacked auto-encoders

CAE, convolutional auto-encoders

RNNs, recurrent neural networks

LTSM, long short-term memory

M-CNN, multi-scale convolutional neural network

ML, machine learning

SML, supervised machine learning

MIL-CNN, multi-instance learning convolutional neural network

LIDC, The lung image database consortium

IDRI, Infectious disease research institute

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Chapter 8

THE GOAL IN THE TREATMENT OF BETA-THALASSEMIA MAJOR: IS THE AWAKENING OF FETAL HEMOGLOBIN?

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1. Introduction

In today, the hemoglobinopathies are the most common inherited disorders of the red cell and are among the world's most common genetic diseases. So that, more than 300,000 children are born with sickle cell disease (SCD) and various forms of thalassemia (thal). Although the hemoglobinopathies originated in the Mediterranean, sub-Saharan Africa, the Middle East, and the southern regions of Asia, because of relatively recent migrations, these diseases are now frequently encountered in northern Europe and North America(Keser, 2017; Keser ve ark., 2004). Therefore, the hemoglobinopathies, thalassemias and abnormal hemoglobins, are global problems in the world. It needs to actual and sustainable scientific therapy without side effects.

1.1.The Structure of Hemoglobin

Hemoglobin (Hb) molecule that is the most abundant protein in erythrocytes from mollusks such as some worms to human, whose function is to transport oxygen (O2) to tissues. There are approximately 270 million hemoglobin molecules in a single erythrocyte in human blood. Hemoglobin consists of 4 globin amino acid chains; They consist of 1 heme group covalently bonded to each one and 1 Fe (iron) molecule bound at their centers and have a quaternary protein feature (https://en.wikipedia.org/wiki/Red_blood_cell, Access date: 13.02.2021) (Figure 1 and 2). It is thought that the oxygen-carrying ability of the hemoglobin molecule dates back about 500 million years ago (Miyata et al., 2020). Oxygen transport abilities; it is thanks to the allosteric arrangement of the iron-bound globin chains in their structure against the O2 element (Perutz, 1989). The affinity of hemoglobins to oxygen increases as O2 binds, allowing them to bind more oxygen. Abnormal or dysfunctional globin chains cannot show the ability to bind to O2 or they tend to bind with different affinities. The affinity of globin chains to O2 varies depending on the environmental conditions and the globin genus and the presence of mutations in the globin genes (Damsgaard et al., 2013; Perutz et al., 1981; Ronda et al., 2013) Any condition that reduces the concentration of functional Hb or decreases red blood cell (RBC) mass may cause anemia. This condition was affected by multiple factors including nutritional deficiencies, acute or chronic blood loss, infectious diseases, RBC abnormalities, chronic inflammation, and genetic variants affecting Hb structure, among others. Anemia is caused three basic mechanisms affecting RBC: 1) Reduced production, 2) Increased destruction (hemolysis), or 3) Loss (hemorrhage) due to defects that can be intrinsic to RBCs or their precursors, or extrinsic (Forget, 2011).

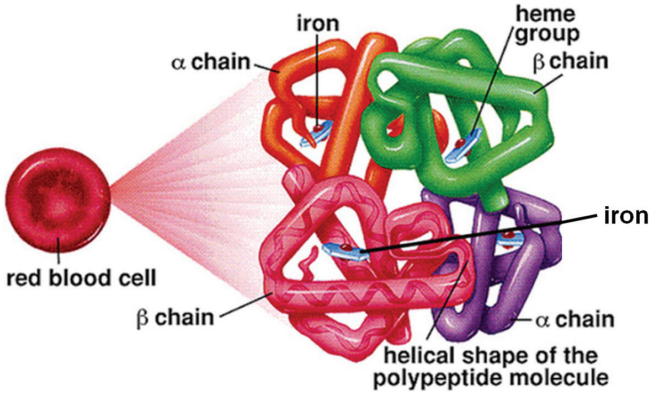

Figure 1. *Structure of Hemoglobin Protein (Hemoglobin.gen.tr, https:// hemoglobin.gen.tr/, Access date: 13.02.2021).*

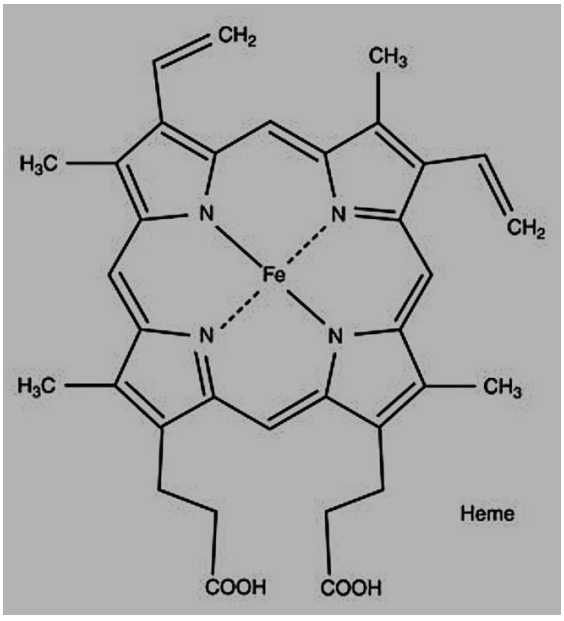

Figure 2. *Structure of the Heme Molecule (Boundless, https: //www.boundless. com/physiology/ textbooks / boundless-anatomy-and-physiology-textbook / respiratory-system-22 / gas-exchange211 / oxygen-transport-1035-2202 /, Access date: 12.02.2021)*

1.2. Hemoglobin Types Depending on the Development Process

During human development, globin gene expressions occur under genetic control. Two switches accompany the expression of genes in

the globin gene cluster. The first of these switches controls fetal globin synthesis from the embryonic globe in the early gestational period, and the second controls the realization of adult globin synthesis from the fetal globe in the near term (Bauer et al., 2013) (Figure 3).

Hemoglobins made in the first 8 weeks of the intrauterine period are called embryonal hemoglobin [Gower1 (ζ2ε2), Gower-2 (α2ε2) and Portland (ζ2γ2)]. After the ninth week, the dominant hemoglobin is fetal hemoglobin [HbF (α2γ2)]. After birth, the amount of HbF gradually decreases and decreases to less than 2% after the age of 1. Adult hemoglobin HbA1 (α2β2); It begins to appear after the first month of gestational life and constitutes 30% of the total hemoglobin at birth, 50% 2 months after birth and approximately 95% after 6 months. HbA2 (α2δ2), on the other hand, begins to appear immediately after birth and continues lifelong at a low level of 1.5-3.5%. HbA1 (α2β2) constitutes 95% of hemoglobin in adults, HbA2 (α2δ2) 2-3% and HbF (α2γ2) less than 1% (Galanello et al., 2010).

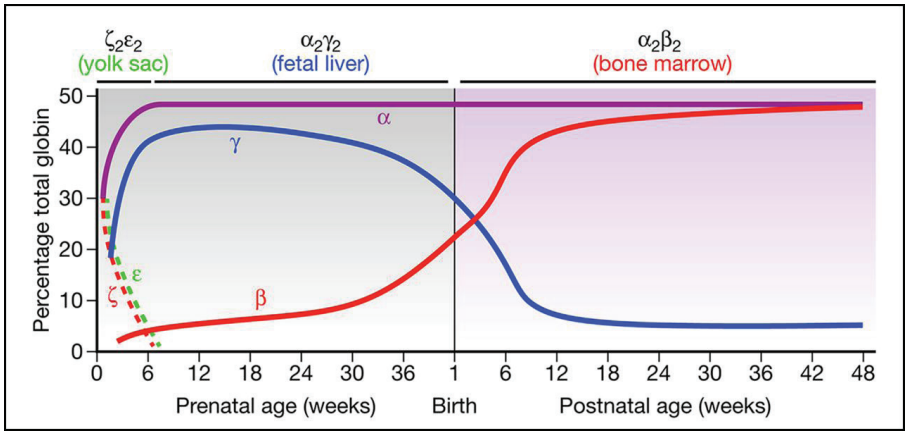

Figure 3. *Approximate timing (weeks) and level of globin of expression of the human α-type (ζ- and α-globin) and β-type (ε, γ, and β) globin genes during embryonic, neonatal, and postnatal development.*

1.3. The Genetics of Hemoglobin Molecule

In the hemoglobin; the genes encoding for α-globin forms (like to α) are on chromosome 16p13.3 region and those like to β (ε, γ, and δ) are on chromosome 11p15.5 region. The globin genes are expressed in the α- and like to β-clusters and their combinations determine the type of produced Hb during human development (Figure 4.). Hemoglobin production is under genetically control. The activation and silencing of the globin genes

are strictly controlled: the α-globin locus is regulated by HS-40, while the β-globin chain is regulated by the locus control region that is an important upstream regulatory region of the beta-globin cluster. The embryonic Hb (ε) form is produced in the initiation of the first trimester from yolk sac−derived erythrocytes. After then, its production begins in enucleated erythrocytes in the stem and progenitor cells in the fetal liver during the second stage of pregnancy. The γ-globin switch on predominantly in the β-like globin cluster. The combination of γ-globin chains and α-globin chains (α2γ2) produces the HbF (fetal Hb). This HbF is dominant in neonatal period of Hb. (Galanello et al., 2009).

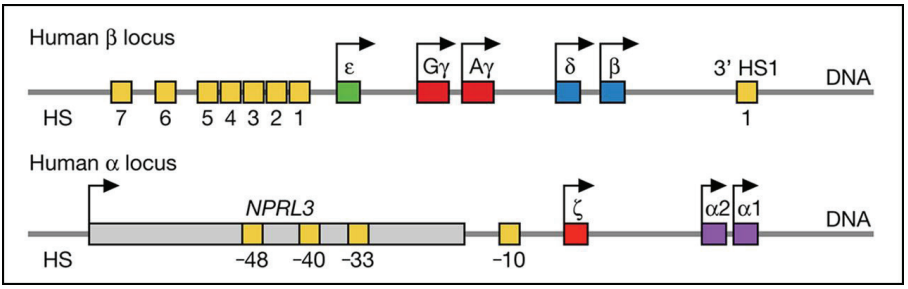

Figure 4. *The diagrams of physical order of the alpha- and beta-like and the major regulatory elements of the alpha- and beta-globin loci.*

2. Hemoglobinopathies

Erythrocytes constitute a significant part of the vital blood, and hemoglobin molecules constitute a significant part of the erythrocytes. Disorders of the globin chains in the structure of hemoglobin, hence hemoglobin, are generally referred to as hemoglobinopathies. Hemoglobinopathies are the most common disease group in the world and approximately 7% of the world population is a carrier of globin gene mutations (Weatherall and Clegg, 2001). Hemoglobinopathies, which are taken under control in many countries, continue to be the most important health problem of the world and our country. Although hemoglobinopathies are single gene diseases, they are characterized by wide clinical and hematological variation. Genetic variations of the different globin chains that make up hemoglobin underlie this variation in hemoglobinopathies. Hemoglobinopathies are one of the most common diseases in the world and are common in certain geographical regions. Hemoglobinopathies, which are characterized by the absence or reduction of globin chain synthesis, generally fall into two groups; They are classified as thalassemias and structural hemoglobin variants (abnormal hemoglobins).

2.1. Thalassemias

The best defined types of thalassemias; α, β, γ, δ, δβ and εγδβ are thalassemias. Common thalassemias are alpha and beta thalassemias, which have distinctive features such as alpha and beta globin chain synthesis imbalance and anemia. More than 400 mutations associated with thalassemia have been identified. The two most common thalassemia syndromes are alpha and beta thalassemias. More than 1650 different mutations underlie the variation only due to alpha and beta globin chains (Weatherall & Clegg, 2001; Williams & Weatherall, 2012).

2.1.1. Alpha Thalassemia

Alpha thalassemias occur as a result of the decrease in alpha globin chain synthesis in the structure of the hemoglobin molecule. Alpha globin chains are synthesized by the α1 and α2 genes located on the short arm of chromosome 16, 16p13.3. There are a total of four alpha globin genes (αα / αα) in a human, with two alpha globin genes in each allele. Alpha thalassemias occur from deletional mutations of one or more α- genes (Keser et al. 2021). If alpha globin chain synthesis is insufficient, β4 and 4 homotetramers with high oxygen affinity are formed. These homotetramers are ineffective oxygen carriers. When β4 and γ4 are oxidized, they precipitate. This disrupts the erythrocyte membrane structure and shortens the life of the erythrocyte cell (Williams & Weatherall, 2012).

2.1.2. Beta (β) Thalassemia

Beta (β) thalassemia is one of the most common hemoglobinopathies, which is located in the short arm of chromosome 11 in the genome, in the region 11p15.5, and is characterized by two copies of the gene and often occurs with point mutations, with carrier, intermedia and major phenotype and clinical reflection. Beta thalassemia occurs generally by point mutations. Hemoglobinopathies are mostly associated with thalassemias, the functioning of many genes is affected due to their systemic disease, and many genes can modify the clinical course and functioning and change the phenotype of the disease (Williams & Weatherall, 2012). If beta globin chain synthesis is insufficient, unpaired alpha globin chains precipitate. This precipitation disrupts the maturation of erythroid precursors and causes ineffective erythropoiesis. It causes anemia and enlargement of erythroid precursors with disseminated hematopoiesis in bones and other organs.

2.1.2.1.The Epidemiology of Beta Thalassemia

Beta (β) thalassemia is one of the hemoglobinopathies and is one of the most common autosomal recessively inherited gene diseases worldwide.

It shows high prevalence in Mediterranean, Middle East, Transcaucasia, Central Asia, Indian Subcontinent and Far East populations (Keser, 2017; Keser, 2010; Altay et al., 1996). It is also relatively common in the African population. The highest incidence is reported in Cyprus (14%), the Italian island of Sardinia (12%) and South East Asia (Weatherall and Clegg, 2001). The high gene frequency of beta thalassemia in these regions is probably associated with selection pressure resulting from Plasmodium falciparum malaria (malaria). It shows a very similar distribution to current or past malaria endemia. Beta thalassemia carriers are relatively protected against invasion by Plasmodium falciparum. However, due to population migration and limited slave trade, beta-thalassemia is also common in Northern Europe, North and South America, the Caribbean and Australia (Weatherall and Clegg, 2001; Williams & Weatherall, 2012).

2.1.2.2. The Clinical Features of Beta Thalassemia

The fact that two alpha and two beta globin chains in adult hemoglobin structure are not synthesized with little (β+) or no (β0) causes beta thalassemia (Galanello and Origa, 2010). The clinical severity of beta thalassemia is related to the extent of imbalance between the alpha globin and non-alpha globin chains. Non-alpha globin chains, in addition to beta globin chains, also contain gamma chains, a specific component of HbF (α2γ2), and are present in small amounts in normal individuals. In individuals with beta thalassemia syndrome, the HbF value is increased but it is found in variable amounts. When beta globin chains are reduced or destroyed in red blood cell precursors, un-combined alpha chains precipitate and oxidative damage of the cell membrane, thus apoptosis occurs (Rund and Rachmilewitz, 2005). Imbalance in beta chain synthesis leads to unstable blood cell formation and hemolytic anemia.

Beta thalassemia, the wide range of clinical manifestations of the disease, reveals different phenotypic groups. These clinical differences include phenotypes ranging from beta thalassemia minor, which is usually asymptomatic, to beta thalassemia major (β-TM), which is severely transfusion dependent. These phenotypes are; they are classified as beta thalassemia minor, beta thalassemia intermedia (β-TI) and beta thalassemia major (β-TM). This phenotypic classification results from either homozygous or compound heterozygous forms of beta globin gene mutations. Beta thalassemia minor is clinically normal phenotype and they are carriers. β-TI is a form of the disease based on the clinical phenotype between beta thalassemia minor and β-TM. Usually there is mild clinical and severe hemolytic anemia compared to the TM clinic. Although β-TI

is a transfusion-independent form of thalassemia, some patients need occasional blood transfusions. Like β-TM, they require careful medical intervention to improve their quality of life (Thein, 2013).

3. Beta Thalassemia Major

Severe hemolytic anemia is usually seen in the β-TM phenotype during the first years of life. As a result of ineffective erythropoiesis, the erythroid precursor cells in the bone marrow are destroyed. They are sick individuals and require regular transfusion and careful medical intervention. β-TM patients have severe microcytic and hypochromic anemia associated with increased red blood cell count, low mean erythrocyte volume (MCV), and mean erythrocyte hemoglobin (MCH). Peripheral blood smears, in addition to microcytosis and hypochromia, show anisocytosis, poikilocytosis (tear drop and elongated cells) and nucleated red blood cells (i.e. erythroblasts). The erythroblast number depends on the degree of anemia and increases significantly after splenectomy. The Hb pattern (by cellulose acetate electrophoresis or high performance liquid chromatography [HPLC]) depends on the type of beta thalassemia. In beta-thalassemia, which is characterized by the absence of beta globin chain synthesis, HbA is absent, HbF 95-98% and HbA2 2-5% (Thein, 2004). The development of β-TM babies gradually decreases. Abdominal enlargement may occur due to nutritional problems, diarrhea, irritability, recurrent bouts of fever, and splenomegaly. Growth and development are normal up to 10-11 years of age if a regular transfusion program is initiated that provides a minimum Hb concentration of 95-105 g/L. After the age of 10-11, affected individuals are at risk of developing serious complications related to post-trans-fusion iron overload, due to their compliance with chelation therapy (Galanello and Origa, 2010).

The classic clinical picture of β-TM is currently only seen in some developing countries, and resources are not available for running long-term transfusion programs in these countries. The most prominent features of untreated or poorly transfused individuals are growth retardation, pallor, jaundice, brown pigmentation of the skin, poor muscle structure, bow leg, hepatosplenomegaly, leg ulcer, the development of masses from extramedullary hematopoiesis, and skeletal changes resulting from joint enlargement. These skeletal changes include deformities of the long bones of the legs and typical craniofacial changes (skull bossiness, pronounced malar height, depression of the nasal bridge, mongoloid propensity of the eye, and hypertrophy of the maxilla). It tends to expose the upper teeth. People who are not regularly transfused usually die before the age of thirty

(Galanello and Origa, 2010).

The clinical severity and course of the disease differ in beta thalassemia major patients with the same mutation. This clinical course ranges from almost asymptomatic findings to life-threatening conditions. This clinical diversity is due to other modifying genes in the genome that affect the genes responsible for the disease (Thein, 2018).

4. The Modifier Genes

Recently, human genome studies have revealed that the clinical differences in single gene disease with the same mutation are due to modifier genes (Haldane, 1949). Until today, many modifier genes have been identified that affect beta-thalassemia major phenotype as in the general hemoglobinopathies of single gene diseases. Modifying genes in beta thalassemia are classified as primary and secondary modifiers. Primary modifier genes describe imbalances in the alpha (α) / non-α globin chain ratio.

4.1. Primary Modifiers

1. α-globin genotype, α-thalassemia: Imbalance due to globin chain decrease and α-globin increase

Co-inheritance of extra α-globin genes (ααα /, αααα /, or HBA gene cluster duplication): Excessive α-globin synthesis and globin chain imbalance.

2. Beta-globin genotype (mutation of 1 or 2 beta-chains): The-globin gene is directly affected. Globin causes chain imbalance.

3. QTL (Quantitative trait locus) that control HbF (Menzel et al., 2019), such as BCL11A [B-cell CLL / lymphoma 11A (zinc finger protein)], KLF1 [Kruppel-like factor 1 (erythroid)], HMIPEP [human mitochondrial intermedia peptidase], Xmn1-HBG2 [Xmn1 polymorphism in the gamma globin gene], Like SNPs: Increased chains - reduced globin chain imbalance with excess α-globin

4. Potential modifiers, including variants in the ubiquitin proteolytic pathway; promotes proteolysis of excess α-globin.

5. Alpha-hemoglobin stabilizing protein (AHSP) (Razak et al., 2018).

Secondary modifiers are associated with the level of complications and treatment of the disease.

4.2. Secondary Modifiers

1. HFE (Hemochromatosis) gene mutations; for example the H63D variant,

Variants in VDR (Vitamin D Receptor), COL1A1 (Collagen, type1, alpha1), COL1A2 (Collagen type1, alpha2), TGFB1 (Transforming Growth Factor Beta1) genes,

2. Apolipoprotein (APOE) ε4, Glutathione-S-transferase M1,

3. The polymorphism of (TA) n in the promoter of UGT1A1 (UDP glucuronyl transferase-1 family, polypeptide A1) (Thein, 2013).

4.3. Tertiary Modifiers

Other polymorphisms and point mutations that have an improvement or opposite effect in beta-thalassemia complications can be examined in this class. Variations in vitamin D receptor (VDR), estrogen receptor gene (ESR), and collagen genes involved in bone metabolism affect the development of osteoporosis, one of the important complications of beta thalassemia (Guzeloglu-Kayisli et al., 2008). It has been shown that mutations in the C282Y and H63D genes, the HFE gene responsible for hemochromatosis, which play a role in iron metabolism, affect the phenotype. Polymorphisms in genes of bilirubin metabolism (Example: UGT1 gene (TA) 7 polymorphism) and mutations and polymorphisms in immune system genes (such as HLA, TNF, ICAM) have also been shown to affect morbidity and mortality in patients with thalassemia (Özkınay, 2014).

5. HbF: Fetal Hemoglobin

Fetal hemoglobins are synthesized in the early stages of development, in the womb; It is a type of hemoglobin consisting of two γ-globin and tetramers of two α-globin chains. It has a higher O2 carrying capacity than adult hemoglobins (HbA) and its presence in thalassemia cases greatly improves the course of the disease, especially in β0-thalassemias.

The heterozygous forms of beta-thalassemia with HPFH or δ-thalassemia that stimulate HbF production in the bone marrow are included in the intermedia class, which is much less severe than thalassemia major and requires less transfusion.

The presence of HPFH depend on the factors below:

- δ-β thalassemia association,

- Mutations in Hb QTL (Quantitative Trait Loci; Quantitative trait locus) regions such as BCL11A, HBS1L-MYB,

-Bone marrow tumors

-Aplastic anemia

-Fanconi anemia,

-Erythropoietic stress

-Pregnancy status,

- HbF-inducing treatments are considered among the causes of increased HbF.

-Single nucleotide changes or deletions that significantly affect the level of HbF usually occur in the following gene regions or transcription factors that bind to these regions;

-Globin Gene and transcription factors (LDB1 / LMO2 / GATA1 / TAL1 / E2A complex) that bind to LCR,

-BCL11A gene,

- HBS1L-MYB inter-gene region (HMIP),

-KLF1 gene,

- HBG1-HBD intergenic region and HBG1 and HBG2 promoters,

- Binding region of Xmn1 in HBG2 promoter (Xmn1-HBG2 region locus),

- LRF (ZBTB7A) gene (Bilgen et al., 2011a; Bilgen et al., 2013; Deng et al., 2012; Grieco et al., 2015; Hu et al., 2020; Martyn et al., 2019; Razak et al., 2018) (Figure 5.)

The three most important quantitative trait loci (QTLs) are C / T at position 5 '−158 of HBG2 on chromosome 11p15.4 called Xmn1-HBG2 or rs7482144, SNP located in the intergenic region of HBS1L-MYB on chromosome 6q23, and chromosome 2p16. These are gene loci that contain SNPs and mutations in the BCL11A gene. DNA mutations and polymorphisms in these three regions account for about 10% -50% of the variation in HbF levels. This shows that additional loci are involved (Thein, 2018).

Many studies have shown that; The HBG1-HBD intergenic region plays an important regulatory role in γ-β globin gene change. Galarneau et al. Identified an SNP (rs10128556) located in this region and suggested that it has a greater effect on HbF than HBG2-Xmn1 QTL (Galarneau et al., 2010; Hu et al., 2020).

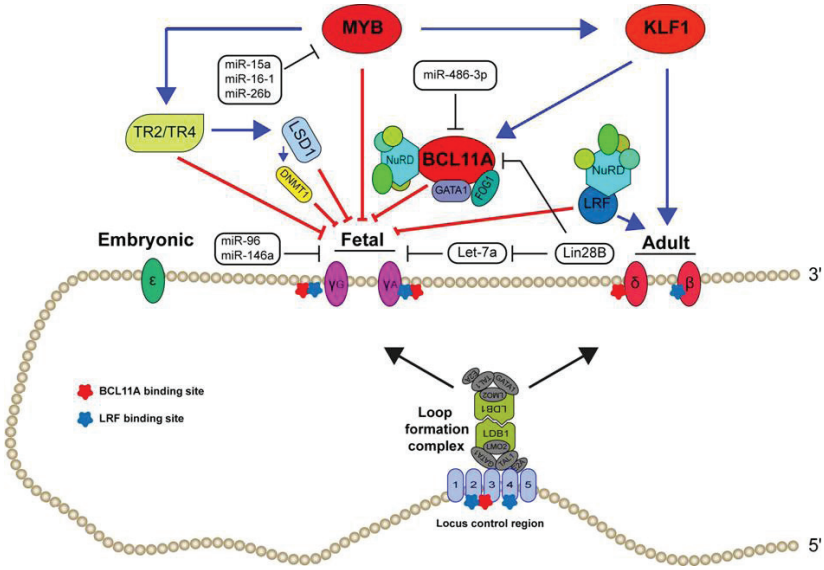

Figure 5. *Major transcription factors that have an effect on the active looping position of the-globin gene (Paikari & Sheehan, 2018). γ-A indicates HBG1 gene, γ-G HBG2 gene, ε HBE gene, δ HBD gene and β HBB gene*

The beta-like globin genes were linearly sequenced on chromosome 11 in the order in which they were expressed during development (HBE1, HBG1 / HBG2, HBD / HBB). The 16 kb long locus control region (β-LCR) consists of four erythroid-specific DNase-I hypersensitive (HS) regions consisting of clusters (loci) of binding regions for transcription activators. LCR is located 40-60 kb upstream of the β-like gene cluster and regulates the expression of β-like genes through looping and direct interaction with β-like globin promoters (Kim & Dean, 2012). The complex of GATA1, TAL1, E2A, LMO2, and LDB1 transcription factor proteins is thought to mediate the loop formation between LCR and globin promoters (Breda et al., 2016). Beta-like globin gene expression; globin genes for gene-autonomic control and LCR, and it is regulated by competitive binding of trans-acting repressor genes that bind to the γ-intergenic region such as BCL11A (Enver et al., 1990).

GWAS (Genome-wide association) and subsequent studies have shown that BCL11A is the key negative regulator of HBG. The BCL11A protein, a ZF (Zinc-Finger) transcriptional repressor, occupies critical regions for HBG activation within the-like globin gene locus and turns off the HBG promoter, allowing the initiation of a long series of interactions between LCR and HBB promoter (Figure 6.). BCL11A can implement this role by interacting with the erythroid major regulators GATA1, SOX6, ZFPM1 /

FOG1 and the NuRD suppressor complex, which includes HDAC1 and HDAC2. In cases where BCL11A is mutated or deleted, LCR that survives BCL11A suppression will initiate γ-globin expression by binding to the released promoter of HBG (Bauer et al., 2013; Paikari and Sheehan, 2018).

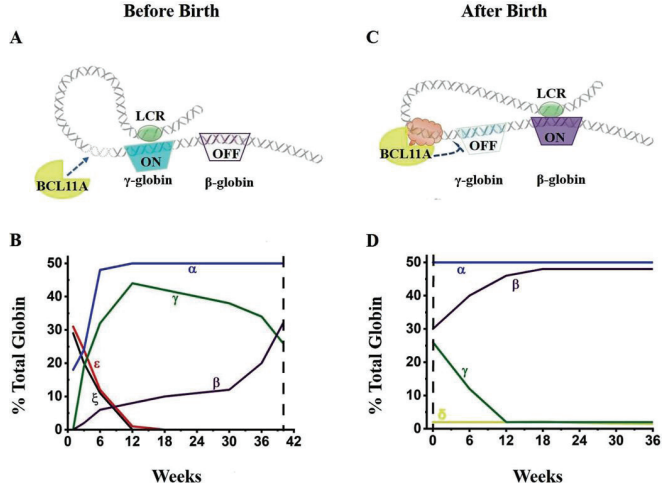

Figure 6. *Illustration showing the change of active / inactive γ-globin and β-globin genes in the developmental process (Ali et al., 2020).*

Genomic studies have also demonstrated mutations and polymorphisms localized in the intergenic region between the GTP binding elongation factor HBS1L on chromosome 6q and the myeloblastotic oncogene MYB, associated with elevated HbF levels. The MYB transcription factor is an important regulatory element of hematopoiesis, erythropoiesis, and HbF levels and modulates erythroid characteristics through two mechanisms: the first directly by activation of other nuclear receptors such as KLF1 and TR2 / TR4, and the second by indirectly altering the kinetics of erythroid differentiation. Low levels of MYB accelerate erythroid differentiation, leading to the production of early erythroid progenitor cells that are larger but still predominantly express-globin (Stadhouders et al., 2014).

KLF1 (Kruppel-like factor 1) is an essential erythroid-specific transcription factor that plays a key role in erythropoiesis. KLF1 affects hemoglobin exchange by both directly activating β-globin gene expression by interacting with the HBB promoter in adulthood, and by increasing the expression of the rep-globin repressor BCL11A by locating in the BCL11A promoter. It has been reported that congenital heterozygous KLF1 mutations result in hereditary persistence of fetal hemoglobin (HPFH) and reduced BCL11A expression of KLF in knock-down adult erythroblast cultures by the lentiviral method (Bank, 2006). Additionally regulation of globin genes; It is epigenetically controlled

by histone modifications, methylation, Lysine-specific Dimethylases (LSDs) and microRNA (miRNAs) (Paikari & Sheehan, 2018).

LDB1 (LIM-Domain Binding 1) Gene; It is the main protein that initiates DNA looping for transcription of β-globin genes. However, it cannot bind directly to DNA, for this it requires GATA elements (β-LCR complex) containing TAL1, LMO2, E2A and GATA1 proteins (Deng et al., 2012). GATA1; KLF1 is also responsible for the activation of MYB genes by binding to the promoter of BCL11A. Therefore, polymorphisms in these genes have the ability to reverse the / β globin regulation by suppressing the self-expression of BCL11A, which has a greater effect on HbF.

In Figure 7., it is shown by Deng and his working group that a ZF (Zinc-finger) oligonucleotide that binds LDB1 instead of GATA1 initiates chromatin looping, but the GATA1 element is needed for the continuation of the transcription.

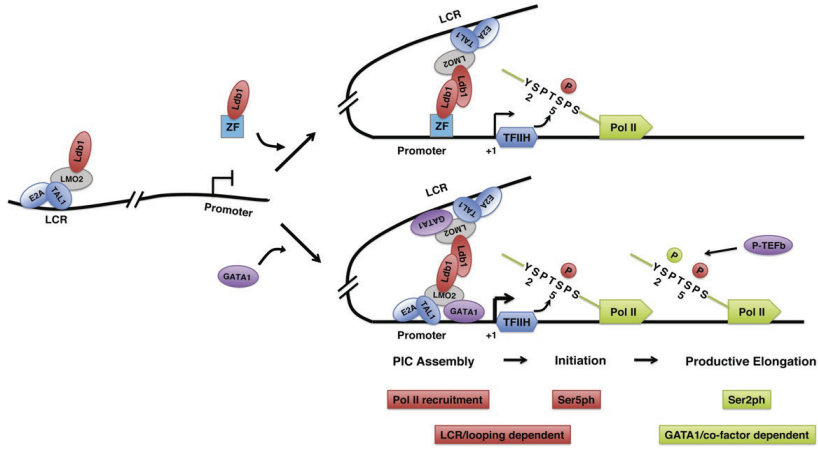

Figure 7. *Illustration of-globin gene transcription via chromatin looping and β-LCR & LDB1 / GATA complex (Deng et al., 2012).*

6. TAL1 Transcription Factor

Protein structured TAL1 (SCL / TAL1, T-cell Acute Leukemia Protein-1, bHLH Transcription Factor-1, Erythroid Differentiation Factor) is a transcription factor involved in hematopoiesis and leukomogenesis processes. It participates in the production of erythrocytes and other blood cells, in the formation of mesoderm in early embryogenesis and regulates hematopoiesis in adulthood. The TAL1 gene was identified 24 years ago for its role in a tumor-specific chromosomal translocation found in human leukemia (Robb et al., 1997). The changes leading to the overexpression of TAL1 are considered to be the most common tumor-specific chromosomal abnormalities found in human T cell acute lymphoblastic leukemia

(T-ALL). In addition, studies have confirmed that TAL1 is involved in malignant transformation and demonstrated that it can act synergistically with other oncogens to accelerate tumor formation (Robb et al., 1997; Schroeder et al., 1968).

TAL1, which has to determine the multipotency of hematopoietic stem cells and keep them in silent phase (G0), also interacts with many transcription factors such as E2A / HEB, GATA1-3, LMO1-2, Ldb1, ETO2, Runx1, ERG, FLI1 and regulates hematopoiesis is obliged. Among these complexes, TAL1 regulates normal myeloid differentiation and proliferation of erythroid progenitors, but also selects the differentiation direction of hematopoietic stem cells (Vagapova et al., 2018).

Transcription complex called SCL complex or TAL complex; It is generally composed of (TAL1 / E2A / GATA1 / LMO2 / Ldb1) main elements, and its members can vary and vary. The type of TFs that TAL1 interacts with and takes place in the complex determines the activation or inhibition of differentiation of myeloid and lymphoid cells, and TAL1 has been directly associated with cancer for many years (Choi, 2014; Gering et al., 1998; Palii et al., 2011; Tan et al., 2019). The transcription factor TAL1 is one of the main regulators of hematopoiesis and contains the HLH (Helix-Loop-Helix) domain that binds to DNA from the regulatory regions, interacts with the E-box sequence (CANNTG) and enables binding to factors such as GATA, Ets, Runx. Studies have shown that inhibition of TAL1 gene expression results in complete cessation of hematopoiesis in the vitellus sac in the early embryonic stage (Robb et al., 1995). In adulthood, the maximum expression of TAL1 indicates the presence of pluripotent hematopoietic stem cells, multipotent myeloid and lymphoid progenitors, erythroid and megakaryocytic cells.

The TAL1 gene produces structurally different long and short transcript variants. While normal TAL1 consists of 6 exons (I-VI exons), short (TAL1-S) and long (TAL1-L) TAL1 transcript variants, isoforms emerge with alternative splicing. TAL1-L and TAL1-S isoforms are synthesized from this normal mRNA. TAL1 plays a major role in chromatin folding in favor of γ-globin in the selection of β-globin / γ-globin in the expression of hemoglobin during the developmental process (Grieco et al., 2015; Vagapova et al., 2018; Wienert et al., 2015; Yun et al. al., 2014). The TAL1 gene is important as the gene encoding the TAL1 protein, which plays a key protein role in all processes from binding of GATA1 to BCL11A, from binding of β-LCR to promoters of β-like genes, activation of KLF1

to activation of GATA1. (Abi Saad et al., 2014)

Basically, its functions can be listed as follows:

- Interaction with bHLH gene family members (such as E2A),

- Interaction with LIM-domain proteins (such as LMO1, LMO2),

-Malignant oncogene activity,

- Binding to DNA from e-box motifs (CANNTG),

- It is involved in vital regulations such as hematopoiesis regulation.

Various network studies have been conducted for TAL1, which has such critical roles, in its behavior towards hematopoiesis, globin gene regulation (especially gamma globin gene expression for HbF) and hemoglobin regulation, for protein and transcription factors with which it is in relation and interaction. In these studies, 3, 6 and multiple connections interacting with TAL1 reveal that TAL1 is more important. At the same time, TAL1 appears to be expressed simultaneously with the interacting LMO2, GATA1-3, MYB genes (Figure 8).

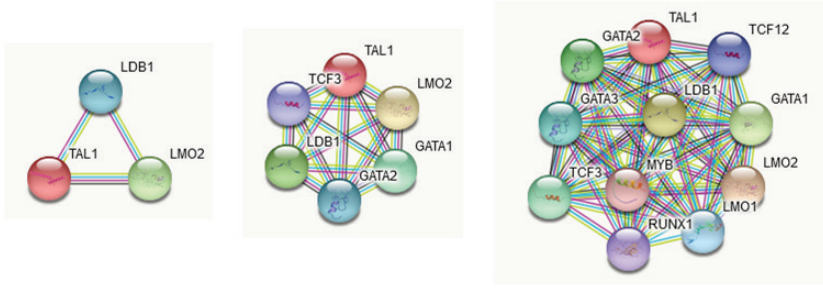

Figure 8. *Protein and transcription factors that TAL1 is associated with (STRING, https://string-db.org/cgi/network?taskId=bbT5HyzABCJl&sessionId=btmjLPM545 nx,Access date: 11.02.2021).*

As results, within the framework of these informations, "Can globin genes turn on and off during normal development be used in the treatment of hemoglobinopathies without any side effects?" the question is asked. The most striking target here is seen as HbF. Because its synthesis continues in the neonatal and postnatal period. Its percentage in total hemoglobin is less than 1%. Since the increase in HbF ratio in beta thalassemia major patients is associated with a good clinical phenotype, it is aimed to awaken the synthesis of gamma globin chain in the HbF structure.

In conclusion, it is clear that there is still a great need for new, alternative, effective, and sustainable therapeutic strategies for treatment

of this life-limiting disease, beta-thalassemia major, to render patients transfusion independent and able to live a normal life. The awaekining of the HbF may be a good target for this purpose.

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Chapter 9

THE IMPORTANCE OF PRİMARY TEETH IN CHILDREN'S HEALTH

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People teething twice, including primary and permanent dentition. As permanent dentition is important in adults, primary dentition is also important in children. In humans, deciduous dentition starts with the appearance of the first deciduous tooth in the mouth at the age of 6 months; It is completed by the appearance of all primary teeth between the ages of 2.5-3. The first deciduous teeth are usually the lower deciduous incisors. When the eruption of primary teeth is completed, there are 20 teeth in total, 10 teeth in the lower and upper jaws. (1)

The main purpose of dental treatment is to eliminate toothache. (2) However, while focuses on pain in decayed teeth, untreated painless decay can cause serious dental and systemic problems in primary teeth. Untreated decayed teeth cause the following problems in children:

1. **Death:** Fatal infections in children caused by dental abscesses (such as brain abscesses), especially with treatments with general anesthesia or sedation. (3)

2. **Dental sepsis, dental abscess:** In addition to pain and discomfort, the consequences of such an infection are twofold: first, a chronic abscess can damage the development of permanent teeth; Secondly, acute abscesses due to primary teeth may rarely cause serious sequelae. orbital cellulitis, brain abscess, and 'unexplained' recurrent fever (4)

Sepsis occurs when the body's immune system responds to infection (such as an odontogenic infection) exaggeratedly. This can cause a range of reactions, from shock to organ failure and even death. Generally, there may be an underlying systemic disease. (5)

Toothache: Decayed primary teeth that are not treated or have reached the pulp are the cause of pain. (6)

3. **Premature loss of primary teeth:** Loss of place occurs due to the neighboring teeth being mesialized and distalized. Malocclusion causes a decrease in alveolar arch length, ectopic eruption, and impact of the permanent tooth. (7)

4. **Impairment of the quality of life:** Psychological problems, Malnutrition, problems in school education, decreased desire to play, etc. (8,9)

It is one of the biggest mistakes that can be made that the problems in these teeth are not taken seriously by the parents because the primary teeth will change completely. Because primary teeth are important because

they are space savers for eating, phonetic, aesthetic, and also permanent teeth. Problems that may occur in primary dentition; Psychological, educational disruption in school-age children, problems in the child's self-development, loss of time for the parents, and the child's growth and development are negatively affected. Primary teeth stimulate the growth of the jawbone and protect the places of permanent teeth that will then be replaced by them. Primary teeth are also very important in terms of speech. Especially in the absence of anterior teeth, many voices cannot be said exactly. Also, the lack of anterior teeth can cause serious aesthetic anxiety in children and can cause many psychological problems. (10.11)

Generally, problems in the form of pain and swelling in the primary teeth can cause distress in the child, and may even affect the appearance of the child. Children's dental health is affected by the education, beliefs, and knowledge of their families. Since parents are the primary caregivers of their children, they must have sufficient knowledge about the health and care of primary teeth. In particular, the more positive the mother's attitude towards dental health, the better the oral hygiene of the child. (12)

The development of deciduous teeth is an indicator that reflects the physiological maturity of infants and young children, together with indicators such as height, weight, and head circumference. Normal growth and development of deciduous teeth not only helps the baby's nutritional intake and absorption, but is also closely related to jaw-face development and tongue development. The number of deciduous teeth and the age of falling are affected by many factors such as genetics, environment, and living conditions, and there are obvious ethnic and regional differences. (13)

If we examine the importance of primary teeth from several aspects; First of all, these teeth are of great importance in terms of providing continuous guidance to the teeth. By helping the development of the face and jaws, they significantly affect the shape of the face. Because teeth make a great contribution to a person's appearance and thus help to increase the self-confidence of the person. Children need to properly bite and chew to properly digest and develop food. However, this process takes place thanks to healthy teeth. (14)

Early loss of deciduous teeth is considered an important problem in oral health. The most common consequences of early deciduous tooth loss are lack of space in the permanent dentition, eruption problems in the permanent tooth, malocclusion, and midline mismatches. Early

loss of deciduous teeth can adversely affect the child's quality of life, aesthetics, eating, speech development, and arch integrity. There may be difficulties in expressing some words during the speech. Also, the early loss of the primary front teeth causes problems in the eruption and appearance of permanent teeth. Besides, problems such as antagonist tooth extrusion, displacement of the adjacent tooth, increased overjet-overbite and midline deviation occur. The most common causes of early deciduous tooth loss are dental caries, trauma, periodontal diseases, and early root resorption. (15-19)

Early loss of deciduous anterior teeth is greater in the maxilla than in the mandible. Early loss of primary molar teeth is greater in the mandible. The most important cause of early loss is early childhood caries (ECC). (20-22)

ECC, which causes premature loss of primary teeth, is the most common chronic childhood disease. ECC is an illness that should be taken into account due to its physical, social, and psychological consequences on children and their families. ECC starts as White spot lesions in the upper anterior teeth and spreads to the molar teeth. Due to ECC, children experience decreases in their quality of life such as loss of appetite, weight loss, insomnia, decrease in school success, and school absenteeism. (23-25)

The first strategy in the treatment of ECC in children is to prevent the transmission of mutans streptococci, the bacteria responsible for caries, to children from their relatives. The second strategy is to reduce the cariogenicity of the diet; The third is to increase the decay resistance of new teeth by applying topical fluoride. (26)

Health and intellectual development problems in children due to dental caries can be improved with dental treatments. (27) The quality of the restorative process on deciduous teeth affects the success or failure of the restoration. Effective and evidence-based restorative interventions should be taken as a basis for the survival of primary teeth. However, failures are inevitable in wrong and faulty restorations made without considering the pulp's condition. (28)

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Chapter 10

BREAST CANCER AND AUTOIMMUNE THROID DISEASE

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INTRODUCTION

Breast cancer is the most common malign tumor found in women all over the world and following lung cancer, it is the second most common cause of cancer related deaths in women. The natural course of breast cancer varies among patients. In some patients with the same tumor diameter, tumor recurrence occurs in a very short period of time while others remain healthy. For this reason, prognostic factors such as tumor size, axillary lymph node involvement, histologic tumor type, histologic grade, hormone receptors (estrogen and progesterone receptors (ER, PR)], tumor proliferation rate, molecular factors such as enzymes, HER2 (Human Epidermal Growth Receptor 2) and onco-suppressor genes [Breast Cancer Susceptibility (BRCA), p53, cathepsin-D] are used in order to identify clinical and biological behavioral differences [1].

The most powerful prognostic factor in breast cancer is axillary lymph node involvement. [2] Nowadays, in addition to axillary lymph node involvement, other important factors such as steroid hormone receptor status efficient in prognosis and treatment, HER2, are used for this purpose. Additionally, the effects on the prognosis of the differences in molecular definitions have become the new targets of the researchers. [3] As a result of the developments in quantitative revert transcriptase polymerase chain reaction and microarray technology, the molecular gene expression profiles of breast cancers have been exposed and breast cancer has been classified according to this heterogeneous pattern. According to the new molecular classification of breast carcinoma, carcinomas carrying the ER are located in the group with the good course. It also benefits to a great degree from the Anti-estrogen treatment [4]. The worst prognostic group is HER2 (+) and the triple (HER-2, ER, PR) negative breast carcinomas.

In some studies, the prevalence of autoimmune thyroid disease (AITD) was found to be higher in patients with breast cancer [5, 6]. Both breast cancer and thyroid diseases are women suggest that a number of common factors may be effective in the etiology of these two diseases. Genetic predisposition to the Hashimoto's thyroiditis (X chromosome), sex steroids, decreased T and B cell function with a significant increase in CD24 + and CD25 + T cells during pregnancy, immune hyperactivity caused by stress induced hypercortisolemi or high corticotropin releasing hormone levels, viral infection, excessive iodine uptake, radiation exposure after the Chernobyl nuclear accident and fetal microchimerism are known as possible risk factors for Hashimoto thyroiditis. There are

not enough studies showing the prognostic significance of autoimmune thyroid disease in patients with breast cancer in the medical literature.

The aim of our study was to determine the breast cancer molecular subgroup frequency and to examine the relationship between AITD and the breast cancer in terms of prognosis and predictive factors.

MATERIAL AND METHODS

One hundred and one women who were followed up at Kirikkale University Medical Faculty Research and Practice Hospital, Medical Oncology outpatient clinics with a diagnosis of breast cancer were included in our study [mean age 57.93±13.44 (min 33- max 89) years]. Patients were diagnosed with other types of cancer outside of breast cancer, those with an autoimmune disease other than autoimmune thyroid disease, immunodeficiency, those with thyroid hormone replacement therapy was excluded from the study. The postoperative pathology reports and medical record in study population were reviewed retrospectively.

Autoimmune thyroid disease was diagnosed when the level of anti-TPO was above 34 IU / mL and thyroid parenchyma image on the thyroid ultrasound was heterogeneous.

A retrospective review was conducted of the post-operation pathology reports of the patients concerning the phase of the American Joint Committee on Cancer (AJCC) phase classification, [7] diameter of the tumor, the histologic grade of the tumor according to the Modified Scarff-Bloom-Richardson grading system, lymphovascular and neural invasion status, hormone (estrogen and progesterone) receptor status, HER2 expression and the axillary lymph node involvement. The axillary lymph node involvement was considered positive in all axillary lymph nodes in the same side of the tumor with a metastasis greater than 0.2 mm. Invasion of the tumor of the surrounding lymphatic and vascular structures was considered as a lymphovascular invasion. As for the HER2 evaluation, while tumors detected with 3+ through immunohistochemistry or those detected with 2+ and subject to a fluorescence in situ hybridization (FISH) test in which an amplification was observed were accepted as HER2 positive while other tumors were accepted as negative. Tumors with a molecular subtype classification of estrogen and/or positive progesterone receptor as well as those with negative HER2 tumors were grouped as “Luminal A”, while those with an estrogen and/or positive progesterone receptor as well as positive HER2 tumors were grouped as “Luminal B”; in addition to tumors with

an estrogen and/or negative progesterone receptor as well as positive HER-2 tumors which were grouped as “HER-2 overexpression” and those tumors with estrogen and/or negative progesterone receptors as well as negative HER-2 tumors which were grouped as “Triple negative”. A written approval was granted by the Kirikkale University, Faculty of Medicine, Clinical Research Ethics Committee through the 01/09 Numbered and 19.01.2016 Dated Decision in order to conduct this study.

Free T3 and free T4 were determined by electrochemiluminescence emission technique and TSH levels by electochemiluminescence Immuno Assay (Roche Diagnostics, Meinhaim, Germany).

Statistical analysis:

The data of the study has been recorded with the program Statistical Package for the Social Sciences (SPSS) version 20 (IBM corporation, New York, United States) and statistically analyzed has been carried out. Descriptive statistics are depicted as number, percentage, mean and standard deviation. The chi-square test was implemented to compare qualitative data. The normal distribution suitability of the variables was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov, Shapiro-Wilk tests). Numerical variables determined according to normal distribution, were compared between the two groups using Independent T test. Numerical variables with no normal distribution were compared between the two groups using the Mann Whitney U test. The relationships between variables were evaluated with Spearman’s correlation analysis. In the analysis results, it is considered significant that the value of p was less than 0.05.

RESULTS

While the mean age of breast cancer patients with autoimmune thyroid disease was 58,58±13,94 years, the mean age of breast cancer patients without autoimmune thyroid disease was 57.73±13.37 years. There was no significant difference between the groups in terms of age distribution. Serum thyroid hormones, TSH and anti TPO levels in our study population were demonstrated Table 1. The prevalence of autoimmune thyroid disease in our study was 23.8% (n = 24) in breast cancer. In addition, 79.2% (n=19) of women with breast cancer diagnosed with AITD were found to be euthyroid, 8.3% (n=2) subclinical hypothyroid, 8.3% (n=2) overt hypothyroid and 4,2% (n=1) subclinical hyperthyroid. 79.2% (n=61) of women with breast cancer diagnosed without AITD (n=77) were found to be euthyroid, 10,4% (n=8) subclinical hypothyroid

and 3,9% (n=3) overt hypothyroid, and 2,6% (n=2) hyperthyroidism.

It has been found that invasive ductal carcinoma in 91.6% (n=22) of breast cancer patients with AITD, 4,2% (n=1) invasive lobular carcinoma, 4.2% (n=1) other subtypes. Invasive ductal carcinoma in 93.5% (n=72) of breast cancer patients without AITD, invasive lobular carcinoma in 3,9% (n=3) and other subtypes in 2,6% (n=2). No significant difference was found in histological subtype distribution among the group with autoimmune thyroid disease and the group without autoimmune thyroid disease. We show cytopathologic feature of breast cancer in study population in Table 2. No significant differences were found between the group with autoimmune thyroid disease and the group without autoimmune thyroid disease in terms of tumor size, tumor stage, molecular subgroup distribution, hormone receptor positivity and tumor grade (Table 2). However, axillary lymph node involvement in breast cancer patients without AITD (n=9) was higher than in breast cancer patients with AITD (n=47) [(61% vs. 37.5%, respectively (p=0.043)] (Table 2).

On the other hand, there was no significant difference in terms of neural invasion and peritumoral lymphovascular invasion among the groups (Table 2). It has been determined that in 54.2% of the breast cancer patients with autoimmune thyroid disease (n=13) Luminal A, 20.8% (n=5) Luminal B, 16.7% (n=4) HER-2 overexpression. In 8.3% (n=2) triple negative breast cancers. It has been observed that in 49.4% (n=38) of breast cancer patients without autoimmune thyroid disease, Luminal A, 31.2% (n=24) Luminal B, HER-2 overexpression in 15.6% (n=12) in 3.9% (n=3) triple negative breast cancer. There was no significant difference between the groups in terms of autoimmune thyroid disease and molecular subgroup distribution (p=0,681).

Table 1: *Serum thyroid hormones, TSH and TPO levels in study pooplation with breast cancer*

	Breast Ca with AITD n:24	Breast Ca without AITD n:77	***P***
FreeT3 (pg/mL)	2,70±0,52	3,03±0,52	0,009
Free T4 (ng/dL)	1,17±0,19	1,26±0,22	0,08
TSH (µU/mL)	3,47±3,67	2,56±1,96	0,256
AntiTPO (IU/mL)	216,39±167,54	9,96±5,24	0,0001

Table 2: *Cytopathologic feature of breast cancer in study pooplation*

	Breast Ca with AITD n:24	Breast Ca without AITD n:77	***P***
Tumor size (cm)	2,47±1,17	2,79±1,69	0,585
Stage Stage 1 Stage 2 Stage 3 Stage 4	6 (25,0%) 13 (54,2%) 1 (4,2%) 4 (16,7%)	16 (20,8%) 39 (50,6%) 9 (11,7%) 13 (16,9%)	0,744
Histological subtype: Invasive ductal carcinoma Invazive lobuler carcinoma Other	22 (91,6%) 1 (4,2%) 1 (4,2%)	72 (93,5%) 3 (3,9%) 2 (2,6%)	0,922
Estrogen receptor Positive Negative	18 (75,0%) 6 (25,0%)	60 (77,9%) 17 (22,1%)	0,766
Progesterone receptor Positive Negative	16 (66,7 %) 8 (33,3%)	55 (71,4%) 22 (28,6%)	0,656
HER-2 overexpiration Positive Negative	9 (37,5%) 15 (62,5%)	36 (46,8) 41 (53,2)	0,426
Grade Grade 1 Grade 2 Grade 3	5 (20,8%) 15 (62,5%) 4 (16,7%)	10 (13,0%) 40 (51,9%) 27 (35,1%)	0,207
Axillary lymph node involvement Positive Negative	9 (37,5%) 15 (62,5%)	47 (61,0%) 30 (39,0%)	0,043
Neural invasion Positive Negative	3 (12,5%) 21 (87,5%)	8 (10,4%) 69 (89,6%)	0,720
Peritumoral lymphovascular invasion Positive Negative	5 (20,8%) 19 (79,2%)	13 (16,9%) 64 (83,1%)	0,761

There was a positive correlation between serum TSH levels and anti thyroid peroxidase (TPO) levels **(r=0.240, p=0.016).** More importantly, there was a significantly negative correlation between anti TPO levels and axillary lymph node involvement in patients with breast carcinoma **(r= -0.245, p=0.014).**

DISCUSSION

Determine of prognostic and predictive factors in breast cancer are very important in the high-risk group, because the differences of the clinical and biological behavioral of breast cancer vary among patients. According to our finding the presence of thyroid autoimmunity in our patients with breast cancer may show a positive prognosis due to low axillary lymph node involvement which is an important poor prognostic parameter independent of other clinical outcome. More importantly, we found a significantly negative correlation between anti TPO levels and axillary lymph node involvement in patients with breast carcinoma**.** According to the limited number of studies in medical literature, axillary lymph node involvement rate in patients with breast cancer accompanied by autoimmunity was lower than other group without autoimmunity (22% vs.46%, p=0,007) (özmen). On the other hand, Cengiz et al. [8] found that the number of lymph nodes involved in breast cancer patients with thyroid disease was greater than that of breast cancer patients without thyroid disease (p=0,005). Unlike previously reported studies, we firstly demonstrated that there was a significantly negative correlation between anti TPO levels and axillary lymph node involvement in patients with breast carcinoma. It seems that antithyroid peroxidase antibody positivity may be associated with a lower incidence of axillary metastases in newly diognosis breast cancer.

Although the pathogenesis is not fully understood, it is suggested that combined iodine-selenium deficiency may facilitate the development of breast cancer. Both thyroid and breast tissue shows similar properties in terms of iodine and selenium metabolism. Both tissues contain the sodium-iodide symporter carrier. On the other hand, excessive iodine uptake and selenium deficiency may contribute to autoimmune thyroid disease. It was reported that increase of selenium level in the diet to cause a decrease in anti-TPO levels. The effect of thyroid autoimmunity and dysfunction is contradictory in the course of patients with breast cancer. Various studies showed that hypothyroidism was associated with the risk of breast cancer development. On the other hand, other studies showed that primary hypothyroidism decrease breast cancer

incidence. Various authors believe that hypothyroidism, autoimmune thyroiditis, and positive serum anti-TPO levels have good outcomes in breast cancer cases. On the other hand, in a recent meta-analysis, it was found that the risk of developing breast cancer in people with AITD was high and an interesting reciprocal link.(odds ratio:2,92) [9]. According to another theory, natural and inducible CD4+ CD25+ Forkhead box P3 (FOXP3)+ regulatory T cells (Tregs) and their co-regulators T helper 17 cells which inhibit autoreactive immune clones and regulate TH1/ TH2 shift in autoimmun disease via IL-17A, IL-17F, IL-21, IL-22 and IL- 26, may play important roles in maintaining immune self-tolerance and the inhibition of both inflammation and cancer. We consider that the advanced immunologic study may clarify possible understanding mechanisms for effect of AITD in prognosis of breast carcinoma.

Interestingly, Muller I et al.(10) previously detected a TPO protein in neoplastic breast epithelium by immunofluorescence. According to their opinion, there was a shared antigen that indicate target for humoral or cell-mediated immune activity associated with both of breast cancer and thyroid (etc.the sodium/iodide symporter, cross-reacting epitopes in TPO or TPO itself and lactoperoxidase). This may explain the high frequency and protective role of TPOAb in breast cancer.

Mucinous, papillary, medullary, adenoid cystic, tubular breast carcinomas have better prognosis compared with the invasive ductal carcinoma and invasive lobular carcinoma. However, micropapillary and metaplastic carcinoma have worse prognosis. In our study, there was no difference in terms of histological subtypes of breast cancer in patients with and without autoimmune thyroid disease.

It was well known that tumor size correlated with lymph node involvement in breast cancer, but their prognostic importance was independent of each other. In a cohort study involving 24,740 patients with breast cancer, 5-year survival rates were found to be 91%, 80% and 63% when the tumor size is 2 cm, 2-5 cm, and > 5 cm, respectively. In our study, although the mean tumor diameter of breast cancer patients with autoimmune thyroid disease was smaller than the mean tumor diameter of breast cancer patients without autoimmune thyroid disease (2.79 ± 1.17 cm versus 2.79 ± 1.69 cm, respectively), no statistically significant difference was found in terms of tumor size between groups (p=0,585). Similarly, Özmen et al. reported that there was no significant difference in terms of tumor size in breast cancer patients with and without autoimmune thyroid disease (2.49 ± 1.45 cm vs 2.46 ± 1.38 cm)(p=0,889)

[11]. Unlike our results, Cengiz et al.[8] have shown that tumor size is greater in breast cancer patients with thyroid disease (p=0,023).

In studies in the medical literature have been shown that estrogen receptor-positive cases live longer than estrogen receptor negative cases. In estrogen / progesterone receptor positive breast cancers within the first 5 years, disease-free survival is higher than receptor-negative breast cancers and recurrence rate is lower. Often the estrogen receptor carries a strong predictive value for disease-free survival; whereas progesterone receptors are associated with overall survival, as hormone therapy is more likely to respond in the event of disease recurrence. In our study, the proportion of patients with estrogen receptor-positive patients was found to be 77,2% (n=78) and the proportion of patients with progesterone receptor-positive patients was found to be 70,3% (n=71), which was slightly higher than the general literature. In a study conducted by Freitas et al. (146) there was no significant difference in terms of hormone receptor status (ER and PR) between breast cancer patients with autoimmune thyroid disease and breast cancer patients without autoimmune thyroid disease. (p=0,052). When our study examined the hormone receptor status of breast cancer patients with autoimmune thyroid disease and without autoimmune thyroid disease, there was no significant difference in terms of hormone receptor positivity between the groups.

HER-2 overexpression is a poor prognostic factor in patients with positive or negative lymph node involvement [12]. Previous studies indicating that HER2 positivity shows reduced survival even when axillary lymph node involvement is positive. In a study conducted by Freitas et al. [3], there was no significant difference in terms of HER2 overexpression between breast cancer patients with autoimmune thyroid disease and breast cancer patients without autoimmune thyroid disease. In our study, there was also no significant difference between study groups in terms of HER2 overexpression (p=0,426).

The presence of peritumoral lymphovascular invasion is a poor prognostic indicator, especially in high-grade tumors. Özmen et al. [11] reported that peritumoral lymphovascular invasion was 58% in patients without autoimmune thyroid disease, and 43% in patients with breast cancer accompanied by autoimmune thyroid disease (p=0,116). In our study, peritumoral lymphovascular invasion rate was lower (17.8%), and there was no significant difference in terms of peritumoral lymphovascular invasion between the groups.

In the conclusion, it has been observed that in cases of breast cancer

with autoimmune thyroid disease axillary lymph node involvement is less common than breast cancer with no autoimmune thyroid disease. Thus, autoimmune thyroid disease may be a positive prognostic indicator in breast cancer surveillance. It is recommended that this positive prognostic marker be taken into account in breast cancer surveillance analyzes. For a bette1r understanding of this positive display, prospective studies have to be done.

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Chapter 11

ORAL PIGMENTED LESIONS

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ORAL PIGMENTED LESIONS

Pigmented lesions; it is one of the common conditions of the oral mucosa. The term pigmented lesion occurs when one or more pigments cause discoloration in the mucosa. Pigmented lesions in the oral mucosa can be divided into two sub-categories: those containing melanin and those containing other pigments. The color of these lesions may show yellow to brown / black writing depending on the depth of pigmentation. Lesions ranging from amalgam tattoos to oral malignant melanoma can give a similar appearance. Diagnosis is usually made by anamnesis and clinical examination / follow-up. Biopsy is used for definitive diagnosis (1-4).

This article aims to provide information about the clinical manifestations and treatment approaches of pigmented lesions in the oral mucosa.

Classmates belonging to the clinical appearance of the lesions, history and examinations play an important role in narrowing the diagnostic class and on the way to diagnosis. Regezi et al. (1) reported the following class of blowing for pigmented lesions of the oral mucosa (Table 1).

Table 1: Classification of Pigmented Lesions

Melanocytic Lesions	Nonmelanocytic Lesions
• Physiological Pigmentation • Smoking-Associated Melanosis • Oral Melanotic Macule • Cafe-au-Lait Macules • Pigmented Neuroectodermal Tumor of Infancy • Melanocytic Nevus • Melanoacanthoma • Melanoma	• Amalgam Tattoo • Drug-Induced Pigmentations • Heavy Metal Pigmentations

MELANOSITIC LESIONS

Melanocytes are cells that emerge from the neural crest embryologically, migrate to the epithelial surfaces where they are located between the basal epithelial cells and produce melanin. Melanocytes produce packaged pigment granules known as melanosomes. Normally, melanocytes do not contain melanosomes, but instead are sent to surrounding keratinocytes and sometimes to adjacent macrophages through dendritic processes. Light, genetic and hormonal factors affect the number of pigment generated (5-9).

Melanocyte cells already are present in the oral mucosa., but they are often unrecognized microscopically due to the relatively low pigment production levels. They appear clearly with the cytoplasm that is not stained on routine histological sections. In the clinical presentation of melanocytic lesions, their color changes from yellow / brown to black and blue depending on the amount of melanin produced and the depth of pigment according to the surface. Usually superficial pigmentation is yellow / brown, allthough deeper pigmentation turns from black to blue as a result of Tyndall effect. The darkening of a pre-existing lesion that is not stimulated by known factors indicates that the pigment cells are generating invading deeper tissue or more melanin (1, 5, 7).

While actively producing or reproducing pigment, they may be responsible for several different entities in the oral mucous membranes ranging from physiological pigmentation to melanoma.

Physiological Pigmentation

Etiology

These are pigmentations that occur with the increase of melanin pigment produced by melanocytes, and are more common in dark-skinned individuals and can be observed with age (10).

Clinical Features

In the oral mucosa, it is most frequently observed in the gingiva (not affecting the marginal gingiva) as a well cicumscribed, simetrical, brown band (11).

It can also be seen in the palate, lip, buccal mucosa and tongue. The color of the lesion shows information from light brown to black. Intensifying color can lead to hormonal conditions, smoking and systemic drug use (12, 13).

Diagnosis and Treatment

It is clinically diagnosed, does not require treatment, but a biopsy can be taken in cases with a systemic condition such as Addison's disease. Although procedures such as gingivectomy, laser therapy and cryotherapy have been applied on the lesions due to aesthetic concerns, the lesions may recur (12).

Smoking-Associated Melanosis

Etiology

One of the smoking chemicals is thought to be formed to stimulate melanin production (14, 15).

Clinical Features

It is observed as multiple and adjacent brown macules in the second mandibular labial gingiva area. Pipe smoking-related pigmentations occur in the buccal mucosa and palate (10).

Diagnosis and Treatment

Clinical findings and smoking are important in diagnosis. Physiological pigmentation, systemic diseases and drug-related pigmentations can be considered in the differential diagnosis (16).

Pigmentation is expected to decrease within 3 years after quitting smoking. Biopsy may be indicated if the lesion is raised from the mucosa, its expected location or increased pigment density (11, 15).

Oral Melanotic Macule

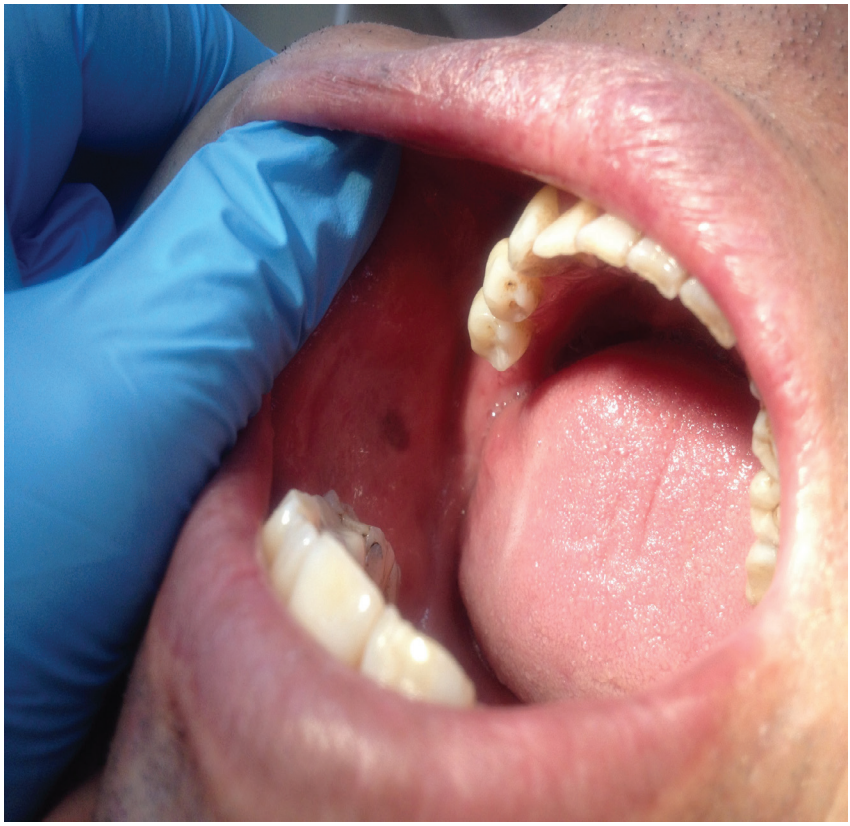
Etiology

The etiology of these lesions is not fully known. However, local trauma and chronic inflammation are considered to be a possible inducing factor of melanotic macules (17). Typically it is the result of increased melanin accumulation, but there may also be an increase in melanocyte count. Melanotic macule is not due to sun exposure (16).

Clinical Features

Melanotic macule is brown to black, small (less than 1 cm in diameter), flat, well circumscribed, asymptomatic lesion. Vermillion border is the most common region (31%). Approximately 91% of this lesion is in the lower lip, and only 6% is in the upper lip. Other common places are the gingivas, palate, and buccal mucosa (Figure 1) (17).

Figure 1: *Melanotic macule detected in the buccal mucosa of the patient who applied to our clinic for dental examination*



Diagnosis and Treatment

Melanotic macules are asymptomatic. If the diagnosis can be made based on clinical findings, treatment or clinical follow-up is not required. They have no malignant potential. However, some lesions develop rapidly and it can mimic melanoma. In order to distinguish the possibility of melanoma, biopsy indication may arise.and in this case, the diagnosis is made histopathologically. This is especially true for palate lesions, where melanomas are the most common (18).

Cafe-au-Lait Macules

Cafe-au-Lait Macules are skin spots with irregular edges and contain uniform brown melanin pigment. It is noticeable at birth or after birth, can be seen in normal children or with a syndrome. Individuals with six or more large cafe-au-lait macules (> 0.5 cm prepubertal,> 1.5 cm

postpubertal) should be suspected of neurofibromatosis (NF). It can also occur with Café-au-lait macules, Albright syndrome, Noonan syndrome, Watson syndrome, Bloom syndrome, ring chromosome syndromes and other syndromes (1).

Clinical Features

Cafe-au-lait macules (CALMs) are common hyperpigmented and smooth skin lesions found in the general population. They can grow in size and number. The color varies from light brown to dark brown and can be found anywhere on the body, but the most common locations are the extremities and trunk (19).

The appearance of melanocytes is normal with microscopic thinness. In microscopic examinations, the appearance of melanocytes is normal, but an increase in their number can be seen (1).

Pigmented Neuroectodermal Tumor of Infancy

Etiology

Pigmented (melanotic) neuroectodermal infancy tumor is a rare, rapidly growing biphasic tumor consisting of cells containing melanin and neuroblast-like cells. Like melanocytes and nevus cells, these cells have their origins in the neural crest (1).

Clinical Features

It is most common (more than 90%) in children under one year old. Although typically seen in the maxilla, it can also be found in the mandible, brain, epididymis and skull. It is in the form of a dark pigmented, non-ulcerated mass. It gives a radiolucent image with irregular borders that includes radiologically developing teeth (1).

Diagnosis and Treatment

Early childhood malignancies such as rhabdomyosarcoma, and "histiocytic" tumors, neuroblastoma may be considered in their differential diagnosis. Good results have been achieved in treatments with wide, local surgical excisions. Recurrent cases can be seen between 10% and 20%. Follow-up is recommended after excision (1).

Melanocytic Nevus

They are benign lesions that are composed of melanocytes, contain melanin, with distinct borders, and can appear as brown-black colored macules, papul or papules (20). They can be of acquired or congenital origin. When examined histologically, it is grouped as intradermal

(intramucosal), junctional, compound and blue nevus according to the localization of the nevus cells (21, 22).

Etiology

Melanocytic nevus are the clustering of nevus cells that are typically nested in round or polygonal shape. It is thought that nevus cells originate from resident melanocytes that have migrated from the neural crest to the epithelium and dermis or changed (1).

Clinical Features

They are benign lesions that can appear as brown-black macules, papul or papules with clear borders (20). 15% of the cases can be observed as non-pigmented, this is usually seen in intramucosal nevus and makes clinical diagnosis difficult (17). Intraoral nevus are most common in the palate. In addition to these, less common areas are gingiva, buccal/labial mucosa, alveolar ridge, and vermilion (1).

Diagnosis and Treatment

Clinical follow-up is usually sufficient. However, since nevus are frequently located on the palate like melanomas and exhibit a similar appearance to the early stage of melanomas, they should be surgically removed in suspicious cases (11).

Melanoacanthoma

Etiology

It is a rare reactive lesion of the oral mucosa that occurs due to trauma or local irritation. It is associated with an increase in melanocytes and melanin (11, 23).

Clinical Features

This lesion is usually solitary, flat or slightly raised black or brown, but; bilateral and multiple melanoacontomas have been reported. These lesions sizes can vary from a few millimeters to several centimeters. This asymptomatic lesion is mostly observed intraorally in the buccal mucosa, but it can also be seen in other regions such as lips, palate, gingivas and alveolar mucosa (24-26).

Diagnosis and Treatment

In the differential diagnosis; lentigo simplex, freckle, melanocytic nevus, malignant melanoma, amalgam tattoo, smoking and drug-induced melanosis. Biopsy distinguishes it from oral melanoma. The treatment

of melanoacanthoma that is diagnosed is not necessary, the lesion may regress after biopsy (3, 4, 18, 23).

Melanoma

Etiology

Mucosal malignant melanomas are thought to arise from melanocytes in the basal layer of the mucosa. They constitute 0.5% of all oral cancers and 0.07% of all head and neck cancers. It is known that these very rare lesions are most common between the ages of 40 and 70. It has been reported that factors such as smoking and alcohol consumption and the use of prostheses may be etiological factors (27, 28). It can also be seen in mucous membranes such as larynx, rhinopharynx, conjunctiva, vagina and anus. Although malignant melanoma cases develop primarily, they may also develop from a previously existing benign lesion that involves melanocytes in the skin or mucosa (23).

Clinical Features

It is usually observed between the ages of 50-60 and affects both genders equally. Oral melanomas have a wide range that can be clinically gray, black, purple and reddish. Tumors are asymmetrical, have irregular borders, and can sometimes be seen more than once. Surface shapes may vary as macules, ulcers or nodules (23, 29). Non-pigmentation amelanotic malignant melanomas may be whitish or mucous in color. Oral melanomas are observed in 80% of the upper jaw, usually on the hard palate, gingivas, and less frequently on the lip mucosa, buccal mucosa, tongue and mandibular alveolar mucosa. If the diagnosis is delayed, it invades the alveolar bone and loss of teeth may occur (20).

Diagnosis and Treatment

In differential diagnosis for oral malignant melanoma, smoking-associated melanosis, oral melanotic macule, melanoplakia, pituitary-based Cushing's syndrome, drug-induced melanosis (antimalarial drugs and Minocycline), inflammatory pigmentation, melanocanthoma, melanocytic nevus of the oral mucosa, blue nevus, Addison disease, PeutzJeghers syndrome, Kaposi Sarcoma, physiological pigmentation, amalgam tattoo, pigmentation associated with the use of heavy metals, and many other conditions that share some macroscopic features with oral malignant melanoma are important. At the same time, such as poorly differentiated carcinoma, and large cell anaplastic lymphoma it must be distinguished from other malignancies (29).

Treatment approach in oral melanoma requires a detailed evaluation. Treatment may include surgical resection with or without neck dissection, immunochemotherapy, and radiation therapy, and various combinations of all of these. It has been reported that clinical stage, vascular invasion, gender, postoperative radiotherapy and response to treatment significantly affect the prognosis (30).

NONMELANOCYTIC LESIONS

Exogenous color changes such as foreign materials containing tattoo pigment, amalgam filling material, pencil, colored foods (such as liquor, red wine, coffee, tea) can also be seen in the oral mucosa. These pathologies are called nonmelanocytic lesions. Common non-melanocytic lesions encountered by dentists are amalgam tattoo, drug-induced pigmentation and heavy metal pigmentation (11).

Amalgam Tattoo

Etiology

They frequently result from iatrogenic mucosal implantation of amalgam particles during dental applications. Amalgam tattoo usually occurs by embedding the amalgam aerosol, which is released during the extraction of the restorated tooth, during amalgam filling/removal, or polishing, into the soft tissue (1, 12).

Clinical Features

It may appear in blue, gray, black colors, but the mucosa is not actually pigmented. The coloration is due to the tracing of metallic particles embedded in the lamina propria and / or submucosa. They are usually small and are frequently seen macules near amalgam-restorated teeth or in areas where these teeth were previously found. It can be observed in the gingivas, alveolar mucosa, floor of the mouth and buccal mucosa (12, 23, 31).

Diagnosis and Treatment

Treatment is not indicated, but if aesthetically uncomfortable is felt, good results are obtained with the Q-switched alexandrite laser or Q-switched ruby laser. If the patient has a history of dental intervention prior to blue-gray pigmentation in the oral mucosa, diagnosis is easy. However, if there is no such story, biopsy may be required for differential diagnosis (11).

Drug-Induced Pigmentations

Etiology

Pigmentation due to drugs; It may be due to increased melanin production, accumulation of drugs or metabolites, and sometimes postinflammatory changes. Phenothiazines, antimalarials, clofazimine, minocycline, cyclophosphamide, busulfan, ACTH, anti HIV drugs and amiodorone are drugs that can cause oral pigmentation. Tetracycline used in pregnancy or early childhood can also stain teeth (32).

Clinical Features

Drug-induced pigmentation can affect any mucosal area. The gingivas, tongue and hard palate are frequently affected (12).

Diagnosis and Treatment

If a temporary link is established between the use of a drug and the pigmentation observed, drug-induced melanosis can be diagnosed. Biopsy is indicated if drug-induced melanosis cannot be properly diagnosed. Except for aesthetic concerns, it is clinically insignificant. Discontinuing the drug may resolve pigmentation. Malignant transformation of drug-related melanotic lesions has not been reported (12).

Heavy-Metal Pigmentations

Etiology

Discoloration of the oral mucosa may occur due to the increased blood level of heavy metals such as lead, bismuth, mercury, silver, arsenic and gold. In adults, this situation often occurs in persons occupationally exposed to vapors of these metals due to prolonged exposure.. It can also be monitored in cases treated with heavy metals. In children, it can be seen due to lead-contaminated water or drugs containing mercury / silver (11).

Clinical Features

These heavy metals can accumulate in both the skin and the oral mucosa. Blue-black pigmentation is prominent along the gingival margin. It can also be seen in other areas of the oral mucosa. (especially the gums). The characteristic color is gray to black and the distribution is linear when found along the gingival rim (11).

Lead and Bismuth staining of gingival tissue is known as bismuth line and guideline respectively. This staining is proportional to the amount of gingivitis and appears to be the result of the reaction of the

heavy metal with bacterially produced hydrogen sulfide at the inflamed areas. Systemic findings may be seen depending on the type of metal deposited and the exposure time (11).

Diagnosis and Treatment

Metallic deposits on the oral mucosa are actually relatively minor. Because of the harmful effects of systemic toxicity, the underlying cause should be investigated. Early diagnosis is important in terms of preventing toxic effects (1,11).

For dentists and dental staff, chronic exposure can occur if dental amalgam is used carelessly and without proper precautions. and this situation is now accepted as an occupational disease. However, dental patients, they experienced during routine office visits are not at risk because apparently relatively short exposure times (1, 11).

CONCLUSION

Pigmented lesions can be encountered in a variety of clinical models ranging from only physiological changes to oral manifestations of malignancies and systemic diseases. Color changes in the oral mucosa can be attributed to the accumulation of endogenous or exogenous pigments as a result of various mucosal diseases. Some of these are localized deposits of harmless melanin, hemosiderin or exogenous metal; others are indicators of systemic or genetic diseases; and some may be associated with life-threatening medical conditions that require urgent intervention (2, 33, 34).

The differential diagnosis for any pigmented lesion is extensive and can include endogenous and exogenous pigment deposits. Although biopsy is a helpful and necessary aid in the diagnosis of focal pigmented lesions, the lesions with diffuse presentation require a comprehensive history and laboratory studies to make a definitive diagnosis.

Dentists should have knowledge about lesions in order to differentiate these pigmentations, to form an appropriate diagnosis and treatment planning, and increase their awareness for early diagnosis of life-threatening lesions, especially malignant melanoma (2, 33).

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Chapter 12

A CHRONOLOGICAL REVIEW OF BIOINFORMATICS SCIENCE IN PLASTIC SURGERY

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INTRODUCTION

After the revelation of the DNA double helix structure by Watson and Crick in the year 1953, a spectacular chain of progressions has been made in the understanding of the human genome organization and function. Genes involved in diseases such as Huntington and Cystic Fibrosis had already been identified and mapped before the Human Genome Project (HGP) started in 1989. But it was not until the year 2013, which coincided with the 50th anniversary of the discovery of DNA structure, when the HGP had been finished and the final version of the human genome got public. Although this event alone led to a huge step forward in medical research, scientists are still on track in solving the nature of many diseases. Meanwhile, plastic surgeons had little interest on those achievements; although, molecular biology and genomics might also provide answers to many problems of this specialty. Nevertheless, by pioneering research some progress has also been made in this field and this review aims to reveal how far and dense this area has gone today.

MATERIAL AND METHODS

Literature search on PubMed with MeSH terms 'Plastic Surgery' and 'Bioinformatics' first revealed a total of 540 articles published until January 2021. After exclusion of all those articles which were irrelevant to either 'Plastic Surgery' or 'Bioinformatics' a final of 100 articles were left for further analyzation. Main topics discussed in those articles were tagged with one of the following headlines: 'Keloid', 'Oncologic Comparison', 'Burn', 'Hand Surgery', 'Weight Loss', 'Craniomaxillofacial (CMF) Surgery', 'Transplantation' (TX), 'Stem Cell', 'Skin Biology', 'Wound Healing (WH)', 'Vascular Tumors (VT)', "Breast Reconstruction (BR)", "Aesthetics", "Trauma", "Diabetic Ulcers (DU)", "Microsurgery", 'Microtia', 'Platelet Rich Plasma' (PRP), 'Ischemia-Reperfusion' (I/R), 'Trigeminal Neuralgia' and 'Review'. Oncologic Comparison papers were also further categorized into 'Malignant Melanoma' (MM), 'Squamous Cell Carcinoma' (SCC) and 'Others'. All articles were also categorized according to the year of their publication.

RESULTS

From the 255 articles analyzed in this survey, 69 articles could be organized under the topic oncologic comparison and further subdivided into malignant melanoma, SCC and other malignancies with 41, 17 and 11 articles each, respectively. CMF article count was 29 and Keloid's was 22. After them, the most favorite topic was WH with 19 articles. Following,

Stem Cell had 17, BR had 16 and Burn had 14 articles. Aesthetics had 9, Skin Biology and VT both had 7, Hand Surgery, Microtia and Reviews had 6 articles in each. Weight Loss, DU 3 and Microsurgery had 3 articles in each. Trauma, Tissue Comparison, PRP had 2 articles and I/R and Trigeminal Neuralgia had only 1 article.

All articles were later analyzed according to the year or their publication. The first article to be published was a review in the year 2002, followed by another review article in 2003. A small rise in the number of publications could be observed in 2005 with the third review article published together with one article each under the topics Keloid, WH, and other malignancies.

Between 2007 and 2009 one article per year was published under the topics Burn, Tissue Comparison and again Burn, respectively. After that a continued rise in publication numbers could be seen with two articles published in 2010 under the topic Burn; five articles published in 2011 with two of them dealing with Keloid and one each with Hand Surgery, MM and SCC; three articles in 2012 dealing with Keloid, Burn, and Skin Biology; and four articles in 2013 with two of them dealing with Keloid and one each with Skin Biology and 'Other' Oncologic Comparison.

The border of more than ten articles published per year could be exceeded in 2014 with a total of thirteen articles published as follows; three articles each in MM and TX, two articles in Keloid, and one article each in SCC, Stem Cell, WH, Trigeminal Neuralgia, and other malignancies. Similarly, fourteen articles were published in 2015 including two articles each in CMF and WH; and one article each in I/R, MM, SCC, TX, Keloid, Burn, Skin Biology, Microtia, Weight loss, and 'Other' Cancer Research.

In 2016 a total of twenty one articles were published with five articles in SCC; four articles in Stem Cell; three articles each in CMF and 'Other' Cancer Research; two articles in MM; and one article each in WH, Hand Surgery, VT, and PRP.

In 2017 twenty five articles were published five articles in MM; four articles in CMF; three articles in TX; two articles each in SCC, Keloid, VT, and Microtia; and one article each in Stem Cell, Hand Surgery, Weight Loss, PRP, and other cancers.

In 2018, nine MM, two SCC, one other malignancies in oncological group, seven CMF, four keloid and burn articles each, two WH and TX each, and one stem cell articles were published. Microsurgery, BR and Aesthetics groups drew attention during this year finally, and came up with

two, one and one articles were published on these topics, respectively.

In 2019, nine MM, three SCC and one other malignancies, ten BR, eight CMF, two keloid, four WH and stem cell each, three aesthetics, two hand surgery, two microtia, one burn, one TX, one skin biology, three reviews, one DU article was published. Both trauma and the last tissue comparison study was published in this year.

In the last year of our study, on 2020, eleven MM, two SCC and one other malignancy studies, five CMF, six keloid, eight WH, six stem cell, five BR, four burn, two TX, five aesthetics, three skin biology, one VT, one microtia, one weight loss, two DU, and one microsurgery articles were published.

Following, categories with less than five papers were excluded from this study.

DISCUSSION

After the DNA structure discovered, identification strategies came into agenda, and genomic researches became one of the favorite topics at the beginning of the 21st century. More than 350000 articles are published in genomic research area. Among them, only 255 are related to Plastic Surgery practice.

Cancer research articles which were tagged with "Oncologic Comparison", were the largest group of this research. Malignant Melanoma (MM) had the main focus among these cancer articles. The earliest study of bioinformatics in MM was Mithani et al.'s study on 2011, which was focused on use of bioinformatic methods to reveal tumor-suppressor genes of MM (Mithani et al., 2011). After a relatively long vacant period on this topic, on 2014, Charbel et al. published their study, in which genetic mutations of giant melanocytic nevi were discussed (Charbel et al., 2014). Also, at the same year, Zhang et al.'s study revealed that melanoma proliferation and invasion was related to a mutant serine/threonine kinase and a miRNA is the inhibitor of it (Zhang J et al., 2014). Liu et al. demonstrated that gallic acid may have apoptotic effects on melanoma cells via glycolysis on 2014 (Liu C et al., 2014). Following, Liu et al. studied metastatic genome properties of melanoma and showed a micro RNA to inhibit metastases (Liu P et al., 2015). On 2016, Li et al.'s study showed another gene to promote an enzymatic pathway to drive melanoma to grow and spread (Li L et al., 2016). Dating back at the same year, Yu and Yang studied another miRNA to be a tumor suppressor gene product (Yu et al., 2016). On 2017, Zhang et al. screened melanoma genetics and summarized differences of

genetic, miRNA and long-noncoding RNA profile (Zhang Q, et al., 2017). Uveal melanoma was examined and classified as Robertson et al.'s study showed differences of mutation pathways between subtypes (Robertson et al., 2017). Friedman et al. tested the idea of using cellular culture of primary biopsy specimens as a target for combinatorial drug therapy and found to have the possibility of being effective (Friedman et al., 2017). Song et al. studied the effects of another miRNA in melanoma and found to be a possible inhibitor of the disease (Song X-F et al., 2017). Likewise, Bu et al.'s study showed another miRNA to be an inhibitor of migration and proliferation of melanoma cells (Bu et al., 2017). Following, Luan et al.'s study showed a long non-coding RNA, which known as an oncogenes lncRNA in some other human tumors, to be an enhancer of melanoma metabolism (Luan et al., 2018). Liu et al.'s article was another study on this topic next year, revealing miRNA675 to inhibit melanoma growth and invasion (Liu K et al., 2018). Low-risk of sentinel lymph node positivity in patients with T1-T2 melanoma was tested with gene expression profiling by Vetto et al. (Vetto et al., 2018) Wang et al. identified several different circRNA profiles between metastatic melanoma cell types. (Wang et al., 2018) Pisanu et al. discovered that BRAF-mutated melanoma stem cells are selected by a pathway which is controlled by Stearoyl-CoA desaturase-1 (Pisanu et al., 2018). Malicherova et al. compared different sequencing techniques to determine BRAF V600E mutation in melanoma (Malicherova et al., 2018). Luan et al. long ncRNA H19 promotes glucose metabolism and cell growth in malignant melanoma via miR-106a-5p/E2F3 axis (Luan et al., 2018) Luan et al. defined a new RNA pathway that facilitate melanoma growth (Luan et al., 2018). A year after, Yu et al. revealed a new mechanism for LICN00518 in the metastasis of melanoma (Yu et al., 2019). Zhang et al. MACC1 was shown to be a target of miRNA-338-3p, as it suppresses cell proliferation, migration and invasion in human malignant melanoma (Zhang et al., 2019). Effects of microRNA-708 was shown to be related with LEF1 through the Wnt signaling pathway in melanoma cells at the same year, by Song, Wang&Huo (Song, Wang&Hou, 2019). Burjanivova et al. showed that BRAFV600E mutation can be used to determine the treatment effectivity and developed a novel technique to measure this mutation in blood samples quantitively (Burjanivova et al., 2019). In another study, PLCB2 expression was shown to shorten melanoma cell line-life (Zhang et al., 2019). Luan et al. discovered a new RNA pathway that facilitate melanoma metastasis (Luan et al., 2019. Differential expression analyses were held to identify roles of ATK1 and CDK2 in melanoma (Wei et al., 2019). Xia et al. probed the possible genes

for melanoma carsinogenesis and progression (Xia et al., 2019). Wang et al. discovered that melanoma proliferation is advanced with higher-expressed miR-106a, suppressing connexin 43 expression, in the final study of that year (Wang et al., 2019). In 2020, Zhou et al. revealed that the miR-200b-3p is down-regulated in melanoma cell lines when compared with benign nevoid cell (Zhou et al., 2020). Huang et al. defined new chemokins of melanoma behavior (Huang et al., 2020). Xiong et al. melanoma relation with microRNA profiles amd target genes of plasma extracellular vesicles were established (Xiong et al., 2020). Possible genes, which include CXCL8, THBS1 and KIT, were found to be responsible for melanoma metastasis (Su et al., 2020). Validity of sentinel lymph node biopsy in thin melanomas was tested with nomograms and concluded this technique to be a decision-making one (Maurichi et al., 2020). Zhou et al. showed that SNHG6 and progression of melanoma was related, and this pathway to be a novel therapeutic target for melanoma (Zhou et al., 2020). Sheng et al. (46): focused on identification of prognostic differentially expressed genes (DEGs) between primary and metastatic melanoma (Sheng et al., 2020). Xue et al. (110): aimed to systemically analyze the bioinformatics of the alternative splicing events at a genomewide level using The Cancer Genome Atlas (TCGA) melanoma data (Xue et al.,2020). Importance of EAF-2 family in melanoma was determined by expression analysis in another study (Han&Shen, 2020). Long ncRNA SNHG7 was shown to promote malignant melanoma progression through negative modulation of miR-9s (Wang et al., 2020). Finally, Louveau et al. suggested a panel to investigate and determine treatment responses in melanoma patients (Louveau et al., 2020).

Following MM, SCC was another main topic under this class of studies. First report of Remmerbach et al. on 2011 experimented a novel screening method of oral cancers (Remmerbach et al., 2011). Following years was similar to MM, but less productive.

“Other” subcategory of Oncologic Comparison articles included eleven articles focused on other soft tissue malignancies. Earliest was from Lehnhardt et al., investigating fibrosarcoma treatment response rates with changes in apoptotic pathway regalations (Lehnhardt et al., 2005). Interestingly, less emphasis has been placed on other skin cancers, given that they are less common. Even rhabdomyosarcoma drew attention and was adressed proteomically (Sun et al., 2014). Finally, Nagashima et al. aimed to establish a national cancer genome atlas for cancer in Japanese nation (Nagashima et al., 2020).

CMF articles were mostly focused on craniosynostosis and related syndromes. Twigg et al's work showed a mutation to be associated with coronal craniosytosis and mental difficulties on 2015 (Twigg et al., 2015). In three studies beginning from 2015, Goos et al. found three different genes to be responsible for syndromic craniosynostosis and a facial cleft syndrome (Goos et al., 2015, 2016, 2017). On 2016, Miller et al. discussed the efficiency of bioinformatics on craniosynostosis and concluded with possibility of early diagnosis with several gene testing (Miller et al., 2017). Fenwick et al. discussed and concluded a genetic pathway to be ended up with a craniosynostosis syndrome the same year (Fenwick et al., 2016). On the following year, 2017, Shaw et al.'s work revealed relation of a rare muscular dystrophy to be associated with craniosynostosis and arhinia (Shaw et al.,2017). Schwerd et al.'s study demonstrated a similar relation between IL6ST gene and craniosynostosis (Schwerd et al., 2017). Similarly, Bae et al.'s animal study demonstrated delayed cranial suture closure to be related with a genetic pathway (Bae et al.,2017). Masotti et al. reported a gene of which mutation can result with cleft lip-palate (Masotti et al.,2018). Commander et al. was focused on hard and software preparation for twin separation operations (Commander et al., 2018). Chen et al. studied hemifacial microsomia etiology in monozygotic twins by genome sequencing (Chen et al., 2018). Sharif-Askary et al. discussed the communal factors of follow-up loss in CL/P patients (Sharif-Askary et al., 2018). Chen et al. investigated the relationship between the EPHA3 polymorphisms and non-syndromic cleft lip/palate (Chen et al., 2018). Zhou et al. revealed that Saethre-Chotzen syndrome results from haploinsufficiency of TWIST1 (Zhou et al., 2018). Masotti et al. studied the method of sampling techniques for gene expression studies (Masotti et al, 2018). Carlson et al. changes of GWAS signals were compared among orofacial cleft patients (Carlson et al., 2019). Goos et al. demonstrated that the BCL11B p.R3S substitution is causally associated with craniosynostosis (Goos et al., 2019). Kehrer et al. studied factors that affect cleft patients to have secondary rhinoplasty to achieve better results (Kehrer et al., 2019). Butali et al. utilized genome-wide association analyses to compare cleft palate only and cleft lip (Butali et al., 2019). Wang et al. indicated several commonly differentially expressed proteins (DEPs) to be created in mouse models on which 2,3,7,8-tetrachlorodibenzo-p-dioxin and retinoic acid was used (Wang et al., 2019). Frank-Ito et al. compared differences in nasal airway obstruction (NAO) of unilateral cleft lip nasal deformity subjects with noncleft subjects experiencing NAO (Frank-Ito et al., 2019). Shaffer et al. researched the low-frequency genetic variants in regulatory

regions in patients of nonsyndromic orofacial clefts (Shaffer et al., 2019). Wu et al. aimed to identify genetic variants influencing craniofacial morphology using whole-exome sequencing (Wu et al., 2019). Yoon et al. studied the variations of sequenced genomic material to reveal the CRS pathogenesis (Yoon et al., 2020). Calpena et al. examined SMAD6 variants in craniosinostosis (Calpena et al., 2020). Le et al. intended to discuss cleft lip/nose operational modalities using bioinformatical methods (Le et al., 2020). Xiong et al. using bioinformatical analyses, DEGs and differentially expressed miRNAs (DEMs) between patients with bone non-union and those with bone union, demonstrating hsa-miR-193a-3p to be a viable biomarker of BN in 2020 (Xiong et al., 2020). Finally, Lee et al. showed Esrp1 mutation to disrupt normal facial developing and cause orofacial clefting (Lee et al., 2020).

Among keloid studies, the first published report came in 2005, focused on proteomic content of hypertrophic scars (Wang et al., 2005). For a long time, studies under this category mostly aimed to determine the pathophysiology of keloid and hypertrophic scars. Chen et al.'s work was the only study among keloid-tagged studies in the early years, which focused on treatment changes of keloid after hydrocortisone injections (Chen et al.,2015). Secondly, Zhang et al. investigated the effects of hydrocortisone on keloids (Zhang et al., 2019). Thirdly, and lastly, Pang et al. aimed to investigate the effect of resveratrol on fibroblasts (Pang et al.; 2020). All of the papers used bioinformatics science on transcriptomic, metabolomic, or even genomic data. However, these entities still remain as a blank page, because a truly effective treatment is yet to be discovered.

Beginning from 2018, BR piled up the attention. As late as in 2018, in the first paper on this topic, Geers et al. aimed to find out if autologous BR affect oncological status (Geers et al., 2018). Atkins et al. discussed the long-term outcomes of immediate BR with acellular dermal matrix outcomes in patients with postmastectomy radiotherapy (Atkins et al., 2019). Orr et al. established the relationship between preoperative anxiety and revisions using bioinformatic methods, while correlating postoperative bleeding with timing and laterality of breast reconstruction, in another study (Orr et al., 2019; Orr et al., 2019). Krucoff et al. surveyed the satisfactionary and well-being of young mammoplasty patients (Krucoff et al., 2019). Glener et al. discussed the utility and benefits of abdominal-based breast reconstruction (Glener et al., 2019). Shammas et al. analyzed if immediate reconstruction of breast delays radiotherapy and have an impact on survival (Shammas et al., 2019). Sergesketter et al. discussed if different epidemiologic properties affect postmastectomy breast reconstruction (Sergesketter et al.,

2019). Sharif-Askary et al. evaluated different methods of postoperative management in patients with abdominal-based microvscular BR (Sharif-Askary et al., 2019). Riggio et al. compared the complication ethiologies of expander and implant-based BR (Riggio et al., 2019). Sergesketter et al. questioned if preoperative marital/relationship status affect the type of BR (Sergesketter et al., 2019). Shammas et al. researched the approaches in imaging modalities of post-mastectomy patients which had reconstruction (Shammas et al., 2020). Anolik et al. shared about their postoperative observations, of which Enhanced Recovery after Surgery method was used postoperatively (Anolik et al., 2020). Nakhlis et al. studied the differences between outcomes of immediate and delayed BR in patients with inflammatory breast cancer (Nakhlis et al., 2020). Cason et al. probed if fat grafting has any negative effects on imaging of breast reconstruction patients (Cason et al.,2020). Sharif-Askary et al. studied the temperature changes after different methods of BR (Sharif-Askary et al., 2020).

Aesthetics is also a new topic for bioinformatics. Similar to BR, this topic began to draw ttention on 2018. Firstly, Kaur et al. discussed using computational techniques to reconstruct facial shape (Kaur et al., 2018). The both years that followed was very rich on papers of this topic. Aging was the most remarkable subject under this headline, including molecular analyses.

The earliest TX study belonging to Kienzl-Wagner & Brandacher, discussed the use of proteomics in transplantation science (Kienzl-Wagner & Brandacher, 2014). Kuo et al. 's study demonstrated proteomic environment of allograft tolerance which gained with adipose stem cells supported by immunosuppressive agents (Kuo et al.,2014). At the same year, Wolfram et al. used computational science to reveal possible treatment targets for intervention on transplant rejections (Wolfram et al., 2014). Next year, a further study by Wolfram et al. revealed gene expression pathways by an animal study (Wolfram et al., 2015). Win et al.'s study pointed out that in face transplantation, preoperative crossmatch tests and allograft biopsies during the follow-ups are feasible to detect antibody-mediated rejections (Win et al., 2017). Noyan et al.'s study presented a way to prevent allograft rejections by modulated T cells (Noyan et al., 2017). Zhang's study showed the increment of survival rates of skin allografts by gene upregulation and occasionally apoptosis suppression in fibroblasts (Zhang, 2017). Kollar et al. designed a pilot study to investigate rejection biomarkers (Kollar et al., 2018). Shubin et al. aimed to analyze blood proteome changes to discover a rejection biomarker (Shubin et al., 2019). Hautz et al. analyzed immunological and prognostic outcomes of

hand transplant patients retrospectively (Hautz et al., 2020). Gok et al investigated the effects of cold storage conditions on transplant muscular tissue in (Gok et al., 2020).

Stem cell researches have gained speed in recent years. The earliest one was published in 2014 by Gong et al., for proteomic examination of an engineered biomedical mesh (Gong et al., 2014). In 2016, 4 of other stem cell researches comes. Quan et al. studies genomic and proteomic changes in differentiation of stem cells. (Quan et al., 2016) Another one of Lough et al. introduced stem cell originated hair bearing tissue restoration (Lough et al., 2016). Allori et al.'s study present a new method of tissue engineering (Allori et al., 2016). Guneta et al.'s study is a comparison study which compares adipose-derived and marrow-derived stem cell genomics (Guneta et al., 2016). Lopez et al.'s study is also a comparison study a year after, comparing stem cell genomics (Lopez et al., 2017). Yu et al. searched the effects of cell-free extracts of fat tissue as a therapeutic agent for ischemic disorders (Yu et al., 2018). Bi et al. studied Stromal Vascular Fraction effects on WH (Bi et al., 2019). Myneni et al. compared age differences of MSC effects on immunosuppression (Myneni et al., 2019). Xu et al. investigated the miRNA profiles of exosomes to effect on angiogenesis (Xu et al., 2019). Hu et al. investigated the effects of mesenchymal stem cell (MSC)-conditioned medium on WH (Hu et al., 2019). Conti et al. investigated cellulite morphogenesis and the role of stem cells in this progress (Conti et al., 2020). Zhang et al. investigated the role of a specific RNA particles' osteogenic effects on adipose derived stem cells (ADSCs) (Zhang et al., 2020). Taha et al. compared gene expression profiles of ADSCs and white adipose cells under tumor necrosis factor alpha effect (Taha et al., 2020). Tirza et al. found out that reduced temperatures of cultural environment has positive effects on ADSC expansion and differentiation (Tirza et al., 2020). Pepin et al. studied ADSC epigenetics to extract data about regenerative capacity (Pepin et al., 2020). Xiong et al. compared ADSCs and fibroblasts on proangiogenic capacity (Xiong et al., 2020).

Among burn articles, Pollins et al. started this topic on 2007, demonstrating the proteomic environment of human burn wounds (Pollins et al., 2007). Following articles used proteomic data, similarly, or used transcriptomic or genomic data to explore the micro-environment of burn wounds, generally. WH articles were another emerging topic in this area. First article was from Takahashi et al. on 2005. Their study was a cell culture study comparing effects of FGF-2 supported insulin and IGF-1 on cartilage cell medium (Takahashi et al., 2005). Year-by-year, WH articles continued to be published, mostly focusing on the processes and changes on metabolomic, transcritomic or genomic data.

WH articles were another emerging topic in this area. First article was from Takahashi et al. on 2005. Their study was a cell culture study comparing effects of FGF-2 supported insulin and IGF-1 on cartilage cell medium (Takahashi et al., 2005). Yang et al., showed proteomic changes in enhanced WH by extracorporeal shock wave therapy in diabetic rats in 2014. (Yang et al., 2014) Following, Shin et al.'s study showed HMGB-1 to be a mediator of estrogen-induced karatinocyte migration in 2015 (Shin et al., 2015). At the same year, Shanmugam et al. came out with benefits of fluorescent imaging in WH (Shanmugam et al., 2015). Taverna et al.'s study enriched the literature by presenting a novel protein extraction technique in pressure ulcer biopsies (Taverna et al., 2016). Kim et al. investigated keratinocyte and fibroblast migration and proliferation in adipose-derived stem cells to and found galectin-1 to be responsible for it (Kim et al., 2018). Kurita et al. showed that programming the cells of wound in vivo would generate a healthy skin epithelium (Kurita et al., 2018). This topic gaining popularity back on 2018. Luo et al. used adipose-derived stem cells to enhance the healing of a full-thickness skin defect (Luo et al., 2019). Icli et al. specified endothelial cell transcriptomic data of angiogenesis and tissue repair (Icli et al., 2019). Henn et al. conceived the miRNA profiles that affect the angiogenesisi and vascular remodelling (Henn et al., 2019). Icli et al. probed transcriptomic data of a tissue injury model to reach miRNA profiles that play role in angiogenesis (Icli et al., 2019). Böttger et al. defined a novel diagnostic method to diagnose chronic wound infection with transcriptome analysis (Böttger et al., 2020). Wang et al. probed the effects of telocytes on burn WH (Wang et al., 2020). Sorkin et al. investigated post-injury inflammatory response microenvironment using an experimental model of traumatic heterotopic ossification (Sorkin et al., 2020). Ashrafi et al. exhibited the changes of metabolomic and microbiomic microenvironments of WH (Ashrafi et al., 2020). Loretelli et al. demonstrated enhanced healing of diabetic wounds with embryonic stem cells in mice (Loretelli et al., 2020). Gudjonsson et al. discussed the role of plasma cells and B cells in hidradenitis suppurativa pathogenesis using specific markers (Gudjonsson et al., 2020). Icli et al. revealed a novel RNA that regulates angiogenesis in endothelium (Icli et al., 2020). Finally, He et al. revealed a sepsific protein in amniotic stem cells to promote keratinocyte differentiation and migration (He et al., 2020).

Among skin biology articles, the earliest one is Zollner et al.'s at 2012, which presented a new mathematical model for expanding skin's biomechanical properties. (Zöllner et al., 2012) On 2013, Yan et al.'s study emerged for transcriptome analysis of aging skin (Yan et al., 2013). On

2015, a hair growth study by Warshauer et al. tied in a micro RNA and p63 gene with a protein deficiency in a syndrome (Warshauer et al., 2015). After a long pause, Byrd et al. probed their database of hidradenitis suppurativa specimens to achieve an appropriate method to help researchers that plan to study pathophysiology of this disorder (Byrd et al., 2019). Dyring-Andersen et al. unearthed the proteomic properties of skin (Dyring-Andersen et al., 2020). Lin et al. studied the structural properties and transcriptomic pathways to enlight hair regeneration, similar to Yan's study on 2013 (Lin et al., 2020). Wang, Liu&Xu Identification of hub genes, key pathways, and therapeutic agents in Hutchinson-Gilford Progeria syndrome using bioinformatics analysis (Wang, Liu&Xu, 2020).

When these papers are considered, the earliest one was published in 2002 (Cole & Isik, 2002). After then, there had been only 99 papers was published on this topic until 2018. As the "disease of the century", cancer research was the main subject of these researches on that time. Remarkably, more paper was published between 2018-2020 than before 2018 (**Fig. 1**). Similar to pre-2018 term, cancer research, especially MM was on the focus. However, as BR and aethetics draw attention, while WH and other up-trending topics like keloid continued to stay in focus, paper count raised and became this high. Moreover, it looks like the novel topics have surpassed most of the older topics such as PRP. Nonetheless, this is only a promising statistic, showing a tendency of this scientific area is becoming a trend. Hopefully, near future may show us more scientists that work together and enlighten more dark points.

CONCLUSION

Since the completion of the Human Genome Project, more than 350000 articles have been published about genomic or bioinformatic researches. Among those, a very little part of them belong to the Plastic Surgery science. Nevertheless, relatively higher counts of papers in last years give the hope of faster achievements. Studies focusing on different areas of TX, Hand Surgery, Keloid, micro-changes in BR, Aesthetics and Oncological researches seem promising as future research areas.

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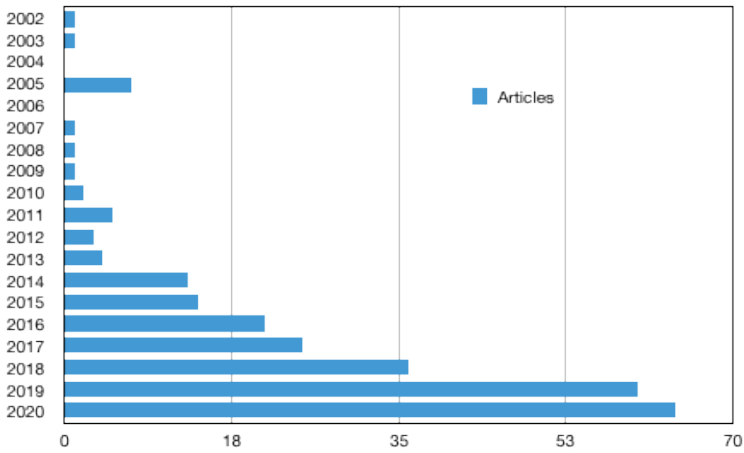
Fig. 1: Schematic diagram of the articles per year.



Tag	Papers	Count
Oncologic Comparison		69
MM	Mithani et al., 2011; Charnel et al., 2014; Zhang et al., 2014; Liu et al., 2014; Liu et al., 2015; Li et al., 2016; Yu&Yang, 2016; Zhang et al., 2017; Robertson et al., 2017; Friedman et al., 2017; Song et al., 2017; Bu et al., 2017; Luan et al., 2018; Liu et al., 2018; Liu et al., 2018; Vetto et al., 2018; Wang et al., 2018; Pisanu et al., 2018; Malicherova et al., 2018; Luan et al., 2018; Luan et al., 2018; Zhang et al., 2019; Song, Wang&Huo, 2019; Burjanivova et al., 2019; Zhang et al., 2019; Luan et al., 2019; Wei et al., 2019; Wang et al., 2019; Zhou et al., 2020; Huang et al., 2020; Xiong et al., 2020; Xia et al., 2019; Su et al., 2020; Maurichi et al., 2020; Zhou et al., 2020; Louveau et al., 2020; Han&Shen, 2020; Wang et al 2020; Sheng et al., 2020; Xue et al., 2020.	40
SCC	Remmerbach et al., 2011; Schmidt et al., 2014; Tian et al., 2015; Zeng et al., 2016; Kuang et al., 2016; Shen et al., 2016; Sand et al., 2016; Seddon et al., 2016; Li et al., 2017; Tabatabaeifar et al., 2017; Bai et al., 2018; Pan et al., 2018; Wang et al., 2019; Yu et al., 2019; Shi et al., 2019; Qian et al., 2019; Ishikawa et al., 2020; Xiong, Jiang&Li, 2020.	18
Other	Lehnhardt et al., 2005; Ponti et al., 2013; Sun et al., 2014; Tetzlaff et al., 2015; Lupu et al., 2016; Tetzlaff et al., 2016; Veneroni et al., 2016; Zeng et al., 2017; Adzavon et al., 2018; Fitzmaurice et al., 2019; Nagashima et al., 2020.	11
CMF	Twigg et al., 2015; Goos et al., 2015 ; Goos et al., 2016; Goos et al., 2017; Miller et al., 2016; Fenwick et al., 2016; Shaw et al., 2017; Schwerd et al., 2017; Bae et al., 2017; Commander et al., 2018; Chen et al., 2018; Sharif-Askary et al., 2018; Chen et al., 2018; Zhou et al., 2018; Masotti et al., 2018; Carlson et al., 2019; Goos et al., 2019; Kehrer et al., 2019; Butali et al., 2019; Wang et al., 2019; Frank-Ito et al., 2019; Shaffer et al., 2019; Wu et al., 2019; Yoon et al., 2020; Calpena et al., 2020; Le et al., 2020; Xiong et al., 2020; Lee et al., 2020.	28

Tag	Papers	Count
Keloid	Wang et al., 2005; Shih, McGrouther&Bayat, 2010; Huang et al., 2011; Engrav et al., 2011; Liu et al., 2012; Huang et al., 2013; Hu et al., 2014; Ogawa et al., 2014; Chen et al., 2015; Sun et al., 2017; Li et al., 2017; Li et al., 2018; Li et al., 2018; Fernández-Mayola et al., 2018; Wang et al., 2019; Zhang et al., 2019; Shi et al., 2020; Jin et al., 2020; Yuan, Sun&Yu, 2020; Zhou et al., 2020; Pang et al., 2020; Lv et al., 2020.	22
WH	Takahashi et al., 2005; Yang et al., 2014; Shin et al., 2015; Shanmugam et al., 2015; Taverna et al., 2016; Kim et al., 2018; Kurita et al., 2018; Luo et al., 2019; Icli et al., 2019; Henn et al., 2019; Icli et al., 2019; Böttger et al., 2020; Wang et al., 2020; Sorkin et al., 2020; Ashrafi et al., 2020; Loretelli et al., 2020; Gudjonsson et al., 2020; Icli et al., 2020; He et al., 2020.	19
Stem Cell	Gong et al., 2014; Quan et al., 2016; Lough et al., 2016; Allori et al., 2016; Guneta et al., 2016; Lopez et al., 2017; Yu et al., 2018; Bi et al., 2019; Myneni et al., 2019; Xu et al., 2019; Hu et al., 2019; Conti et al., 2020; Zhang et al., 2020; Taha et al., 2020; Tirza et al., 2020; Pepin et al., 2020; Xiong et al., 2020.	17
BR	Geers et al., 2018; Atkins et al., 2019; Orr et al., 2019; Krucoff et al., 2019; Glener et al., 2019; Orr et al., 2019; Shammas et al., 2019; Sergesketter et al., 2019; Sharif-Askary et al., 2019; Riggio et al., 2019; Sergesketter et al., 2019; Shammas et al., 2020; Anolik et al., 2020; Nakhlis et al., 2020; Cason et al., 2020; Sharif-Askary et al., 2020.	16
Burn	Pollins et al., 2007; Zeng et al., 2009; Zhang et al., 2010; Zhang et al., 2010; Liang et al., 2012; Giri et al., 2015; Wu et al., 2018; Lin et al., 2018; Tang et al., 2018; Zhang et al., 2018; Cao et al., 2019; Jiang et al., 2020; Wu et al., 2020; Li et al., 2020; Qi et al., 2020.	15
TX	Kienzl-Wagner & Brandacher, 2014; Kuo et al., 2014; Wolfram et al., 2014; Wolfram et al., 2015; Win et al., 2017; Noyan et al., 2017; Zhang, 2017; Kollar et al., 2018; Shubin et al., 2019; Hautz et al., 2020; Gok et al., 2020.	11
Aesthetics	Kaur et al., 2018; Xiong et al., 2019; Suresh et al., 2019; Thomas et al., 2019; Borsting et al., 2020; Gnad et al., 2020; Kaur et al., 2020; Ma et al., 2020; Al-Hadidi et al., 2020.	9
Skin Biology	Zöllner et al., 2012; Yan et al., 2013; Warshauer et al., 2015; Byrd et al., 2019; Dyring-Andersen et al., 2020; Lin et al., 2020; Wang, Liu&Xu, 2020.	7
VT	Cetinkaya et al., 2016; Li et al., 2017; Fu et al., 2017; Li et al., 2018; Li et al., 2018; Rodriguez-Laguna et al., 2018; Horbach et al., 2020.	7
Hand Surgery	Jiang et al., 2011; Weiss et al., 2016; Baas et al., 2017; Siegel et al., 2018; Potuijt et al., 2019; Major et al., 2019.	6

Tag	Papers	Count
Microtia	Wei, 2015; Lei et al., 2017; Zhang et al., 2017; Chen&Zhang, 2019; Xin et al., 2019; Guo et al., 2020.	6
Review	Cole&Isik, 2002; Pleat, Dunkin&Zitzmann, 2003; Semple et al., 2005; Ascha, Ascha &Gatherwright, 2019; Putra et al., 2019; Potuijt et al., 2019.	6
Weight Loss	Excluded	3
DU	Excluded	3
Microsurgery	Excluded	3
Trauma	Excluded	2
Tissue Comparison	Excluded	2
PRP	Excluded	2
I/R	Excluded	1
Trigeminal Neuralgia	Excluded	1
Total		255