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CONTENTS

CHAPTER 1

ULTRA-PROCESSED FOODS AND HEALTH

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Introduction

Diverse techniques of food preservation, preparation, cooking, and processing have been a central part of human evolution and influenced the development of populations and civilizations (Hotz & Gibson, 2007; Wrangham, 2013). This trend is accompanied by the development of systems that allow fresh foods to be consumed for a more extended period using food processing methods such as salting, drying and smoking. In the second phase, the industrial revolution that took place in the late 18th century influenced food processing through numerous innovations and developments. Steam engines and machinery spread throughout countries, which greatly facilitated transportation and removed trade barriers, and influenced the price and availability of flour and spices as well as food ingredients such as sugars, oils, and salt, which were previously available only in certain regions (Zucker & Giedion, 1949). In parallel with these developments, the properties of certain micronutrients and macronutrients were discovered in addition to the advancements in scientific disciplines such as chemical and mechanical engineering, and the first effort was made in establishing nutrition as a biochemical discipline (Hotz & Gibson, 2007).

In summary, a whole new set of industrial food technologies has led to the development of new, mass-produced industrial food products that are in many ways the prototypes of the foods we still consume today. Advancements in these disciplines have led to changes in food processing and storage systems, enabling the production and consumption of a number of new types of processed foods based on innovative production systems, such as confectionery, buns and cakes, soft drinks, condensed milk, breakfast cereals, and industrial breads.

Increasing the quality characteristics, i.e., nutritional value, color, consistency, smell, and crispness, during the processing, storage, and transportation processes and making use of the food processing methods and additives in that direction ensure food safety and preservation of the nutritional value and quality of the foods and significantly reduce food waste by preventing deterioration. Food processing has positive effects on the shelf life, transportation, and variety of foods but also has adverse effects on consumer health. In recent years, there has been an increasing interest in studying the relationship between the consumption of highly processed foods, which are considered unhealthy, and their impact on health. The concept of processed food, which is more and more referred to as unhealthy food, is increasingly recognized by its negative effect on diet quality and as a risk factor for diet-related diseases, disorders, and conditions (Silva Meneguelli et al., 2020; Carlos A. Monteiro & Cannon, 2019).

Classification of Processed Foods

Processed foods are defined as industrial compositions produced using compounds derived or synthesized from food or food substrates and are characterized by the NOVA classification (Aguayo-Patrón & Calderón de la Barca, 2017; Cunha et al., 2018). NOVA is a food classification system first proposed in 2009 by Monteiro et al. from the University of Sao Paulo Center for Epidemiological Health and Nutrition Research in Brasil. In NOVA, which was later approved by the United Nations and the World Health Organization, foods are subjected to a detailed classification based on the nature, scope, and causes of food processing (C. A. Monteiro et al., 2013; J. C. Moubarac & Levy, 2017). Monteiro et al. argued that the effect of processed foods on health had been overlooked in spheres of both research and government policies. Accordingly, they suggested classifying foods into three groups. Monteiro et al.'s publication is considered a precursor to the NOVA classification; however, subsequent publications have further developed the definitions and expanded the classification scheme by dividing the third group into two different groups of "processed" and "ultra-processed" foods (J. C. Moubarac et al., 2014). In parallel, in current practice, foods are divided into four groups based on the NOVA system according to the nature, scope, and objective of the industrial food processes used to process these foods (J. C. Moubarac & Levy, 2017). The NOVA Food Classification System and the food groups created based on this system are given in Table 1.

Table 1. NOVA Food Classification System

Group 1: Unprocessed or Minimally Processed foods	**Group 2: Fats, Salt, and Sugar**	**Group 3: Processed Foods**	**Group 4: Ultra-Processed Foods**
Peanuts, Nuts, and Other Seeds Without Sugar or Salt	Butter	Tomato Concentrates, Pastes or Extract, With Sugar and/or Salt	Alcoholic Beverages (Distilled) Such as Whisky, Vodka, Rum, Gin, Et etc.
Mushrooms (Fresh And Dried) and Other Fungi/Algae	Lard	Meat or Fish (Salted, Dried, Smoked or Cured)	Margarines and Spreads
Herbs (Fresh And Dried) and Spices	Honey Extracted From Honeycombs	Fruits in Sugar Syrup (With/ Without Added Antioxidants)	Pastries, Cakes and Cake Mixes
Vegetables, Fruits, Potatoes, and other Roots and Tubers (Natural, Packaged/ Cut/ Chilled or Frozen)	Oils Made From Nuts, Seeds, and Fruits, To Include Soybeans, Corn, Olives, Sunflower or Oil Palm	Legumes (Bottled or Canned) or Vegetables Preserved In Salt (Brine) or Vinegar, or By Pickling	Infant Drinks & Formulas, And Meal Replacement Shakes (e.g., 'Slim Fast')
Bulk or Packaged Grains Such as Brown, White, Parboiled and Wholegrain Rice, Wheat Berry, or Corn Kernel	Types of Sugar (White, Brown and Other) and Molasses Obtained from Beet or Cane	Canned Fish, such as Tuna and Sardine (With or Without Added Preservatives)	Dairy Drinks, Including Chocolate Milk

Pasta (Fresh or Dried), Couscous, and Polenta Made From Water and The Grits/ Flakes/Flours Described Above	Syrup Extracted from Maple Trees	Freshly-Made (Unpackaged) Breads Made of Yeast, Wheat Flour, Salt, and Water	Sweetened and Flavored Yogurts, Including Fruit Yoghurts
Lentils, Chickpeas, Beans, and Other Legumes	Salt (Refined or Coarse), Mined or From Seawater	Fermented Alcoholic Beverages such as Alcoholic Cider, Wine, and Beer	Baked Products Made with Ingredients such as Hydrogenated
Dried Fruits	Coconut Fat	Nuts and Seed (Sugared or Salted)	Cola, Soda, and Other Carbonated Soft Drinks
Fresh, Chilled or Frozen Meat, Poultry, Fish and Seafood, Whole or in the Form of Fillets, Steaks, and Other Cuts	Starches Extracted From Corn and Other Plant	Coconut Fat	Chocolates, Candies, and Confectionery in General
Fresh or Pasteurized Milk; Yoghurt without Sugar	Food Combining any two of the above, such as 'Salted Butter'	Freshly-Made Cheeses	Ice Creams and Frozen Desserts
Tap, Spring and Mineral Water		Bacon	Pre-Prepared Pizza and Pasta Dishes
Eggs		Beef Jerky	
Fruit or Vegetable Juices (Pasteurized or Fresh) with No Added Sugar or Other Substances		Pastrami	Pre-Prepared (Packaged) Meat, Fish, and Vegetable
Tea, Herbal Infusions		Soujouk	Breakfast Cereals and Bars

In the NOVA , Group 1 foods comprise unprocessed or natural foods that are obtained directly from plant or animal tissues without being subjected to any physical and biochemical food processing. Group 2 foods comprise minimally processed foods obtained from nature or natural foods through processes such as pressing, grinding, crushing, refining, subjected to fundamental processes such as cleaning, removal of inedible or unwanted parts, drying, shredding, grinding, pasteurization,fermentation, freezing, cooling, but to which no liquid, fat, sugar, salt or other substances have been added, and used for seasoning and cooking food in homes and restaurants, thereby creating various and delicious dishes of all kinds, including broths, salads, soups, desserts, pies, breads, cakes, and jams. Group 3 foods comprise processed foods produced via industrial processes using oil, salt, sugar, or other substances directly derived from food. Group 4 foods comprise UPFs, which are referred to as industrial formulation products derived entirely or predominantly from food-derived substances (proteins, sugar, fats, and starch) and food ingredients (hydrogenated oils and modified starches) (J. C. Moubarac et al., 2014; Carlos A. Monteiro & Cannon, 2019).

Ultra-processed food consumption is in high demand due to many factors such as convenience, low prices, almost unlimited availability, and effective marketing techniques. These foods are produced in factories and

are mostly sold as "packaged food," "ready-to-eat food," as ubiquitous food. UPFs are subjected to multiple processes and modifications before they are consumed and contain significant amounts of additional sugar, salt, saturated fat, and additives per product (Bleiweiss-Sande et al., 2019).

This book chapter focuses on the 4th NOVA group entitled "ultra-processed foods", which are typically defined as industrial coompositions of more than five ingredients. The most well-known examples of UPFs are industrially produced ice cream, confectionery, desserts, biscuits, and cookies; carbonated drinks and sugary drinks; prepackaged noodles and soups; packaged sweet or savory snacks; sweetened milk and fruit drinks; energy drinks; ready-to-eat meals; and sausages, hamburgers, hot dogs, meatballs, other processed meat products with added preservatives (such as nitrites) other than salt. UPFs are made predominantly from traditional culinary ingredients such as oils, oils, and sugars together with unnatural ingredients such as hydrogenated or intermediate esterified oils, soy protein isolate, hydrolyzed proteins, invert sugar, high fructose corn syrup, and maltodextrin, with the addition of color stabilizers, food colors, flavor enhancers, flavors, or sugar-free sweeteners (J. C. Moubarac & Levy, 2017).

UPFs can cause significant health problems such as excessive weight gain and obesity, asthma, wheezing, cardiovascular diseases, Type-2 diabetes, cancer, depression, irritable bowel syndrome in children, and indigestion and fragility (fatigue, muscle weakness) in adults. Further studies are needed on reducing the growing consumer trend towards ready-to-eat processed foods and bringing to the fore the traditional dietary habits based on minimally processed foods and freshly prepared meals to improve consumer health.

Ultra-Processed Foods and Their Effects on Health

Global Changes in Eating Habits

Generally speaking, many food processing methods are beneficial and not a public health concern. Food has been processed in various ways since the fire, salt, dry air, and storage methods such as fermentation and smoking started to be used to prepare and cook food. Food processing has led to increased human evolution and adaptation to settled life (Wrangham, 2013). UPFs, on the other hand, are products with a characteristically high energy density, high fat, sugar, and salt content, extremely tasty, inexpensive, attractive, ready-to-eat, and generally obesogenic, which were increasingly consumed first in high-income countries before and today also in the middle- and low-income countries (C. A. Monteiro et al., 2013).

Food processing is an integral part of industrialization; thus, its nature, scope, and objective have changed in line with the new trends in the industry (Wrangham, 2013). The development of mechanization since the middle of the 19th century has led to more effective and efficient formulations and an increase in mass production, which in turn led to an increase in the distribution and sales of food products such as bread, pies, biscuits, sauces and meat products. These developments have led to a decline in food shortages and food insecurity, which were then the main food-related public health problems globally, including in industrialized countries, until the early 20th century. However, with the increasing consumption of sugary, fatty, and animal foods by the end of the 20th century, cardiovascular diseases began to increase, first in high-income countries followed by the whole world (Popkin, 1993).

Urbanization trends in the societies, the increases in income levels, and the ratio of working women make ready-to-heat and ready-to-eat food products suitable and attractive options. However, the fact that an increase in the consumption of ultra-processed products has been observed not only in high-, but also in low- and middle-income countries indicate that the main reason underlying the increase in the consumption of ultra-processed products is also social as well as economic (C. A. Monteiro et al., 2013).

It is crucial that the effects of industrial processing of foods on diet patterns, including eating and drinking environments, are not overlooked while investigating the relationship between the rapid increase in obesity and obesity-related diseases globally and the global food system (C. A. Monteiro et al., 2013). The replacement of meal ingredients and food with ultra-processed ready-to-eat products is transforming food culture and nutrition patterns, replacing established dietary patterns that are more socially and environmentally appropriate at a global level (Stuckler & Nestle, 2012). Compared to unprocessed or minimally processed foods, UPFs have higher energy density and glycemic loads, harmful dietary fats, simple sugars, and sodium. They may also contain less dietary fiber, micronutrients, and phytochemicals (Monteiro et al., 2011). Taking also into consideration that they are combined with materials such as oil, flour, sugar, and salt during meal preparation, it is necessary to pay utmost attention to UPFs in terms of their adverse effects on public health (J. C. Moubarac et al., 2013).

Noncommunicable Disease Risk

There is increasing evidence that greater consumption of UPFs is associated with an increased risk of obesity and non-communicable diseases (Adams et al., 2020). Consumption of UPFs causes an increase in body mass index, waist circumference, body fat percentage, visceral fat,

and metabolic syndrome in adults (Silva et al., 2018; Fernanda Rauber et al., 2020; Juul et al., 2018; Tavares et al., 2012), and higher waist circumference and dyslipidemia in children (Costa et al., 2019; F. Rauber et al., 2015). The nutrient profiles of UPFs are thought to have detrimental effects on health (Adams et al., 2020). In parallel, the term "empty calories" is used for UPFs because of their high energy and low nutrient density (Fardet & Rock, 2019). These products are energy-dense and have higher free sugar, saturated fats, and sodium as compared to less processed alternatives (Adams et al., 2020).

The Food and Agriculture Organization of the United Nations (FAO) has reported that there is an essential and direct dose-response relationship between excessive intake of UPFs and the levels of free/additional sugar, fats (saturated, and trans), and sodium in the diet, which may lead to adverse health effects. In addition, a significant and inverse dose-response relationship has been reported between protein, fiber, potassium, phytoestrogens, and micronutrients, which are thought to have a protective effect on non-communicable diseases, and the share of UPFs in the diet, concurrently with an increase in the risk of insufficient intake for potassium and fiber (Monteiro et al., 2019).

There is a relationship between excessive intake of added sugar and accelerated pathogenesis of vascular diseases and risk of cardiovascular mortality; high sodium intake and cardiovascular mortality and certain types of cancer, such as stomach cancer; low fiber intake and the risk of cardiovascular diseases and associated mortality, coronary artery disease and cancer (pancreatic and gastric cancer); excessive fat intake and increased risk of obesity resulting in increased cardiovascular and respiratory diseases and decreased levels of serotonin and dopamine, which play a role in regulating neurological reward circuits and mood (Lane et al., 2021).

In summary, UPFs increase the risk of various chronic diseases and related deaths due to their lower fiber and higher sugar, sodium, and trans and saturated fat contents compared to unprocessed or minimally processed foods (Poti et al., 2017).

Factors That Promote Overconsumption

Ultra-processed products do not pose a significant health risk when consumed in low amounts along with other healthy foods. Nevertheless, factors such as their intense flavors due to having higher fat, salt, sugar, and food additive contents, their ubiquitousness as well as aggressive marketing strategies such as offering discounted prices for super-large portions further promote the consumption of UPFs, resulting in the further replacement of fresh or minimally processed foods with UPFs (Monteiro et

al., 2011; Ludwig, 2011). In modern society, food consumption is not done to satisfy physiological hunger and replenish energy stores, but rather to enjoy and satisfy the desire to eat, serving a hedonic goal (Ahima, 2009).

UPFs can lead to lower satiety and fullness levels through multiple mechanisms of action. While satiety is associated with cessation of eating, fullness determines how long after the individual will have his/her next meal. Different satiety and fullness levels are associated with the carbohydrate, protein, and fat levels consumed. Proteins increase both satiety and fullness, whereas fat is thought to contribute the least to both (Morell & Fiszman, 2017; Tremblay & Bellisle, 2015). This is in line with the "protein leverage hypothesis," which asserts that food intake is regulated by protein (not energy) needs in humans (Gosby et al., 2014). It has been reported that excessive energy consumption through the consumption of carbohydrates and fats based on UPFs is sufficient to reach the absolute amount of protein needed and thus supports the protein leverage hypothesis (Martínez Steele et al., 2018).

Ultra-processed products affect endogenous satiety mechanisms and cause excessive energy consumption and thus obesity (Ludwig, 2011; Ahima, 2009). Sugar, salt, fat, and sugar taste perception are referred to as the culprits of modern foods that increase appetite, which cause excessive food intake and weight gain by affecting the neurons associated with reward mechanisms and conscious control of eating (Ahima, 2009). Eating behavior is regulated by many metabolic pathways that provide homeostasis together with neuropsychological factors such as neurotransmitters and hormones and hedonic systems. In short, eating systems are regulated by two different systems: homeostatic and hedonic systems. It is claimed that if nutrition were regulated only by homeostatic systems, then all people would have an ideal weight (Braet et al., 2008; Saper et al., 2002). Hedonic eating is an individual's irresistible desire for delicious foods and consuming these foods with great pleasure. Whether the energy and nutrient content of the consumed food is sufficient and balanced is not important for individuals that adopt a hedonic eating behavior; hence they prefer to consume foods that suit their palate and make them happy by giving pleasure (Lutter & Nestler, 2009).

Dopamine, a neurochemical released from nerve cells, is stored in synaptic vesicles located in the space between two nerve cells. When the stimulus reaches the nerve, the dopamines stored in the vesicles spread to the synaptic space. Dopamine binds to dopaminergic receptors, initiating a series of reactions, and is subsequently withdrawn. A feeling of pleasure occurs when dopamine is prevented from reuptake and remains in the synaptic space for more extended periods. As in other substance addictions, dopamine reabsorption is inhibited in food addiction, and the excess

dopamine remaining in the synaptic space creates a feeling of pleasure similar to the feeling felt by addicts. In the event of repeated consumption, the tolerance threshold increases, therefore requiring the consumption of a higher amount of substance/food in order to provide the same feeling (Öncü & Karakaya, 2013; Gearhardt et al., 2011). Consumption of foods high in fat and sugar is the type of food consumption behavior that aims to increase the release of opioids and dopamine (Serin & Şanlıer, 2018). Excessive consumption of delicious foods in individuals with low dopamine levels is an alternative metabolic pathway to increase dopamine activation. Individuals with high reward sensitivity prefer to consume high-fat and high-sugar foods (Lutter & Nestler, 2009).

Tasty foods high in sugar and fat delay the response of the satiety signal, and insensitivity to satiety signals develops over time. After a decrease in serotonin levels, individuals may want to consume foods with high carbohydrate content in order to feel good (Rogers & Smit, 2000). Sugar is considered an addictive substance since it releases dopamine and opioids associated with neurochemicals related to the addiction and follows the typical addictive pathway: excessive consumption, deprivation, desire to eat, and cross-sensitivity. In the event of long-term consumption of junk food and fast food, dopamine suppresses the brain and leads to a decrease in the number of dopamine receptors. As a result, the need to consume more of these foods arises in order to achieve the same effect (Öncü & Karakaya, 2013).

UPFs are almost always available from any food outlet, many of which are open 24/7, mainly in the form of snacks, beverages, or ready-to-eat/to-be-heated items. They can be consumed anywhere, regardless of location, whether a restaurant, car, home, workplace, street, etc. They usually come in packages that can be easily transported and stored without the need for equipment such as plates. They are mostly consumed while focusing on another task such as working, driving, sitting in front of a computer or television, etc. All these factors make UPFs easy to access and increase their consumption (PAHO, 2015). It has been reported that high sugar, salt, and fat content in processed foods, changes made to the food textures to make it easier to eat faster, and implementation of special packaging and aggressive advertising strategies with a view to manipulating the brain response to consume more of the food may lead to conditioned overeating in some individuals as a result of exposure to an intense stimulus (Ahima, 2009). Large companies allocate huge annual budgets (around billions of dollars) for advertising and promotions, including cross-brands, to make their products more attractive. As with alcoholic beverages and tobacco products, marketing strategies often use seductive ideas, newspeak, and images that specifically target children, adolescents, and other vulnerable

groups, undermining the ability to make rational and healthy choices (PAHO, 2015).

Conclusion

UPFs are products of complex industrial processes involving the addition of fat, sugar, salt, and/or additives and are generally characterized by unfavorable nutritional composition. It is thought that they promote excessive food consumption by affecting satiety, fullness, and eating speed through properties such as food texture, energy density, flavor. Among possible behavioral determinants associated with the consumption of UPFs are cooking skills, knowledge level about nutrition, consumers' attitudes towards food additives/processing/packaging, convenience, ubiquitousness, cost, and marketing strategies. In order for individuals to be aware of the ingredients of foods and avoid the ingredients that act as stimulants for overeating, start associating the perception of eating processed foods with harmfulness instead of pleasure and comfort, and prevent food companies from taking advantage of individuals' biological weaknesses, the society needs to be educated about food ingredients and their potential effects on health.

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CHAPTER 2

SOME IMPORTANT BIOMARKERS AND CLINICAL SIGNIFICANCE

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Introduction

The term biomarker was first used in 1989 in the US National Academy of Sciences Report. This concept is accepted by the World Health Organization, the Organization for Security and Cooperation in Europe and the National Cancer Institute (Akay, 2004; Köse and Maden, 2013). Routine analyzes of biomarkers, which are in blood, body fluids or tissues, an indicator of a normal state or disease and a biological molecule, can be performed in certain body fluids (Köse and Maden, 2015). The definition of a biomarker has been defined as a biological molecule in blood, body fluids or tissues that indicates normal or abnormal states in the body (De Loor et al., 2013). Recently, biomarkers have been frequently used to reveal various diseases and pathological disorders (De Loor et al., 2013) and to evaluate the response to treatment (Aerts et al., 2005; Köse and Maden, 2013). Biomarkers provide important information in monitoring the disease process in humans and animals. Biomarkers used for this purpose have been classified in various ways as pharmacodynamic, diagnostic, prognostic and predictive. In human and veterinary studies, it has been stated that biomarkers have an important role in the diagnosis of diseases and inflammation (Moore et al., 2007; Kuru et al., 2015; Erkılıç et al., 2019; Kuru et al., 2020; Kuru at al., 2021, Sezer and Gökce, 2021; Akyüz et al., 2022). While some biomarkers provide important information in the diagnosis of the disease; while others provide important information about the course of the disease and inflammation (Aronson, 2005; Akyüz et al., 2021). Determining the relationship between external and internal dose, public health studies, determining individual sensitivity levels to chemical and biological substances, monitoring the effectiveness of treatment in the clinic, evaluating the diagnosis and prognosis of diseases are among the uses of biomarkers (De Loor et al., 2013).

Characteristics of Ideal Biomarkers

An ideal biomarker should be inexpensive, measured quickly and reproducibly. In addition, it should be reliable (Cox, 2005; Köse and Maden, 2013). It should be biochemically stable and work in small blood volumes. The sampling time should be large, and it should be determined by a simple and automated analysis method. The results should be comparable between laboratories and the threshold value should be well defined. Must be able to distinguish between bacterial or viral pathogen. Should be able to distinguish between sepsis and non-infectious systemic inflammatory response syndrome (SIRS). It must be associated with the inflammatory response and/or organ dysfunction. It should be able to demonstrate the effectiveness of prognosis and treatment (Dupuy et al., 2013; Köse and Maden, 2013).

Classification of Biomarker

Biomarkers are mainly divided into three important groups: a. Biomarkers induced by exposure. b. Effect-dependent biomarkers. c. Biomarkers of susceptibility/vulnerability (Timbrell, 1998; Akay, 2004; Anetor et al., 2008; Köse and Maden, 2013).

Exposure-Related Biomarkers

Biomarkers of biological exposure are based on the measurement of the chemical substance, its metabolites or reaction products resulting from interaction with the biological molecule in body fluids or tissues. For example; detection of substances such as transforming growth factor-β1, kidney injury molecule-1 in blood or urine, which show the damage caused by nephrotoxic drugs, can be given as examples of exposure biomarkers (Ichimura et al., 2004; Köse and Maden, 2013).

Influence-Related Biomarkers

A biomarker showing the efficacy of any chemical substance can be used in the identification of direct damage and evaluation of possible risks, determination of dose-response relationships, etc. can be used for occasions. Biomarkers in this group are examined in 8 subsections, including hematological, nephrotoxicity, tumor, hepatotoxicity, immunotoxicity, pulmonary toxicity, toxic effects on the reproductive system and development, and neurotoxicity (Anetor et al., 2008; Köse and Maden, 2013). 1-Hematological Biomarkers: Biomarkers in this group are used to monitor leukocyte, erythrocyte and thrombocyte levels in patients treated with cytotoxic drugs (Akay, 2004; Köse and Maden, 2013). 2-Nephrotoxicity Biomarkers: As biomarkers of kidney damage, serum BUN and creatinine, urine proteins, and urinary enzymes are used as biomarkers (Clemo, 1998; Maden and Aslan, 1999; Akay, 2004; De Loor, 2013). In addition, substances such as β2-globulin, cystatin-C low and high molecular weight proteins, albumin, transferrin, retinol binding globulin, rheumatoid factor, immunoglobulin G, matrix metalloproteinase-9 (Han et al., 2008) are also kidney biomarkers in the determination of renal damage. (Barratt and Topham, 2007; Köse and Maden, 2013). 3- Tumor Biomarkers: It is very important that an ideal tumor biomarker has high sensitivity and specificity, enabling early diagnosis of the tumor and initiating treatment applications when the tumor is still small or in asymptomatic patients (Üstüner et al., 2004; Köse and Maden, 2013). Tumor biomarkers are used in the diagnosis and monitoring of the treatment of the tumor, as well as in the evaluation of tumor volume and activity (Henry, 2010; Pang and Argyle, 2010). The Bence-Jones protein is the first tumor marker identified (Akay, 2004). Other biomarkers used in cancer studies include neopterin, osteopontin, nuclear matrix proteins, cancer antigens,

oncofetal proteins, enzymes, hormones, growth factors (Neumann et al., 2008; Köse and Maden, 2013). 4-Hepatotoxicity Biomarkers: The liver is the body's most important detoxification center and also an organ that is open to toxic attacks. Serum activities of enzymes such as alkaline phosphatase and γ-glutamyl transpeptidase are used as biomarkers of liver damage, especially associated with biliary excretion (Akay, 2004). In addition to these enzymes used, parameters such as collagenases, glycoprotein and polysaccharides, collagenases and inhibitors, cytokines are used as biomarkers in determining liver damage (Grigorescu, 2006; Köse and Maden, 2013). 5-Immunotoxicity Biomarkers: The immune system protects the organism against infectious agents and the formation of some neoplasms. Factors such as genetic factors, nutrition, stress, living standards and health status are effective on the immune system (Akay, 2004; Köse and Maden, 2013). Components of humoral immunity and elements of cellular immunity are used as biomarkers (Tesch, 2010). Changes in the levels of lymphocyte subgroups are used to evaluate the suppression of the immune system (Fournier et al., 2000; Köse and Maden, 2013). It is stated that immunoglobulins, Ig-A and Ig-M excreted in the urine can be used as markers that can determine kidney damage (Tesch, 2010). 6-Pulmonary Toxicity Biomarkers: Broncho-alveolar lavage fluid is currently used to detect lung damage, follow the progression of lung diseases and evaluate the effectiveness of the treatment (Maden et al., 2001; Aydın 2007). The most sensitive biomarker for inflammatory responses in the broncho-alveolar region is the neutrophil count in the broncho-alveolar lavage fluid (Akay, 2004). Biomarkers such as carcinoembryonic antigen, neuron-specific enolase, calcitonin, ferritin, sialic acid, and creatine kinase were used for diagnosis in lung cancer. However, when the findings obtained are examined, it has been stated that their use for diagnosis and screening purposes is not appropriate (Aydın, 2007; Köse and Maden, 2013). 7-Biomarkers of Effects on Reproduction and Development: Human chorionic gonadotropin in urine is a biomarker used for the risk of miscarriage in pregnancy. Studies have reported that urine relaxin concentration measurement is a sensitive and specific method for pregnancy diagnosis in both domestic cats and leopards (De Haas van Dorsser et al., 2006). 8-Biomarkers of Neurotoxicity: Biomarkers of neurotoxicity are used as indicators of many conditions ranging from neurophysiological changes or behavioral changes caused by the effects of chemical substances on the nervous system at the cellular or molecular level (Anetor et al., 2008). Biochemical and molecular events underlying neurotoxic effects can provide clues about the mechanisms of action of toxic stimuli (Rajdev and Sharp, 2000). Since acetylcholine esterase in erythrocytes is similar to that in the brain, it can be considered as a biomarker of neurotoxicity (Anetor et al., 2008; Köse and Maden, 2013).

Susceptibility Biomarkers

Susceptibility biomarkers are indicators that develop as a result of hereditary or environmental effects and reflect sensitivity to a special chemical or a group of chemicals with similar properties (Akay, 2004). Oxidizing molecules, which are released after the intake of certain drugs and foods, have a negative effect on hemoglobin and membrane proteins (Yıldız et al., 2010; Köse and Maden, 2013).

Clinical Uses of Biomarkers

It is emphasized that changes in the percentage of glutathione S-transferase enzyme can be used as a biomarker in the investigation of pesticide-induced toxicity (Otitoju and Onwurah, 2007). It has been reported that it can be used as a suitable biomarker for diagnosis by examining the changes in the urine protein profile of sick cattle related to the diagnosis of zoonotic bovine spongiform encephalopathy disease (Simon et al., 2008). In the research conducted for the early diagnosis of hypocalcemia developing after birth in cows, it is stated that there is a significant negative relationship between urine pH and serum calcium and phosphorus, and that urine pH measurements within 48 hours after birth can be used to evaluate the risk of hypocalcemia (Seifi et al., 2004). Biomarkers find a wide range of applications in clinical evaluations. With the new techniques developed in the field of biology over the years, it has been concluded that biomarkers will be extremely effective on issues such as diagnosis and treatment in clinical practice (Aronson, 2005). If we give some examples of clinical studies; In human and veterinary medicine related to cardiac disorders, serum creatine kinase, lactate dehydrogenase, aspartate amino transferase analyzes are used in the diagnosis of cardiac muscle damage (Sönmez and Ağaoğlu, 2010). The creatinine kinase enzyme is found in very high amounts in striated muscles. The increase in its activity in the serum indicates damage to the skeletal or cardiac muscle (Çakıroğlu et al., 2009). In addition, cardiac troponins have become an important diagnostic tool in veterinary medicine in recent years (Sugen and Güneş, 2008). It has been stated that troponin levels are high in dogs with dilated cardiomyopathy (Çakıroğlu et al., 2009), and lambs with white muscle disease, and it is a useful diagnostic tool in demonstrating heart damage (Sugen and Güneş, 2008). In a study conducted in cattle, üroplakin IIIb was shown to be a highly sensitive marker in the diagnosis of urothelial carcinomas of cattle (Roperto et al., 2005). It is emphasized that urinary TGF-β1 level is high in cats with chronic renal failure and it may be a useful clinical marker for chronic renal failure in cats (Arata et al., 2005). Obesity paves the way for many disorders such as decreased quality of life in pets, diabetes mellitus, neoplasia, orthopedic and respiratory diseases. It has been stated that adipokines can be used as a biomarker in obesity cases (German et al., 2010). In recent years, deaths due to sepsis have increased significantly

in both human and veterinary medicine. Early diagnosis and treatment are very important in the fight against sepsis. Therefore, diagnostic methods are needed. For this reason, the use of biomarkers has been emphasized. For this purpose, it has been reported in studies that biomarkers such as interleukins, high mobility group-box 1 protein, osteopontin, tumor necrosis factor were evaluated for this purpose (Pierrakos and Vincent, 2010).

Some Important Biomarkers Used

C-reactive protein

C-reactive protein (CRP) is an acute phase protein that was first discovered in the serum of patients with *S. pneumoniae pneumonia* by Tillet and Francis in 1930 and named as "C fraction protein" (Tillett and Francis, 1930; Akar and Köse, 2016). CRP is a protein belonging to the pentraxin family and consists of five identical polypeptide subunits, each containing 206 amino acids (Pepys and Hirschfield, 2003). CRP is synthesized in the liver under the control of cytokines such as tumor necrosis factor (TNF-α), interlockin (IL)-6 and IL-1β in case of tissue damage and inflammation (Simon et al., 2004, Kaya et al., 2016). Serum levels begin to rise 6-8 hours after the onset of inflammation, reach the highest value in approximately 48 hours, and the half-life varies between 4-9 hours (Clyne and Olshaker, 1999; Akar and Köse, 2016).

Procalcitonin

Procalcitonin is a prohormone consisting of 116 amino acids, a precursor to calcitonin. It is secreted from neuroendocrine cells in the lung and small intestine, especially thyroid gland C cells in the living body (Mehanic and Baljic, 2013; Akar and Köse, 2016; Akyüz and Gökce, 2021; Akyüz et al. 2022). Calcitonin is a hormone involved in calcium metabolism (Whicher et al., 2001). Procalcitonin has both a hormonal effect and has been named as a hormokine because it exhibits cytokine-like behaviors due to inflammatory stimulation. The inflammatory response is usually against microbial toxins. Sometimes this response occurs indirectly, through hormonal and cellular immunity, and tumor necrosis factor-α. In the case of sepsis, procalcitonin derives its source from liver, kidney, adipose tissue and muscle cells and is present in excess in the circulation (Lee, 2013). However, there is no increase in procalcitonin level in viral infections. Therefore, procalcitonin is very important in distinguishing between bacterial and viral infections (Foushee et al., 2012). Neutropenia, immunosuppression and drug use do not affect the procalcitonin level. An increased serum procalcitonin level indicates the severity of the infection and the inflammatory response. Procalcitonin levels halve within 24 hours after infection control (Mehanic and Baljic, 2013).

Neopterin

Structurally, neopterin is a pteridine derivative purine nucleotide synthesized by activated monocytes/macrophages as a result of interferon (IFN)-γ induction (Sönmezer and Tülek, 2015; Akyüz and Gökce, 2021; Akyüz et al. 2022). Neopterin, with a normal plasma level of 10 nmol/L, can be used as a clinical indicator of inflammation and especially gram-negative bacterial infection. It has a similar value with proclitonin in predicting mortality and prognosis in patients with bacterial infection and sepsis (Kozlowska-Murawska and Obuchowicz, 2008; Sönmezer and Tülek, 2015).

Natriuretic Peptides

Natriuretic peptides (NP) are peptides that have important roles in cardiovascular homeostasis and regulation of fluid volume. NP contribute to natriuresis and diuresis, at the same time they have vasodilator effects on vessels and have antimitogenic effects in cardiovascular tissues (Akar and Köse, 2016). NP levels increase in cases of cardiac dysfunction due to congestive heart failure, myocardial infarction, and septic shock (Brueckmann et al., 2005). In scientific studies, hemodynamic and hormonal changes that occur in the inflammatory process affect the cardiovascular system. Plasma BNP levels have a better diagnostic value than plasma ANP levels in terms of assessing cardiac damage in septic patients (Ogawa and de Bold, 2012).

Adenosine Deaminase

Adenosine deaminase (ADA) is especially found in high amounts in lymphoid tissues. It contributes to the differentiation and proliferation of lymphoid cells and maturation of the immune system. Therefore, it is considered a nonspecific biomarker of T-cell activation. It has been stated that ADA enzyme activity changes in diseases such as rheumatoid arthritis and tuberculosis as an indicator of cellular immunity. Since humoral and cellular immunity is affected in patients with ADA deficiency, immunodeficiency findings occur (Bahadır et al., 2011; Akar and Köse, 2016).

Nitric Oxide

Nitric oxide (NO) is associated with almost all systems in the body and its most important functions are the provision of vascular hemostasis, the regulation of neurotransmission and host defense (Mian et al., 2013; Akar and Köse, 2016). Nitric oxide is produced by many cells such as epithelial and vascular endothelial cells and takes part in intercellular interaction in case of inflammation. It is synthesized by NO synthase (NOS). This enzyme has 3 isoenzymes: neural (NOS1), inducible (iNOS, NOS2) and

endothelial (NOS3). These enzymes catalyze L-arginine to L-citrulline and NO in various tissues (Hahn, 2013; Akar and Köse, 2016, Kuru et al., 2018).

Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE) is an enzyme synthesized by endothelial cells and plays a role in the regulation of blood pressure. It catalyzes the conversion of angiotensin I, an inactive protein, to angiotensin II. The angiotensin converting enzyme is found in all endothelial cells in the body (especially in high amounts in the lungs). The source of ACE activity measured in blood is the lungs (Baughman et al., 2011; Akar and Köse, 2016).

Lipopolysaccharide Binding Protein

Lipopolysaccharide binding protein (LBP) is an acute phase protein synthesized from the liver after immune response to endotoxins. LPS binding protein has the feature of increasing the effects of LPS at low concentrations and inhibiting the effects of LPS at high concentrations (Jerala, 2007; Sönmezer and Tülek, 2015). Studies have found that patients with febrile neutropenia have 100% sensitivity and specificity, with a threshold value of 46.3 mg/L, in estimating gram-negative bacteremias (Oude Nijhuis et al., 2003; Sönmezer and Tülek, 2015).

Pentraxin-3

Pentraxin-3 is an acute phase protein similar in structure and function to C-reactive protein. Pentraxin-3 is released from macrophages, dendritic cells, leukocytes and endothelial cells during the inflammatory response. In studies conducted with patients with pneumonia, a positive correlation was found between the severity of the disease and the length of hospital stay and plasma pentraxin-3 level (Kao et al., 2013; Sönmezer and Tülek, 2015).

Adrenomedullin

Adrenomedullin (ADM) is a peptide vasodilator released from many tissues such as adrenal medulla, lungs, central nervous system, vascular smooth muscle cells. Adrenomedullin inhibits the release of proinflammatory cytokines, leads to an increase in the activation of the complement system, decreases reactive oxygen release and limits the formation of inflammatory exudate (Struck et al., 2004; Sönmezer and Tülek, 2015). ADM level increases in lipopolysaccharides, cytokines, hypoxia, oxidative stress and inflammatory conditions (Garayoa et al., 2000; Fahmey et al., 2018; Ghobrial et al., 2020).

Endothelin-1

Endothelin-1 (ET-1) is the most potent vasoconstrictor released by endothelial cells. Prepro-ET-1, which is a precursor protein in endotoxemias, is especially released from the heart and lungs. Therefore, its level increases in heart and lung diseases (Schuetz et al., 2011; Sönmezer and Tülek, 2015).

Lactate

It increases in parallel with the development of anaerobic metabolism in cases where tissue perfusion is impaired due to circulatory disorders such as lactate acidosis and septic shock. The inability to metabolize lactate by the liver as a result of impaired liver perfusion also increases lactate levels (Sönmezer and Tülek, 2015). In a study, it was concluded that cerebrospinal fluid lactate measurement can be helpful biomarkers in the prediction of prognosis and mortality in distinguishing bacterial and viral meningitis by measuring cerebrospinal fluid lactate levels (Viallon et al., 2011; Sönmezer and Tülek, 2015).

New Future Biomarkers: Micro-RNAs

Micro-RNAs (miRNAs) are small biological molecules that have been discovered recently, consisting of about 20 nucleotide sequences released from eukaryotic cells, especially liver, lung and kidney, and are responsible for posttranscriptional regulation (Dupuy et al., 2013). In the case of dysfunction of these miRNAs, which play a role in both positive and negative regulation of gene expression, various cancers, cardiomyopathy and central nervous system diseases can be seen. In addition, these molecules play a role in the defense mechanism against viruses such as Epstein-Barr virus, cytomegalovirus, herpes simplex virus and hepatitis C virus, and infections due to Mycobacterium tuberculosis (Vigorito et al., 2013; Sönmezer and Tülek, 2015).

As a result, biomarkers have an important place in the diagnosis of diseases. Day by day, its importance has begun to be understood and new biomarkers have begun to gain clinical importance. It provides useful information in the early diagnosis of diseases and provides important information about the severity of diseases. I believe that biomarkers will find much more use in the future.

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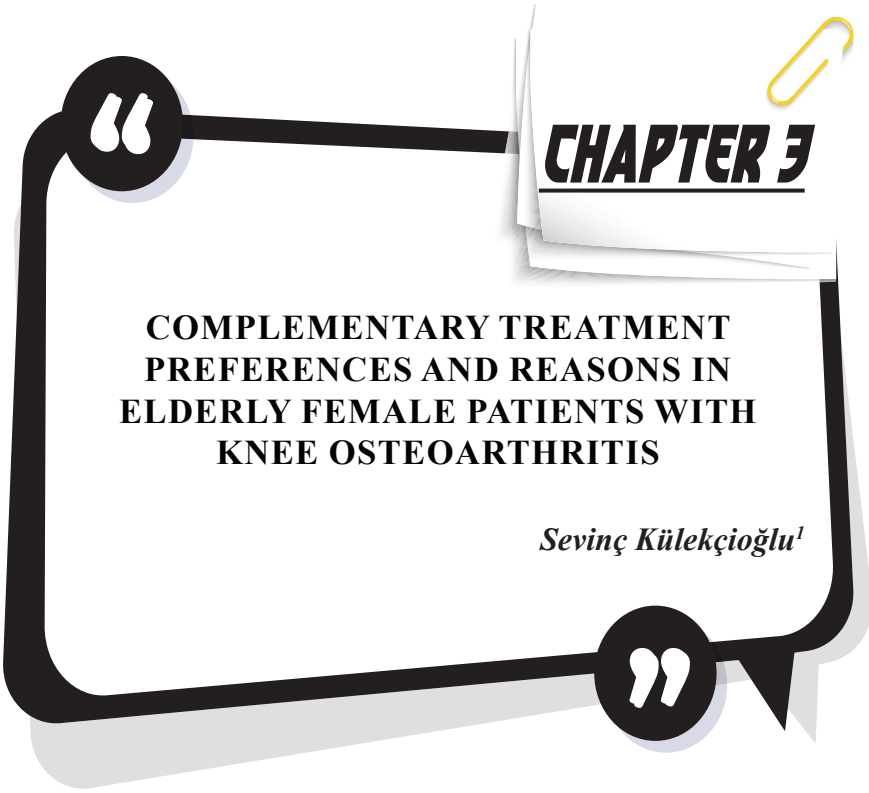

CHAPTER 3

COMPLEMENTARY TREATMENT PREFERENCES AND REASONS IN ELDERLY FEMALE PATIENTS WITH KNEE OSTEOARTHRITIS

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INTRODUCTION

Traditionally, osteoarthritis (OA) is considered the progressive wear and tear of articular cartilage. However, recent evidence suggests that this is an inflammatory disease of the entire synovial joint, involving not only mechanical degeneration of the articular cartilage, but also structural and functional alteration of the entire joint, including the synovium, meniscus (in the knee), periarticular ligament, and subchondral bone (Kan, et al.,2019). Synovial inflammation, abnormal bone structure, and bone marrow lesions are common in knee osteoarthritis (KOA) patients, and this may underlie persistent knee pain (O'Neill, et al., 2018). KOA ranks 11th among global contributors to a disability, and some of its secondary diseases lead to obesity, cardiovascular disease, and higher mortality rates (Cleveland, et al., 2019).

In Turkey, the population aged 65 and over, which is considered advanced age, has increased by 24% in the last five years and reached 8 million 245 thousand 124 people in 2021. According to the data of the Statistical Institute, the proportion of the elderly population in the total population increased to 9,7% whereas 55,7% of this rate is female. OA, being the third highest disability risk factor in seniors, may cause pain, loss of function, and impaired quality of life (Hawker, 2019; Cross et al., 2014). Besides, KOA patients constitute more than 80% of OA patients (Vos et al., 2012). KOA is the most common musculoskeletal system disease that affects almost 4% of the world population, whereas it is the main reason for lower extremity disabilities in seniors (Vos et al., 2012). Females constitute a higher percentage of OA patients (Lee & Kim, 2017), and the female gender is a risk factor that increases the possibility of developing OA (Park & Jung, 2017). A cross-sectional study that was carried out with 9512 participants aged 50 and over shows that the radiographic KOA prevalence is 43.8% in females and 21.1% in males (Vina & Kwoh, 2018). Therefore, effective treatment and prevention of OA in females are important (O'Connor, 2006; Srikanth et al., 2005). In addition, females usually suffer from more pain and more function loss than males, and this may lead to sarcopenia (Glass et al., 2014).

The latest guideline published in the USA in 2020 contains 6 key recommendations for the non-surgical treatment of KOA, being diagnosis, self-management, physical therapy, pharmacotherapy, orthobiology, and complementary and integrative health (Krishnamurthy et al., 2021) (table 1). Pharmacological treatments have been most widely adopted. However, the potential side effects of pharmacological treatment (digestive problems, heart failure, and renal impairment) have limited their use. Some comorbidities may lead to increased side effects as a result of drug interactions in the senior population. In addition, the dissatisfaction with conventional treatments due to medical, psychological, social, and

economic costs leads patients and physicians to seek alternative methods. WHO has reported that 88% of 170 member states use complementary therapy (CT) (WHO, 2019). Evidence-based assessment is often difficult due to the poor methodology of the studies in this area; however, these treatments are considered natural and accepted by patients and physicians. The fact that they are easier to access, less costly, and methods that have been known for a long time increase their usage rates. Various complementary therapies are being used in KOA to relieve pain, improve physical function, and minimize or slow down the progression of the disease.

The aim of this study is to investigate CT usage patterns, experiences, and preferences in elderly female patients with KOA.

PATIENTS and METHODS

This prospective cross-sectional study was performed in Physical Medicine, and Rehabilitation clinic between 01/06/2021-01/11/2021 with the approval of the ethics committee obtained from Bursa Yüksek İhtisas Training and Research Hospital. All procedures were performed according to the Principles of the World Medical Association Declaration of Helsinki.

The criteria for inclusion were:

1.being over the age of 65 and female,

2.having unilateral or bilateral, grade 2-4 KOA according to the Kellgren-Lawrence (KL) rating scale. Numerous variations of the KL classification system have been used in research (Kellgren & Lawrence, 1961). Below is the original description:

grade 0 (none): definite absence of x-ray changes of osteoarthritis

grade 1 (doubtful): doubtful joint space narrowing and possible osteophytic lipping

grade 2 (minimal): definite osteophytes and possible joint space narrowing

grade 3 (moderate): moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and deformity of bone ends

grade 4 (severe): large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone ends

3.having knee pain for at least 6 months and being followed up for 3 months,

4.experiencing knee pain most days in the previous month,

5.having an average daily pain higher than 3 points in the last week (based on the 0-10 point Visual Analog Scale (VAS),

6.being treated with at least one CT (acupuncture, ozone, mesotherapy, prolotherapy, hirudotherapy, reflexology, phytotherapy (lavender oil, fish oil, almond oil, chamomile oil, ginger oil, curcuma, harpagophytum, and ginger, etc.), supportive medication (animal origin glucosamine sulfate, chondroitin sulfate) and collagen), balneotherapy/spa and cupping therapy) the method within the last 2 years for pain or function loss in the knees.

The criteria for exclusion were:

1.severe systemic disease,

2.malignant disease,

3.acute infection,

4.psychiatric and neurological disease that makes communication difficult,

5.knee joint replacement,

6.acute meniscus and ligament injury.

The study was introduced to 148 patients who met the required criteria, and informed consent forms were obtained from patients who agreed to participate. The data of the patients were collected through a face-to-face interview technique by using a questionnaire form. The questions about sociodemographic attributes (age, marital status, education level, whether the participant lives alone), chronic disease status, drug usage (how many types), CT treatments (which they preferred in the first place and benefited from in the first place), the rate of gaining benefit from these treatments, the person who recommended and performed them for the first time, the reasons for preferring these treatments and the time of preference (before or after conventional treatments?) were included in the questionnaire form. VAS was the first parameter used to determine the level of gaining benefit. VAS; A common, simple method with a well-established validity and reliability. The physician was asked to mark the severity of pain before and after the treatment by explaining what each of the numbers means on a 10 cm line (0=no pain, 5=moderate pain, 10=very severe pain). VAS scores were recorded before and after treatment. In the second method to determine the level of gaining benefit from treatments; The patients were asked to mark one of the options: I benefited a lot, I benefited, I benefited a little, I did not benefit at all, I got harmed.

Statistical Analysis

Data analysis was performed with SPSS 26.0 software and the confidence level was 95% frequency (n) and percentage (%) statistics were provided for categorical (qualitative) variables, while average (X), standard deviation (ss), minimum and maximum statistics were provided for numerical (quantitative) variables.

RESULTS

148 female patients with KOA over the age of 65 had previously been treated with at least one CT method. The average age was 71.30 years and the average number of drugs they used daily was five. 84 patients were using NSAIDs every day. The distribution of the characteristics of the patients is shown in Table 2. The average number of CTs applied to date is approximately 2, whereas it has been determined that the most frequently used method is phytotherapy. The distribution of the most preferred and most beneficial treatments is shown in Table 3. The people who recommended and applied the treatments, reasons for preference, and times are shown in Table 4. 22,97% of the people who recommended them and 28,37% of the people who applied them were physicians. Reasons for referral to CT before conventional treatment are shown in table 5. Changes in VAS values were found significant before and after the treatment methods from which the patients gained benefit the most (Table 6). Levels of gaining benefits from CT are shown in Table 7. When it is asked whether they would get the patients to have it done if they could reach them, 89,3% answered yes.

Table 1. Full List of Recommendations

-Diagnosis

-Self- management: Exercise, weight loss and bracing

-Physical therapy

-Pharmacotherapy: a.Topical pharmacotherapy

b.Oral pharmacotherapy (acetaminophen and/or oral nonsteroidal anti-inflammatory drugs), duloxetine, opioids (including tramadol)

c.Intra-articular injections

1.intra-articular corticosteroid injection

2. intra-articular Visco supplementation injection

-Orthobiologics: a. platelet-rich plasma injections

b. stem cell injections (mesenchymal adipose-derived, and bone marrow-derived)

-Complementary and Integrative Health: a.Dietary Supplements, and Nutraceuticals: Avocado and soybean extract, Boswellia serrata, Cannabidiol, Chondroitin, Curcumin, Collagen, Glucosamine, Glucosamine plus chondroitin, Methylsulfonylmethane, Omega-3 fatty acid, Pycnogenol, Rosehip, Traditional Chinese medicine, Vitamin D, Vitamin E, Willow bark extract.

b.Acupuncture

c.Massage

d.Light touch

e.Meditation

f.Tai chi

g.Yoga

Table 2. *Characteristics of Patients*

Characteristics	**n (%) /148**
Marital Status	
Married	67(45,27)
Single	81(54,72)
Education	
Primary	97(65,54)
High School	36(24,32)
University	15(10,13)
Living Alone	
Yes	25(16,89)
No	123(83,10)
Chronic Disease	
Diabetes Mellitus	55(%37,16)
Cardiac Disease	100(67,56)

Table 3. *Distribution of the Most Preferred and Most Effective CT Applications*

	n* (%) /148	n** (%) / 148	n***(%) /n*
Phytotherapy	100 (67,56)	5(3,37)	5(5) /100
Balneotherapy	71(47,97)	20(13,51)	22(30,98) /71
Supportive Medication	66 (44,59)	6(4,05)	4(6,06) /66
Cupping/Bloodletting	58 (39,18)	14(9,45)	14(24,13) /58
Mesotherapy	34 (22,97)	16(10,81)	16(47,05) /34
Acupuncture	31 (20,94)	15(10,13)	15(48,38) /31
Prolotherapy	27 (18,24)	10(6,75)	10(37,03) /27
Hirudotherapy	24 (16,21)	4(2,70)	4(16,6) /24
Ozone	18 (12,16)	6(4,05)	6(33,33) /18
Massage/ reflexology	12 (8,1)	2(1,35)	2(16,66) /12

n*: Distribution of most preferred applications

n**: Distribution of most effective applications

n***: The distribution of the most benefitted application in themselves

Table 4. *Recommenders, practitioners, reasons for preference and timing of CTs*

Suggested By	
Physician	34 (22,97)
Neighbor/relative/friend	69 (46,62)
TV/social media	45 (30,40)
Applied By	
Medical Practitioner	42(28,37)
Non-Physician Health Personnel	25(16,89)
Other	81(54,72)
Time of Resorting to CT	
Before conventional treatment	33(22,29)
After conventional treatment	115(77,70)

Table 5. *Reasons for Preferring CT before Conventional Treatment*

Reasons for Preferring CT	**n (%) / 33**
More effective	3(9,09)
Safer	12(36,36)
Less costly	6(18,18)
Easier to access	12(36,36)

Table 6. *VAS Measurements*

	Max-Min	**Mean±sd**
VAS before Treatment	9-3	6,19±1,06
VAS after Treatment	6-0	4,19±1,3

Table 7. *Level of gaining benefit from CT*

	n (%) /148
I benefited a lot	21(14,18)
I benefited	34(22,97)
I benefited a little	63(42,56)
I did not benefit at all	27(18,24)
I got harmed	3(2,02)

DISCUSSION

This study is aimed to investigate the CT usage patterns, experiences, and preferences in elderly female patients with KOA. 148 female patients with an average age of 71,3 had previously been treated with an average of 2 CT methods. The average number of drugs they used per day was 5, whereas 84 patients were using NSAIDs every day. The first three most frequently used CT methods were phytotherapy, balneotherapy, and

dietary supplements, whereas the most beneficial CTs were, respectively, acupuncture, mesotherapy, and prolotherapy. Less than one-third of patients had been encouraged for CT and treated by a physician.

Even though there are several treatments available to manage KOA, there is no proven cure, and many patients complain of unrelenting pain and chronic disability. Due to the potential side effects of pharmacological and surgical treatments, the American College of Rheumatology reported that it supports the use of pharmacological and non-surgical treatments (Hochberg et al., 2012). 47% of the patients with KOA report that they use various CT methods (Lapane et al., 2012)**.** The leading reasons for preference can be listed as the fact that patients think that these treatments have fewer side effects, they do not want to use other drugs since they use too many drugs and each new drug may cause new side effects, CTs are cheaper and easily accessible, and sometimes the perception that these treatments are more effective.

Elderly patients are using more drugs over time due to various health problems. In a study conducted in Italy, it was found that all (95%) females aged 75 and overused at least one drug, whereas one-third of them used five or more drugs (Nobili et al., 1997). In a study conducted with a group of elderly people living in nursing homes in Turkey, 94.4% of females were found to use at least one drug (Esengen et al., 2000). In line with the literature, only 5 of 148 female patients were not using any medication in our study. 143 (96.62%) patients were using at least one drug. The average number of drugs used daily was five, whereas the rate of those using 5 or more drugs was 34%. Since the average life expectancy in females is longer than males, and chronic diseases and health complaints accompanying advanced age are more common, it can be expected that the use of multiple drugs in elderly females is higher than in males. In our study, nearly half of the patients had lost their spouses, and approximately 17% were living alone. Elderly people who had lost their spouses and/or living alone are more likely to have depression and sleep disturbances. The quality of life reduces in the elderly people living alone due to reasons such as advanced age, chronic diseases such as diabetes and hypertension, social isolation caused by lack of mobility, and depression, and the use of multiple drugs is also more common (Solmaz & Akın, 2009; Ayrancı et al., 2005). Similarly, in other studies conducted in Turkey, multiple drug use was found to be higher in females than in males (Esengen et al., 2000; Arslan et al., 2000). It is known that patients do not gain enough benefit from non-steroidal anti-inflammatory drugs (NSAIDs) that they use for a long time, and they experience problems especially related to the digestive system. Excessive use of these drugs may cause side effects such as gastrointestinal hemorrhage, increased liver enzymes, hypertension, and

kidney failure (Bruyère et al., 2014). It is thought that the patients cannot use NSAID for the aforementioned reasons and seek CT considering that they have fewer side effects.

According to the results of our study, 22% of the patients had recourse to CT before conventional methods, and this is thought to be associated with the idea that CTs are safe and easy to access. Phytotherapy and balneotherapy are the most frequently preferred methods. The fact that the first three most beneficial CTs (acupuncture, mesotherapy, prolotherapy) were not the first choice, may be associated with the fact that they are relatively expensive and more difficult to access. It is thought that this is another reason these methods have to be applied by certified experts. In the general CT questionnaire, approximately 80% of the patients stated that they gained benefit from the applied method. When it is considered that the CT provides a substantial benefit in this respect, it is thought that a need will arise in the following years for more researches to be carried out and for appropriate treatment protocols to be developed.

The interaction of drugs with herbal products is one of the least studied subjects, even though it is an expected and therefore undesired situation. Detailed clinical studies have not been conducted on plant-based products that can be provided over-the-counter or from herbalists. It is not known which active substances enter the systemic circulation at what rate in cases where these products are used, and moreover, it is not certainly known whether the contents of the products prepared by different companies and individuals are the same. Due to such reasons, it is difficult to define drug-herbal product interaction. However, in the light of the information obtained from the undesirable effects of herbal products that are widely used together with drugs, it is thought that the interaction potential is too high to be underestimated. In the light of the rational drug use principles; when it is considered that the use of multiple drugs in seniors is at a rate that cannot be underestimated, it would be a more accurate approach to use herbal products very carefully and under the control of a physician. WHO states that it will promote the integration, safe and effective use of CTs in health systems (WHO, 2019).

In countries such as China, Korea, the USA, Germany, Switzerland, Cuba, Japan, and Chile, 40-86% of the population resort to CT at least once a year (Biçer & Pınar Balçık, 2019). The methods used vary according to the geographical location of a country, ethnic origin, educational and socioeconomic factors, religious beliefs, lifestyles, and cultures. In a study, the rate of CT use in Turkey was found to be 60.5% (Şimşek et al., 2017). In another study, it was found that 48% of cases with OA had resorted to a CT method (Karadağ, 2012). Due to the continuous development of CT studies in recent years, significant results have been obtained in the treatment of

KOA (Zhang et al., 2019; Khan et al., 2016).

Similar to our study, it was determined that the most commonly used CT methods in studies conducted in Turkey were herbal treatments and nutritional supplements (Şimşek et al., 2017; Karadağ, 2012; Ulusoy et al., 2012; Dikici et al., 2015). The manners of access to the CT method used are truly diverse. In the study by Ulusoy et al., it was found that most of the patients were encouraged by their relatives or the mass media, while 13.6% suggested that they resorted to CT in line with the recommendations of the physicians (Ulusoy et al., 2015). Similarly, in the study by Karadag et al., the recommendation of family and social environment took the first place with a rate of 52.5% (Karadağ et al., 2012). In the study conducted by Dikici et al., approximately half of the patients resorted to CT methods under the influence of their family and friends, while 21.8% resorted to these methods upon the recommendation of a physician (Dikici et al., 2015). Similarly, in our study, the highest rate was upon the recommendation of neighbors/relatives/friends (46.62%), while the rate of patients who were treated upon the recommendation of a physician was 22.97%. Despite best practice recommendations, the integration of complementary therapies into KOA management may be controversial, and there is no international consensus on when or how this should be practiced. There is no direct recommendation for CT applications other than a few guidelines. This lack of appropriate clinical advice and information constitutes a challenge to physicians on how to best advise patients. Therefore, understanding the patterns of CT users offers health care professionals an opportunity to make more comprehensive treatment decisions and to improve relationships with patients. The rate of resorting to CT methods is increasing due to the facts such as the modern treatment methods being expensive and difficult to reach, fear of the side effects of modern treatments, people's desire for long and healthy life, getting rid of hopelessness, and the communication networks such as the media and the Internet becoming popular (Hochberg et al., 2012; Iversen et al., 2018). Therefore, it is thought that the use of CT must be taken into consideration in the examinations of patients with chronic pain.

In aging populations, the prevalence of COA is expected to increase; therefore, consensus on non-surgical OA management is needed to improve outcomes and reduce the burden of arthroplasty in patients with OA. Various CT programs for the non-surgical treatment of OA have been shown to be effective in improving pain, physical function, mobility, and quality of life. Long-term follow-up of these programs should be done to further evaluate their results.

Limitations of our study; The collection of information through questionnaires causes the reliability of the data to depend on the

respondent's memory accuracy and retrospective recall of pain levels. The difference in the use of phytotherapy products in the form of oral intake or local application has not been questioned. The beneficial ness of CTs was evaluated collectively, not individually.

Conclusion

In conclusion, aging is an inevitable process and KOA is a worthy cause of morbidity in geriatric patients. Developing strategies related to life and healthy aging in advanced age is important for society. Using CT treatments upon the suggestion of a physician in the safe and effective treatment of pain may help provide good pain control. The results of our study are important in terms of providing a ground for the use of CT methods in the treatment of KOA to reduce the side effects of polypharmacy in elderly female patients and to create an effective and safe treatment protocol. Clear and non-judgmental conversations between CT providers and patients must be encouraged to ensure that the patient's health care needs are covered.

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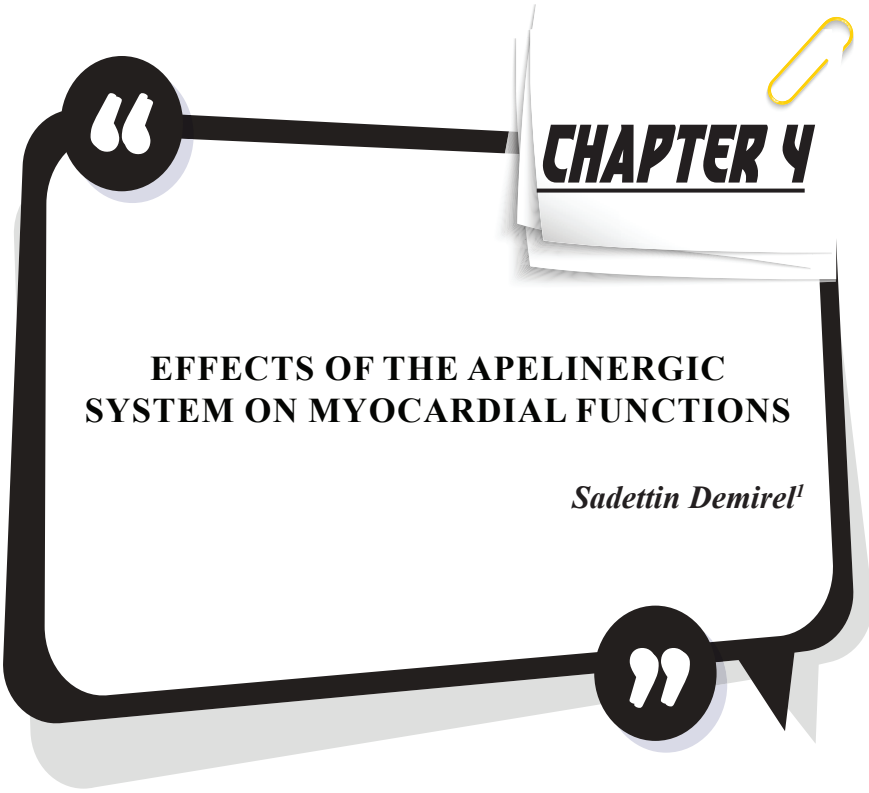

CHAPTER 4

EFFECTS OF THE APELINERGIC SYSTEM ON MYOCARDIAL FUNCTIONS

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Introduction

The apelinergic system consists of apelin receptor (APJ), apelin, and elabela (apela, toddler, ELA). APJ, which is a G protein-coupled receptor, acts through many different G protein subunits and provides activation of various signaling pathways. Apelin and elabela are peptide hormones and are the two endogenous ligands of APJ that have been identified so far. Apelinergic system components are highly conserved among species and are intensely expressed in many tissues. Apelin and elabela have important roles in many physiological processes such as cardiac and vascular contractility, arterial blood pressure, energy metabolism, body-fluid homeostasis, angiogenesis, neuroendocrine stress response, nutrient intake, gastrointestinal motility, and secretion regulation. It also participates in various pathological processes such as diabetes (Huang et al., 2019; Ishida et al., 2004; Japp et al., 2008; Lee et al., 2000; O'Carroll et al., 2003; Reaux et al., 2001; Shin et al., 2017; Szokodi et al., 2002; Taheri et al., 2002; Tatemoto et al., 2001; Wysocka et al., 2018; Zhang et al., 2016). The cardiovascular effects of apelin and elabela are the most remarkable and the most widely studied. It has been suggested that both have cardioprotective, vasodilator, hypotensive, and strong positive inotropic effects (Ishida et al., 2004; Japp et al., 2008; Lee et al., 2000; Sahinturk et al., 2021; Sahinturk, & Isbil, 2022; Shin et al., 2017; Szokodi et al., 2002; Tatemoto et al., 2001; Wysocka et al., 2018). Although the effects of apelin and elabela are similar, their potencies and mechanisms of action differ. Current studies have focused on developing an alternative treatment for diseases such as heart failure through the apelinergic system (Isbil et al., 2020; Sahinturk, & Isbil, 2020). For this purpose, long-acting and potent apelin and elabela analogs and APJ agonists are being developed. Therefore, it is very important to understand the positive inotropic effect of the apelinergic system and its mechanism of action.

APJ

APJ, a G protein-coupled receptor, was discovered in 1993 (O'Dowd et al., 1993). Since its ligand was unknown, it was previously described as an *orphan* receptor. This situation ended in 1998 with the discovery of the first endogenous APJ ligand apelin. The Aplnr gene encoding APJ is located on chromosome 11 in humans (O'Dowd et al., 1993). Consisting of 380 amino acids, the APJ has 7 transmembrane regions (Hosoya et al., 2000). The APJ shows significant sequence homology (31%) with the Ang-II type 1A (AT1A) receptor, with greater similarity in the transmembrane region (54%) (Xu et al., 2018). At the same time, the tissue distributions of these two receptors are quite similar. However, neither Ang-II binds to the APJ nor apelin to the AT1A receptor (Xu et al., 2018). APJ expression is widely observed in many different tissues. In the cardiovascular system,

APJ is extensively expressed in cardiomyocytes, vascular endothelial cells, and vascular smooth muscle cells (Kleinz, & Davenport, 2005). APJ exhibits its effects by activating various signaling pathways such as phosphatidylinositol 3-kinase (PI3K)/Akt/endothelial nitric oxide synthase (eNOS) and phospholipase C (PLC)/protein kinase C (PKC) via G proteins such as $Ga_{i/o}$, $Ga_{q/11}$, and $Ga_{12/13}$ (Zhang et al., 2018). As a result, effects such as suppression of cyclic adenosine monophosphate (cAMP) production, stimulation of extracellular signal-regulated kinase (ERK) phosphorylation, eNOS activation, and calcium mobilization occur (Zhang et al., 2018). It has been suggested that particularly the mitogen-activated protein kinase (MEK)/ERK pathway and phospholipase C activation are important in increasing myocardial contractility (Folino et al., 2015).

Apelin and Myocardial Contractility

Apelin, the first found endogenous APJ ligand, was isolated from bovine stomach extract in 1998 (Tatemoto et al., 1998). The Apln gene encoding apelin is located in the q25-26.1 band of the human X chromosome (Lee et al., 2000). Various apelin isoforms such as apelin-12, apelin-13, apelin-17, and apelin-36 are formed from preproapelin, which is a precursor protein and contains 77 amino acids. Posttranslational modification from apelin-13 produces pyroglutamyl-apelin-13 ([Pyr^1]apelin-13), which is more stable and resistant to enzymatic degradation (Zhang et al., 2018). Apelin fragments shorter than 12 amino acids are biologically inactive, as the 12 amino acid C-terminal is required for apelin to bind to the receptor. The N-terminal sequence regulates the interaction of apelin with the receptor (Folino et al., 2015). Apelin is highly expressed in the heart, brain, pancreas, lung, liver, kidney, placenta, and vascular endothelium (Xu et al., 2018; Zhang et al., 2018). There are differences between the apelin isoforms in terms of tissue distribution and potency. [Pyr^1]apelin-13 is the dominant isoform in human plasma and cardiovascular tissue, with a plasma concentration of 7.7-23.3 pg/mL (Folino et al., 2015; Mughal, & O'Rourke, 2018; Zhen et al., 2013). Apelin-13 and [Pyr^1]apelin-13 are the predominant and more potent apelin isoforms in the cardiovascular system (Folino et al., 2015; Tatemoto et al., 1998; Zhen et al., 2013). Apelin has a very short plasma half-life. It has been reported that the plasma half-life of apelin-13 and apelin 36 does not exceed 8 min (Japp et al., 2008). Angiotensin-converting enzyme 2 (ACE2) and neprilysin are involved in the enzymatic degradation of apelin (Folino et al., 2015). The cardiovascular effects of apelin, which has a role in various physiological processes such as body-fluid regulation, energy metabolism, and nutrient intake, are very important. Apelin is extensively expressed in cardiomyocytes and vascular endothelium (Xu et al., 2018; Zhang et al., 2018). Apelin, which has a very strong positive inotropic and vasodilatory effect, is thought to have a role in various pathological

processes (Folino et al., 2015; Szokodi et al., 2002). Some of these are heart failure, obesity, diabetes, and cancer, which are very common today and are the most common causes of mortality and morbidity (Antushevich, & Wójcik, 2018; Wysocka et al., 2018).

Perhaps the most important effect of apelin is its strong positive inotropic effect. This effect has been clearly demonstrated in *in vitro* and *in vivo* studies. Szodoki et al. (2002) found that apelin infusion dose-dependently increased DT (*developed tension*) in isolated rat hearts, and reported the role of PKC, PLC, Na^{+}-Ca^{2+} exchanger (NCX), and Na^{+}-H^{+} exchanger (NHE) in this effect. In this study, it was determined that apelin has a strong positive inotropic effect as well as endogenous substances such as endothelin-I (ET-1) and adrenomedullin, as well as 69% of the maximum effect of isoproterenol. In the same study, it was observed that the effect due to apelin appeared in 2 min and reached a maximum in 24 min. Again in the same study, the role of AT1 receptors was investigated due to the sequence homology with APJ in the effect of apelin, but it was seen that it had no effect. However, in this study, which showed that endothelin receptors, adrenergic receptors, and NO do not play a role in the positive inotropic effect of apelin, it was suggested that apelin may exert a positive inotropic effect through its own receptor (Szodoki et al., 2002). Berry et al. (2004) showed an increase in Pmax (maximum left ventricular pressure), dP/dtmax (maximum left ventricular pressure change over time: indicates systolic function), dP/dtmin (minimum left ventricular pressure change over time: indicates diastolic function), and cardiac output as a result of apelin-16 infusion in rats in vivo. In the same study, it was determined that the stroke volume increased without changing the left ventricular end-diastolic volume (Berry et al., 2004). Atluri et al. (2007) reported a significant increase in cardiac output, stroke volume, dP/dtmax, and Pmax values due to apelin-13 administration in rats in vivo. In the same study, the effects of apelin-16 and apelin-13 were compared and it was suggested that apelin-16 was stronger (Atluri et al., 2007). Principe et al. (2008) showed that intravenous (IV) apelin-13 administration in rats significantly increased the cardiac index. Maguire et al. (2009), in the study they determined that [Pyr^{1}]apelin-13 is the predominant isoform of apelin in the human heart, suggested that [Pyr^{1}]apelin-13, apelin-13, and apelin-36 increase the contractile force in human atrial strips, with a stronger positive inotropic effect than ET-1, and apelin is one of the most potent endogenous positive inotropic agents. In addition, apelin-mediated endothelium-dependent vasodilatory effects and direct vasoconstrictor effects were also demonstrated in the same study (Maguire et al., 2009). Japp et al. (2010) demonstrated that systemic infusion of [Pyr1]apelin-13 and apelin-36 increases cardiac output, cardiac index, and heart rate

while reducing peripheral vascular resistance in humans. Barnes et al. (2013) determined that long-term systemic IV [Pyr^{1}]apelin-13 infusion in humans increased cardiac index and heart rate, but decreased peripheral vascular resistance. Pang et al. (2014) showed that infusion of [Pyr^{1}] apelin-13 caused a significant increase in +dP/dtmax (rate of maximum increase in left ventricular pressure during systole) and -dP/dtmax (rate of maximum decrease in left ventricular pressure during diastole) in rats. Perjes et al. (2014) reported that apelin-16 administration increased contractile performance in the isolated rat heart by a mechanism involving PKCε, ERK1/2, and myosin light chain kinase (MLCK) activation. In this study, which showed a significant increase in DT due to the application of Apelin-16, it was suggested that the activation of PKCε and ERK1/2 occurred independently but in parallel (Perjes et al., 2014). Yang et al. (2017a) showed that [Pyr^{1}]apelin-13$_{(1-12)}$, the ACE2 metabolite of [Pyr^{1}] apelin-13, increases the contractile force in mouse right ventricle and human atrial strips. In the same study, [Pyr^{1}]apelin-13$_{(1-12)}$ was reported to induce human saphenous vein contraction, increase human forearm blood flow, and reduce blood pressure in anesthetized rats (Yang et al., 2017a). In another study, Yang et al. (2017b) determined that [Pyr^{1}] apelin-13 infusion significantly increased cardiac output, ejection fraction (EF), stroke volume, and dP/dtmax, while it reduces systolic and diastolic blood pressure in rats in vivo. It was determined that systemic infusion of the APJ receptor agonist MM07, developed by Brame et al. (2015), increased cardiac output in a dose-dependent manner and this effect was significantly stronger than [Pyr^{1}]apelin-13. Gerbier et al. (2017) reported that the administration of apelin-17 and a more stable apelin-17 analog, P92, significantly increased the dP/dtmax value in isolated rat hearts.

There are calcium-dependent and calcium-independent mechanisms in the positive inotropic effect of apelin. The calcium-dependent mechanism begins with the binding of apelin to the APJ in the cell membrane of cardiomyocytes. Then, the activated G protein activates the PLC. Inositol triphosphate (IP3) and diacylglycerol (DAG) are formed from phosphatidylinositol biphosphate. IP3 stimulates Ca^{2+} release from the sarcoplasmic reticulum, while DAG activates PKC. PKC enables NHE to be activated. Due to the increased intracellular Na^{+} concentration, NCX is stimulated and the cytoplasmic Ca^{2+} concentration increases. Thus, there is an increase in the force of contraction of the heart. It is thought that mechanisms such as increased sensitivity of myofilaments to calcium or increased affinity of troponin C to Ca^{2+} play a role in the calcium-independent positive inotropic effect. After the activation of PKC, the cytoplasmic pH increases due to the induced NHE, and an increase in the Ca^{2+} sensitivity of myofilaments occurs. However, it is suggested

that MLCK/RMLC (*regulatory myosin light chain*) and MEK1/2-ERK1/2 activations are other mechanisms that increase Ca^{2+} sensitivity (Folino et al., 2015; Rastaldo et al., 2011b; Szokodi et al., 2002; Wang et al., 2008; Yang et al., 2015).

Apelin and its analogs are prescribed as an alternative treatment method for many cardiovascular diseases due to their strong positive inotropic effects. In many studies, it has been reported that in pathological conditions such as heart failure where cardiac performance is reduced, the parameters showing the heart contractility are improved due to the application of apelin. Dai et al. (2006) determined that apelin-12 increased the force of contraction by increasing the amount of Ca^{2+} in cardiac trabeculae of rats with failure. Jia et al. (2006) showed that apelin-36 was protective against isoproterenol (ISO)-induced myocardial damage and heart failure, and reported that +LVdP/dtmax (maximum rate of increase in left ventricular pressure during systole), –LVdP/dtmax (maximum rate of decrease in left ventricular pressure during diastole), left ventricular end-systolic pressure (LVESP), and mean arterial blood pressure (MABP) values were significantly higher while left ventricular end-diastolic pressure (LVEDP) was significantly lower, in the ISO+apelin-36 group compared to the ISO alone group in rats. In the same study, a decrease in MABP and LVEDP was observed with an increase in ±LVdP/dtmax and LVESP due to IV apelin-36 administration in the control group, but these effects were found to be stronger when apelin-36 was administered after ISO (Jia et al., 2006). Atluri et al. (2007) detected a significant increase in cardiac output, dP/dtmax, and maximum ventricular pressure values due to apelin-13 administration in rats with ischemic heart failure in vivo. Zeng et al. (2009) reported that apelin-13 was protective against ischemia-reperfusion injury in rats and provided a significant increase in ±dP/dtmax values. Rastaldo et al. (2011a) showed that apelin-13 infusion applied after ischemia in an isolated rat heart caused a decrease in infarct size and a significant increase in ±dp/dtmax values. Hekmat et al. (2011) and Najafipour et al. (2012) determined that apelin-13 provided an antihypertensive effect at a dose of 20 µg/kg in two-kidney-one-clip hypertensive rats with signs of heart failure, as well as a significant increase in ±LVdP/dtmax values. Japp et al. (2010) showed that systemic [Pyr^{1}]apelin-13 infusion improves cardiac index and cardiac output in people with heart failure, while it reduces peripheral vascular resistance and MABP. Barnes et al. (2013) demonstrated that long-term systemic IV [Pyr^{1}]apelin-13 infusion increases cardiac index, fractional shortening, and left ventricular EF in humans with heart failure, while it reduces MABP and peripheral vascular resistance. Wang et al. (2013) determined that IV infusion of apelin-13 increased EF in dogs with heart failure. Zhang et al. (2014) reported

that apelin-13 application provides an improvement in cardiac functions deteriorated after myocardial infarction and a significant increase in ±dp/dtmax values in rats. In this study, apelin-13 was also shown to reduce the size of the infarct. Pang et al. (2014) showed that infusion of [Pyr^{1}]apelin-13 in rats with hypertensive heart failure resulted in a significant increase in ±dp/dtmax values. Azizi et al. (2015) observed that [Pyr^{1}]apelin-13 administration improved the contraction and relaxation functions, which were impaired after myocardial infarction in rats, although it could not restore them to the previous level, and determined that there was a significant increase in ±dp/dtmax values. Chung et al. (2016) reported that after myocardial infarction in mice, apelin-13 infusion protected cardiac performance by reducing infarct size and increasing left ventricular EF.

Elabela and Myocardial Contractility

Less is known about another endogenous APJ ligand, elabela, as it is newer. Elabela was found by two different research groups in 2013 and 2014 (Chng et al., 2013; Pauli et al., 2014). The Apela gene encoding elabela is located on chromosome 4 in humans (Pauli et al., 2014). The isoforms of elabela formed from the precursor peptide with 54 amino acids are the mature forms ela-32, ela-21, and ela-11 (Chng et al., 2013; Zhang et al., 2018). Elabela is extensively expressed in cardiovascular endothelium. Elabela is required for normal heart development and angiogenesis (Zhang et al., 2018). Similar to apelin, elabela also causes vasodilation and has a positive inotropic effect (Kuba et al., 2019; Perjés et al., 2016; Sahinturk et al., 2022; Wang et al., 2015; Zhang et al., 2018). It has been suggested that the vasodilator and positive inotropic effect of elabela are more potent than apelin (Wang et al., 2015; Yang et al., 2017b). The role of ERK1/2 activation in the positive inotropic effect of elabela has been demonstrated (Perjés et al., 2016). It has been suggested that apelin and elabela work together to antagonize the renin-angiotensin system, which has an important role in the control of arterial blood pressure, thus being effective in preventing pathologies such as cardiac hypertrophy and cardiac fibrosis (Kuba et al., 2019; Zhang et al., 2018).

A limited number of studies have shown that elabela increases myocardial contractility. Murza et al. (2016) reported that ELA-32 and its analog (analog 3, ELA(19-32)) caused an increase in LVDP (*left ventricular developed pressure*) similar to apelin-13, and the effect of ELA-32 was stronger than apelin-13 in the isolated rat heart. In this study, apelin-13, ELA-32, and analog 3 were used at doses ranging from 0.001 to 0.3 nM, and the strongest effect was seen at 0.3 nM. In the same study, the effect of analog 3 was shown to be weaker than apelin-13 and ELA-32. Again in the same study, it was reported that apelin-13, ELA-32, analog 3, and analog 4 (ELA(22-32)), which is another analog of elabela, administered

intravenously to rats decreased MABP (Murza et al., 2016). Yang et al. (2017b) determined that in vivo infusion of ELA-32 in rats significantly increased cardiac output, EF, stroke volume, and dP/dtmax [Pyr^{1}]apelin-13. In the same study, it was also shown that the administration of ELA-32 and [Pyr^{1}]apelin-13 reduced systolic and diastolic blood pressure. Again in the same study, it was determined that ELA-32 and [Pyr^{1}]apelin-13 were short-acting and their effects ended in 10 to 20 min (Yang et al., 2017b). Perjes et al. (2016) reported that the application of elabela increased DT in isolated rat hearts. In this study, it was shown that elabela administered at doses of 0.1-10 nM provides a significant increase in cardiac contractility from 0.3 nM and the maximum effect is seen at a dose of 10 nM. The effect of elabela started at the 2nd min, reached the maximum at the 15th min, and continued in the same way until the 20th min. The maximum increase in heart contraction force was determined as 46±4%. The effect, which decreased slightly after the 20th min, continued until the 30th minute as approximately 70% of the maximum effect (Perjes et al., 2016). Researchers stated that this pattern seen in the duration of effect is similar to apelin (Perjes et al., 2016; Szokodi et al., 2002). In the same study, it was determined that the MEK1/2-ERK1/2 pathway has a role in the positive inotropic effect of elabela. Again in the same study, it was reported that similar to apelin, elabela also decreased the perfusion pressure in a dose-dependent manner (Perjes et al., 2016). Sato et al. (2017) showed that continuous infusion of elabela prevented the reduction in cardiac fractional shortening in pressure-loaded mice with transverse aortic constriction. However, in this study, it was reported that the application of elabela suppressed cardiac dysfunction, fibrosis, and hypertrophy due to pressure overload. In the same study, it was suggested that elabela provides protection against heart failure due to pressure overload by suppressing ACE expression and preventing pathological Ang-II activation (Sato et al., 2017).

Conclusion

In conclusion, apelin has a positive inotropic effect in physiological conditions as well as in pathological conditions such as heart failure. Similarly, elabela has a positive inotropic effect and is claimed to be more potent than apelin in this respect. Due to their strong positive inotropic, antihypertensive, and cardioprotective effects; apelin, elabela, their analogs, and APJ receptor agonists may be promising agents for the treatment of cardiovascular diseases. Despite the positive inotropic effect of the apelinergic system, it is an important advantage that it does not cause pathological hypertrophy. The major issue here is that apelin and elabela have short half-lives, thus reducing their therapeutic potential. Therefore, longer-acting and more potent apelin analogs, elabela analogs,

and APJ agonists are being developed. Since a better understanding of the positive inotropic action mechanisms of the apelinergic system will guide the development of alternative agents in the treatment of heart failure, further studies are needed on this subject.

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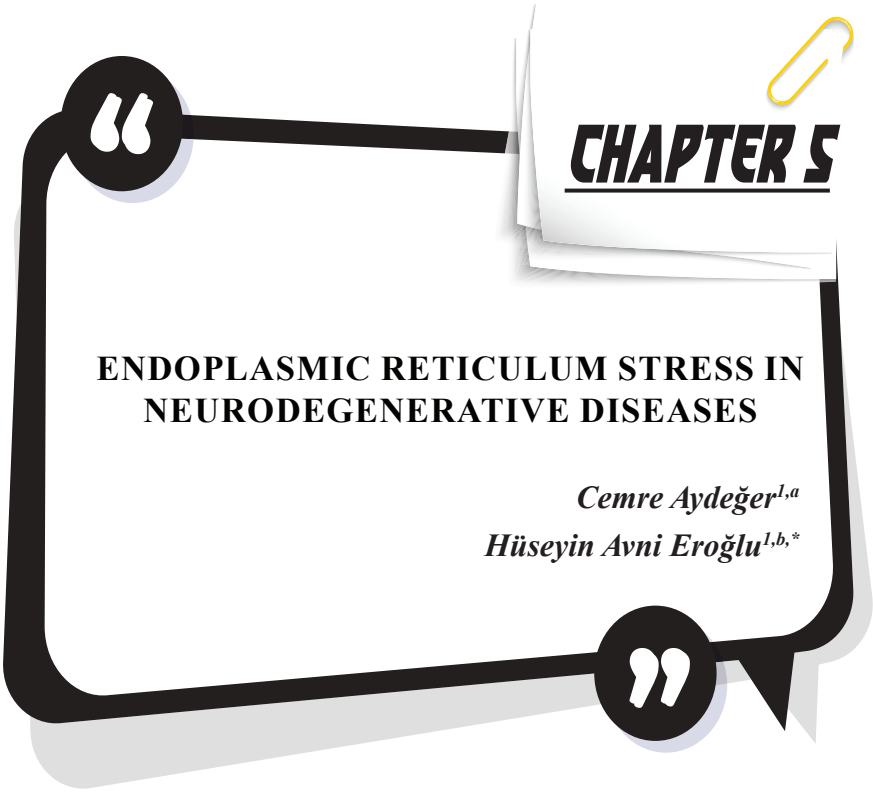

CHAPTER 5

ENDOPLASMIC RETICULUM STRESS IN NEURODEGENERATIVE DISEASES

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The Endoplasmic Reticulum (ER) is a membranous organelle found in cell organelles. According to the presented area, ER is called rough, straight, or external nuclear sheath. (Gorman, Healy, Jäger, & Samali, 2012). ER is a multifunctional organelle, all those functions can be classified into four main titles:

1. Secretory protein synthesis
2. Fold of proteins which are secretory or transmembrane
3. Storage of intracellular calcium

Synthesis of lipids and steroids (Schröder, 2008).

Protein homeostasis (proteostasis) include transcription, translation, folding, and degradation processes of proteins. In ER, two of those processes take place in order to synthesize membranous or extracellular proteins. It is known that ER is important for proteostasis (Chadwick & Lajoie, 2019; Schröder, 2008). In ER, four main modifications are eventuated by these processes:

1. Adding carbohydrates with covalent bonds and processing (glycosylation)
2. Composing disulphide connections
3. Folding polypeptide chains properly and occurring proteins, which consist of many subunits,
4. Proteolytic specific cuts.

Some of these modifications are actualized by the apparatus of Golgi apart from ER. As a result, proteins regain their natural structure and become more appropriate for extracellular space. Also, modifications of glycosylation is indispensable for specialization of different cells (Lodish et al., 2020).

The ER is linked to the mitochondria thus optimal conditions which are essential for protein folding such as ATP, calcium, and the oxidized environment. Changes of cellular energy levels, redox state, or calcium concentration are the highlights for enhancement of protein folding capacity in ER. (Gorman et al., 2012). Under appropriate conditions, enzymes fold into proteins, which are placed in ER lumen. Oxyreductases and also chaperons and foldalases which charge from glycocylation are the main responsible enzymes of correct folding, consequently correct folded protein transfers from ER to Golgi apparatus. However, not always correctly folded proteins produce from ER. When misfolded proteins are produced, ER control systems became active and defines the origin of error. Chaperones, called at the same time as BIP proteins, make definition of this misproduce with a process that needs ATP to work. Further misfolded

proteins cross through cytosol by a pathway called as retrotranslocation or dislocation. Dislocation occurs with the Sec61 complex, which is the same translocator used in the synthesis of protein and some other helper proteins. If a misfolded protein crosses through cytosol, it means it is addressed to the proteasome for degradation (Alberts et al., 2002; Chadwick & Lajoie, 2019; Oakes, 2020; Schröder, 2008).

Commonly, misfolded or unfolded proteins are involved one third of all folded proteins in ER. Various physiological and pathological conditions could be the main cause of incorrect folding. All these process reduce ER function and it is called as ER stress (Chadwick & Lajoie, 2019; Oakes, 2020; Schröder, 2008). When proteins accumulate and degradade, cell function becomes limited and inadequate. Moreover this restriction leads to cell death (Oakes, 2020). Cell scavenges system become active against disfunctioning for cell survive. Cells improve two different stress pathways: heat- shock protein response (HSR) and unfolding protein response (UPR) (Chadwick & Lajoie, 2019).

The defending system is termed as HSR which was investigated against heat stress. However, HSR becomes active in various cases, including membrane perturbation, exposure to heavy metals, and oxidative stress (Przekora, 2020). One of the most deleterious and activating event is ER stress (Chadwick & Lajoie, 2019). The response is characterized as protection of protein against accumulation by heat-shock proteins (HSPs). HSPs help folded proteins, refolded denaturated proteins and degradaded misfolded proteins which are not usable or translatable permanently (Dukay, Csoboz, & Tóth, 2019). sHSPs have a small molecular weight and are localized in the cytosol or nucleus, activate as foldolase or disaggregase. Also, HSP60, HSP70, HSP90, and HSP100 are subtypes of them. sHSPs link with related proteins and form a sHSP- substrate complex with them. If this complex consists of enough sHSP, the substrate becomes soluble, and its aggregation could be restrained. At that point, mainly HSP70 and helper HSP100 take the last decision about the substrate protein. If the protein is appropriate HSP70 leads for refolding. Otherwise, HSP70 mediates its degradation with ATPase related proteases (Mogk, Ruger-Herreros, & Bukau, 2019) (Figure 1).

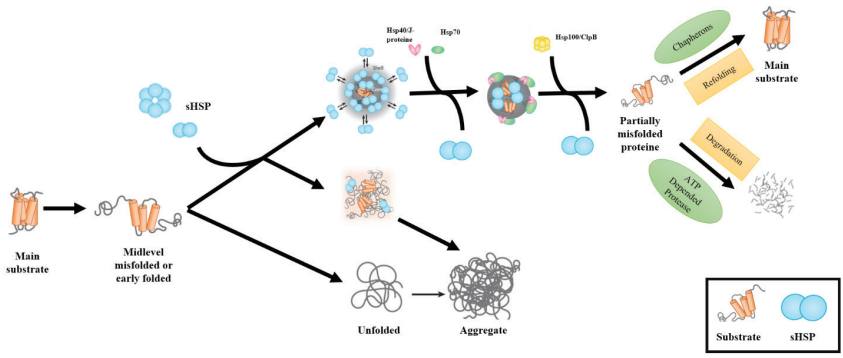

Figure 1. *Representative mechanism of HSR response by HSPs for misfolded proteins (Mogk et al., 2019).*

UPR pathway is located inside ER membrane and activates especially during in ER stress. (Chadwick & Lajoie, 2019). The UPR pathway is affected by hormones, growth factors, small ligands and protein folding in ER (Hetz, Zhang, & Kaufman, 2020). Changes in oxygen or nutrition, as well as carcinogenic or oncogenic mutations, are frequent causes of UPR that related with autophagia, signals of hypoxia, mitochondrial biogenesis, and reactive oxygen species (ROS) (Senft & Ronai, 2015).

Under normal conditions, the sensor of UPR is termed Binding Immunoglobulin Protein (BiP) or Heat Shock Protein Family A (Hsp70) Member 5 (HSPA5) or Glucose-Regulated Protein 78 (GRP78). This protein provides inactivation through placing on luminal side. Accumulation of misfolded proteins leads to separation of GRP78 which takes place in UPR (Liang et al., 2021). Three main cascades are affected by UPR: Inositol-requiring transmembrane kinase endoribonuclease-1α (IRE1α), Protein Kinase R-like ER Kinase (PERK), and activating transcription factor 6α (ATF6α) (Hetz et al., 2020; Liang et al., 2021). These cascades organise autophagia and ER-associated protein degradation (ERAD) pathways for prevention of ER stress (Hetz et al., 2020) (Figure 2).

Autophagia is the first outcome of ER stress effecting proteins, cytoplasmic components, and organelles catabolism. The process is controlled strictly by approximately 30 autophagia-related genes (ATG). Accumulation of misfolded proteins increased during autophagia. Thus, enhancement of UPR augments autophagia either. PERK- eIF2α is the main signalling pathway of autophagia. The pathway mainly enhances level of autophagia triggering ATG with ATF4 and C/EBP Homologous Protein (CHOP). Another pathway is IRE1α. IRE1α leads to phosphorylation of Bcl-2 protein being in charge of apoptosis, degradation of Beclin-1 and activation of Phosphoinositide 3-kinases (PI3K) complex. At the same time, IRE1α effects other pathways, induce other stressors, such as c-Jun NH2-

terminal kinases (JNK) or nuclear factor κB (NF-κB) trying to prevent ER stress. On the other hand, previous studies about IRE1α investigated the increase of protein flow by regulation of mRNA degradation. Lastly, transcription factor X box-binding protein 1 (XBP1) is activated by IRE1α and it modulates ER stress (Senft & Ronai, 2015).

ERAD is a structure having lots of components and identifies and targets misfolded proteins and marks misfolded proteins with ubiqutine and helps degradation by 26S proteosome (Vembar & Brodsky, 2008). There are three types of ERADs exist which are ERAD-L, -M, and -C. ERAD-L locates in ER lumen and consist of Ring (interesting new gene) type ligase-Hrd1 with three membrane proteins which are Hrd3, Usa1 and Der1, and a luminal protein known as Yos9. ERAD-M localises in ER membrane. Similarly to ERAD-L, ERAD-M has another type of Ring ligase complex-Doa10 (Ruggiano, Foresti, & Carvalho, 2014; Wu & Rapoport, 2018). In addition, ERAD substrates have cascade enzymes termed E1, E2 and E3. E1 which activate ubiquitin. E2 is an enzyme conjugating with ubiquitin. The last component of cascade is E3 ligase. There are two E2 enzymes in ERAC-C. Ubr1 ligase in Doa10 complex is responsible for mainly conjugation. When misfolded proteins link this area ERAD complex makes process begin (Sun & Brodsky, 2019). Linked protein retrotranslocate to cytosol from ER after, it is transferred to ubiquitination machines (Lopata, Kniss, Löhr, Rogov, & Dötsch, 2020). At the same time, UPR determines which could be autophagia or ERAD. When huge amounts of aggregated proteins become hard to fix, an autophagosome takes in them for autophagia. If a protein could be fixable, ERAD breaks the protein (Hwang & Qi, 2018). One of other way for degradation contains ubiquitin ligase which involves to Asi 1, 2 and 3. This complex degrades misfolded proteins into inner membrane proteins (Wu & Rapoport, 2018).

Aforementioned mechanisms all provide cell survival. The survival process can be divided into two main subject: against survival (anti-survival) and supportive of survival (pro-survival) (Figure 2). The anti-survival response, CHOP activation increases and induce JNK with caspases and enhances the expression of PUMA, BAX, and NOX proteins. On the other hand, it also inhibits protein translocation, increase ER chaperone levels and degrade ER-related proteins (Enogieru, Omoruyi, Hiss, & Ekpo, 2019).

The UPR has responsible for various physiological and pathological conditions. Several tissues and organs are affected under those conditions (Figure 3). Brain is the most affected organ by UPR. At homeostatic conditions, UPR improves brain synaptic plasticity in a behavioural and structural manner (Hetz et al., 2020). Some pathological changes in UPR

are related to neurodegenerative diseases which has important role in proteostasis of brain tissue. Ranging diseases augment chronic ER stress via several secretory proteins (Hetz, 2021). Chronic ER stress increases brain pressure and causes neuronal lose and inhibits synaptic protein synthesis. At that way, this situation results with deleterious effects on cognition and memory (Hetz & Saxena, 2017).

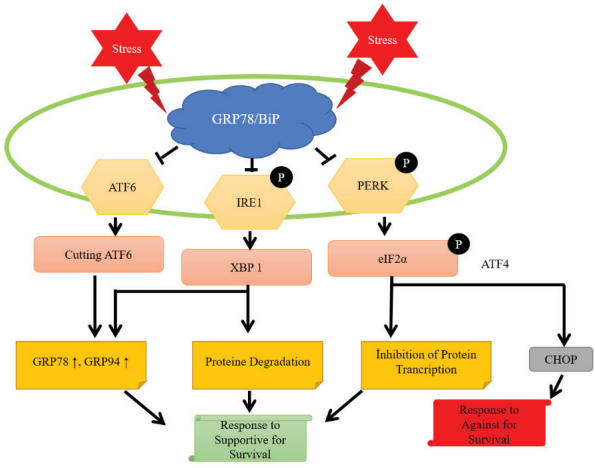

Figure 2. *A schematic diagram representing the ER stress pathways (Enogieru et al., 2019).*

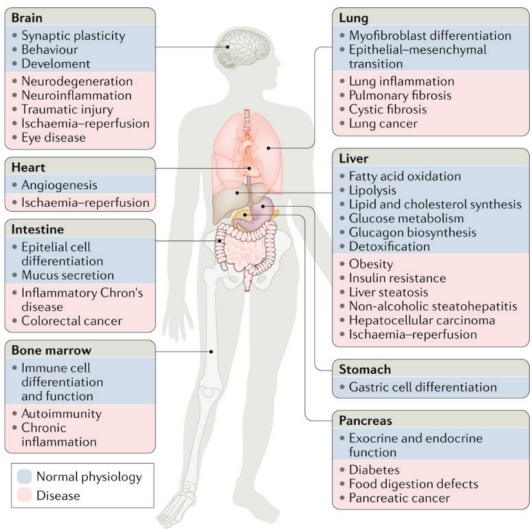

Figure 3. *Role of UPR in physiologic and pathologic conditions (Hetz et al., 2020)*

Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington' Disease (HD), amyotrophic lateral sclerosis (ALS) and prion-related diseases (PrD) are all related with ER stress. Amyloid- β peptides, α-synuclein, TDP43 and FUS proteins increased expression levels are respectively related to AD, PD and ALS. All those related proteins accumulate and aggregate (Figure 4). For this reason, neurodegenerative diseases are linked to ER stress (Hetz & Mollereau, 2014).

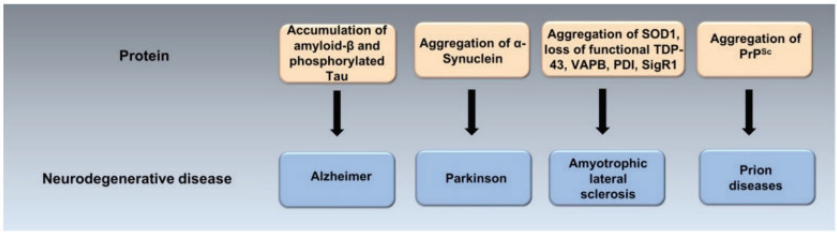

Figure 4. *Some proteins' aggregating in cell and relationship neurodegenerative disease (Ghemrawi & Khair, 2020).*

Misfolded proteins lose their physical activities and become neurotoxic improving brain damage. Aggregates trigger to ER stress and activate UPR. First response to those disorders is to adaptive. However, when ER stress continue it shortens neuron survival activating apoptosis (Doyle et al., 2011; Ghemrawi & Khair, 2020). In addition to this, the upstream of UPR response improves the inflammation, reduces oxidative stress and ameliorates mitochondrial dysfunction. It can be strongly suggested that ER stress has mainly suspicious member of neurodegenerative diseases and neuroinflammatory pathogenesis (Morris et al., 2018) (Figure 5).

One of most common neurodegenerative disease is AD characterized by cognitive impairment in which beta amyloid plagues accumulated (Hashimoto & Saido, 2018). These plagues are originated from amyloid precursor protein (APP), which is a transmembrane protein with 37-43 amino acids. Studies suggested that accumulation of APP products increase ER stress. Genetic mutations are one of the reasons (Bermales, Soto, & McCullagh, 2012). IRE1/XBP1 signal axis activation has also a role in AD. XBP1 promoter polymorphism is accepted as a risk factor for AD (Remondelli & Renna, 2017). Best of the our knowledge it can be considered that ER stress has a complex role in AD (Bermales et al., 2012). As we know ER is a calcium storage, if a disruption occurs in ER like ER stress calcium reuptake might be affected. AD disorder gets worsened because of calcium metabolism dysfunction and provides neuronal death.

Another neurodegenerative disease which commonly occurs is PD. PD is characterized by neuronal loss from pars compacta of substantia nigra. In the pathology of PD, neuronal death occurs with alpha synuclein aggregation. However, the underlying mechanism is unclear, it is suggested

that genetic factors, environmental factors, and some cellular processes might have a role. One of the reasons of cellular processes is ER stress. UPR and autophagia, results of ER stress, have important roles in PD pathogenesis (Ren, Zhai, Lu, & Wang, 2021). Accumulated evidences support this suggestion. Also increase in alpha synuclein may be a result of ER stress directly or/and indirectly. In addition, it is reported that microinflammation plays a role in PD's pathogenesis and is related to ER stress (Colla, 2019).

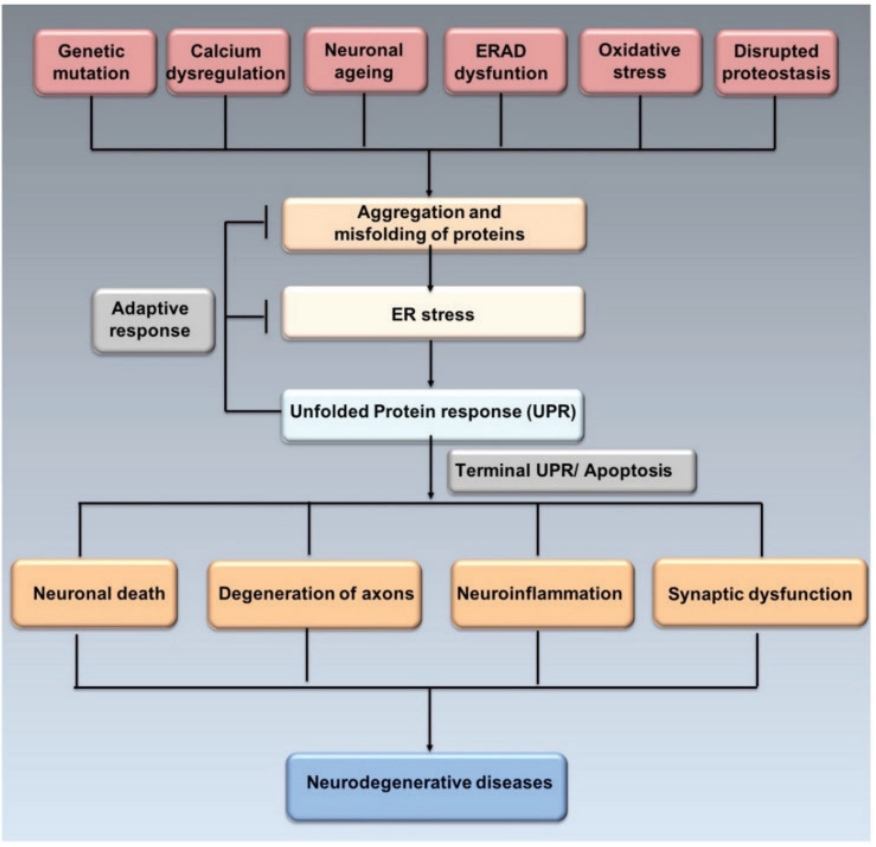

Figure 5. *Schematic representation of protein aggregation effects on neurodegeneration (Ghemrawi & Khair, 2020).*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease which results in mortality and characterized motor neuronal loss in motor cortex, brain steam and spinal cord linked with ER stress (Lautenschlaeger, Prell, & Grosskreutz, 2012). It is stated that familiar and sporadic types of ALS are related with ER stress either. In ALS, activation of impaired calcium homeostasis and activates NF-κB signalling pathway (Prell et al., 2014).

ER stress is also an important factor for Huntington's disease which is one of a neurodegenerative disease (Chen, 2021). PolyQ disease also related with Huntington's disease. Repetitive transcription of Cytosine-adenine-guanine (CAG) code results in PolyQ. Overexpression of the code decrease functional protein levels and cause accumulation of them based on ER stress. So reducement of ER stress is a main target against Huntington's disease (Fan et al., 2014; Remondelli & Renna, 2017).

Prion related diseases (PrD) are a group of diseases which progress with the accumulation of prion proteins, have limited proteolysis property and are related to ER stress. (Richardson, Fitsanakis, Westerink, & Kanthasamy, 2019). Overexpression and accumulation of prion proteins occur as a result of infection (Joshi-Barr et al., 2014). The accumulation cause an imbalance in ER homeostasis and inhibition of UPR (Park et al., 2017). Still the relationship between PrD and ER stress remains lacking, further studies should be beneficial for the investigation of specific mechanisms (Xu & Zhu, 2012).

Consequently, ER is a crucial organelle for cell survival. ER damage results in ER stress playing a key role in both physiological and pathological conditions. One of the conditions are neurodegenerative diseases that are closely connected with misfolding proteins. So, the association reveals more beneficial results for treatment of these diseases.

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CHAPTER 6

MESENCHYMAL STEM CELLS AND THEIR APPLICATIONS IN MEDICINE

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Stem Cell

Stem cells are simply the source of life. The zygote formed as a result of the union of the ovum and sperm; are the cells that have the most competent differentiation ability in the living world. In a short time, they first lead the embryo and then all the tissue formations. Because; at the beginning of this chain, in which each cell consists of a single cell, is the fertilized ovum. These cells have the ability to self-renew and transform into different cells (Andrades vd., 2011).

The blastocyte is formed by dividing the zygote five or six times in succession and the blastocyte cells show the potential to differentiate into approximately 200 cell types. The outer layer of the formation called blastocyst forms the placenta, while the inner cell mass forms the embryo (Pittenger vd., 1999).

Stem Cell Division

Although the genomes of all proliferating cells are the same; while many genes are active; gene expression is low. In differentiated cells; gene expressions are higher. In other words, an increase in protein synthesis is observed in a differentiated cell. After division, one of the daughter cells remains close to the microenvironment (niche) and preserves its stem cell ability. The other daughter cell begins to differentiate as it loses its connection with the microenvironment (Morrison & Kimble, 2006).

Stem Cell Differentiation

Stem cells have high differentiation abilities. While the stem cell is protecting its own backup; on the other hand, they turn into cells that progress in the direction of differentiation. In the process called differentiation; cells stop dividing, while preparing to respond to stimuli from the environment (Fuchs & Segre, 2000).

Differentiation and proliferation time do not occur at the same time. The cell first proliferates and reaches a sufficient amount, and then the process related to the differentiation mechanism begins by closing the pathways related to self-renewal. Certain physical and chemical conditions are required for stem cells to be differentiated in vitro or the genetic program of the cell needs to be changed (Hwang, Varghese, & Elisseeff, 2008).

Stem cells are classified according to their differentiation capacity as totipotent, pluripotent, multipotent, oligopotent and unipotent (Chagastelles & Nardi, 2011).

Stem Cell Markers

Today, the most commonly used method to determine the stem cell type is to use stem cell markers. These markers are often referred to as 'CD' (Clusters of Differentiation) and are either cell specific or very common in that cell. In this way; stem cells are differentiated from other cells (Mokhtarzadeh vd., 2017).

Types of Stem Cells

Stem cells, which are the source of life, are divided into embryonic and adult (Bacakova vd., 2018).

1. Embryonic Stem Cells

Embryonic stem cells, one of the stem cell types, are obtained from the inner cell part of the blastocyst. These stem cells have the potential to transform into all cell layers and organs, but they do not have the potential to completely transform the whole organism. Tumor cell transformation is also possible due to the high telomerase enzyme activity of these types of cells (Rippon & Bishop, 2004) .

2. Adult Stem Cells

Non-embryonic stem cells are cells that are undifferentiated in a differentiated tissue, have high regeneration abilities, and can differentiate into specific cells of the organ they are in. The main functions of adult stem cells are to ensure the repair and integrity of the tissue they are in (Bojanić & Golubić Cepulić, 2006). Mesenchymal stem cells (MSCs) are non-embryonic stem cell types and were first described by Fridenstein in 1976 (Friedenstein, Gorskaja, & Kulagina, 1976).

Mesenchymal stem cell (MSC)

These cells, which show adherent properties in cultures made by Fridenstein using FBS, resemble fibroblasts morphologically, and can differentiate into bone and adipocyte cells, were named MSCs (Reinisch vd., 2015).

In general, they are used in a wide variety of uses in medicine, as they have support cell properties. It can be obtained from many tissues. However, they are durable cells whose numbers are increased by making the necessary passages since they are few in number in the tissues from which they are obtained (Andrades vd., 2011).

MSC Division

MSCs have to be produced under laboratory conditions since they are less available from tissues. Cultured MSCs are spindle-shaped and form fibroblast-like cell aggregates. If the concentration of the cells to

be cultured is low, they multiply as colonies, while the cells with high concentrations line up and reproduce (Akgün, 2016).

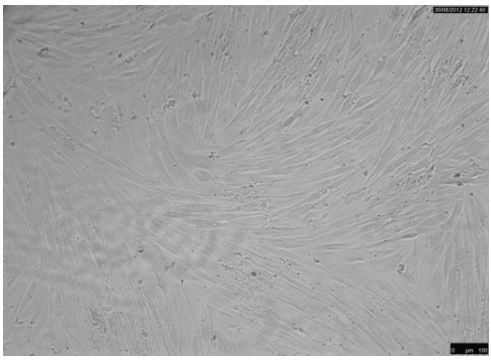

Figure 1. Bone marrow-derived MSC (Özen & Sancak, 2014).

Very specific conditions are not required to culture MSCs. It can grow adherently inside the flasks in cultures containing 10-15% FCS (Ozen, Sancak, Rechenberg, & Koch, 2013; Pittenger vd., 1999).

MSC Differentiation

It has been observed that MSCs can differentiate into fat, bone, cartilage, muscle, tendon and even neuron under appropriate stimuli under laboratory conditions. In 1966, Friedenstein et al. showed that when mouse bone marrow stroma is transplanted to another location, it can transform into osteocytes, adipocyte and chondrogenic (Friedenstein, Piatetzky-Shapiro, & Petrakova, 1966; Phinney & Prockop, 2007). MSCs can easily be induced to differentiate in the culture medium. Differentiation into these three different series is essential for MSCs (Horwitz vd., 2005).

It is determined using immunofluorescence, histochemical, and immunohistochemical techniques to determine whether there is differentiation into the intended cells. Differences are also confirmed by gene expression analysis (Vater, Kasten, & Stiehler, 2011).

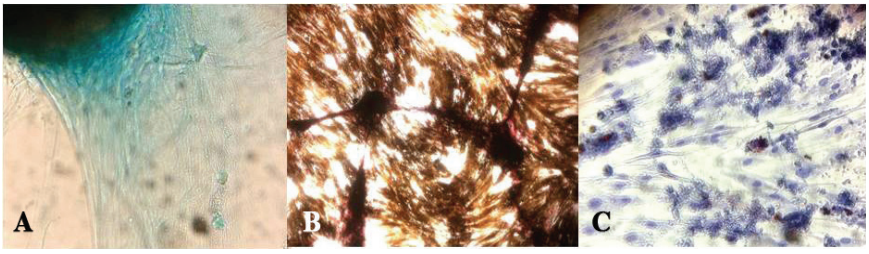

Figure 2. (a) Chondrogenic appearance at day 21 (Alcian blue staining), (b) Osteogenic appearance at day 21 (Von kossa staining), (c) Adipogenic appearance at day 14 (C staining) (Özen & Sancak, 2014).

Special dyes can be made for osteogenic, adipogenic and chondrogenic. Alcian blue is used for the chondrogenic appearance, Von Kossa is used for the osteogenic appearance, and Oil red O is used for the adipogenic appearance (Caplan & Dennis, 2006; Lettry, Hosoya, Takagi, & Okumura, 2010).

Acquisition of MSC

There are also hematopoietic and endothelial cells in the bone marrow, which is the main source for MSC. MSCs can be obtained from many tissues other than bone marrow. It is possible to identify adipocyte tissue, cord blood, dental pulp, liver, muscle, bone, placenta, synovial and amniotic fluids, cord stroma and peripheral blood by making use of their adhesion to the surface when cultures are made (Fernández-Francos, Eiro, González-Galiano, & Vizoso, 2021).

MSC Markers

There is no definitive cell marker to distinguish MSCs. In order to distinguish MSCs from other cells in a heterogeneous culture, they must not carry hematopoietic stem cells and tissue-specific antigens from which they are obtained. It is known that MSCs do not carry hematopoietic stem cell antigens, but express stroma-specific antigens at high levels. Immunophenotypic properties of MSCs were investigated by flow cytometry (Le vd., 2021).

According to the International Cell Therapy Society (ISCT) data, it has been reported that MSCs CD73 (SH3/SH4), CD90 and CD105 (SH2) must be positive. In addition, markers with CD9, CD10, CD29, CD44, CD71, CD106, CD124, w4a5, STRO-1 should be present on their surfaces (Atoui & Chiu, 2012; Pittenger vd., 1999).

Unlike hematopoietic stem cells, they are $CD34^{-}$ and $CD45^{-}$. It has also been reported that it does not express vWF, CD4, CD11a, CD11b, CD14, CD19, CD31, CD79α or HLA-DR (Dominici vd., 2006).

In a study, it was observed that CD73, CD90 and CD105 were highly expressed in early cultures in in vitro culture medium, while the expression of these biomarkers decreased in the late period (Menssen vd., 2011). With this result, it was determined that cultures of MSCs showed better biomarker expressions in the early period. It was determined that viable cell counts gave similar results at the same time intervals. In a study by Harting M. et al., it was observed that CD73, CD105 and Stro-1 gene expressions of MSCs decreased after the tenth passage. Expressions of CD34, CD45 and CD11b, which are used as negative biomarkers for MSCs in culture, were also examined and gene expressions could not be determined in both early and late periods (Gratwohl vd., 2008; Nakamura vd., 2003).

Advantages of MSCs

MSCs inhibit T and B lymphocytes and stimulate regulatory T cells. It has immunomodulatory effects on dendritic cells, NK cells, B lymphocytes, especially on T cells. It has been determined that it has an immunosuppressive effect by inhibiting the proliferation of T and B lymphocytes. Due to its immunosuppressive properties, it is not rejected in therapeutic use (Tocci & Forte, 2003).

In addition to their immunomodulatory effects, MSCs also have antigenic, antifibrotic and antiapoptotic abilities. Having these qualities explains the functions of these cells in repair of damage (Sun, Chen, & Pei, 2018).

MSCs are on their way to damaged tissue. It is the changing microenvironment of the damaged tissue that is responsible for this. SDF-1, MCP-1, and complement C3 secreted from the damaged microenvironment have been reported to play important roles. In this way, these stimulants secreted from the damaged area enable the stem cells to migrate there. At the same time, MSCs that migrate towards damaged tissue can repair damaged tissues by secreting many cytokines, chemokines such as SDF alpha-1, monocyte chemoattractant protein, and growth factors, since they are found as support cells in many different tissues. HLA tissue compatibility is not essential for clinical use of MSCs, and MSC treatment can be successful even from completely incompatible individuals. Therefore, it has been reported that positive results were obtained by using MSC four hours before the infusion of hematopoietic cells in transplantation. It has been reported that MSCs support hematopoiesis by synthesizing cytokines such as GM-CSF, G-CSF, CSF, and IL-6 (Gnecchi, Danieli, Malpasso, & Ciuffreda, 2016).

The gene transfer processes of these cells are easy and therefore suitable for gene therapy. Thus, it has the potential to be used for many diseases. In addition, since these cells have the ability to secrete enzymes, they can be a remedy for enzyme deficiencies in diseases (Marofi, Vahedi, Biglari, Esmaeilzadeh, & Athari, 2017).

It also produces monocyte stimulating factor (M-CSF), Flt-3 ligand, which is necessary for hematopoietic stem cells. MSCs are also low in immunogenicity (Molaeipour vd., 2016).

Disadvantages of MSCs

It can cause some differences in the cell with the effect of chemicals exposed as a result of passage of cells. Since the majority of studies in basic research are done using cells in in vitro environments, they are far from the characteristics of cells in vivo. Therefore, it creates question

marks in the mind and creates a disadvantage for clinical applications (H. J. Kim & Park, 2017).

There is a risk of cell senescence, cytogenetic impairment, and a low probability of malignant transformation, which occurs with passage of MSCs in culture. In addition, in order to be used in the clinic, some advanced technological investments are required. There should be a GMP laboratory at the beginning. Providing such a working environment requires serious technology, infrastructure, experience and cost (Fekete vd., 2012).

One of the clinical problems of MSC is the difficulties in obtaining products such as FCS/FBS. There may be some difficulties in maintaining the viability and function of these cells in a living thing given MSC. Therefore, repeated inclusions may be required. In addition, it has been determined that the microenvironment in the tissue given MSC can be effective in directing the effects of this stem cell. For this reason, it is necessary to determine the patient, cell dose, time and dose intervals in which these cells will be used (H. J. Kim & Park, 2017).

Clinical Uses of MSCs

The first clinical trial of MSCs was administered to patients with hematological malignancies in 1995. The number of MSC-based clinical trials is over 1,200 as of 11 October 2020. Most studies to date are phase 1 and phase 2 studies and evidence of therapeutic efficacy is still lacking (Zhuang vd., 2021).

The most common indications for MSC-induced cellular therapy include graft-versus-host disease, osteoarthritis, spinal cord injury, multiple sclerosis, and ischemic heart disease. In addition, a large number of MSC-derived studies have been recorded against the coronavirus disease-19 (COVID-19) outbreak. Thus, MSC holds great promise in the treatment of immune and inflammatory diseases (Zhuang vd., 2021).

It has been shown that clinical improvement is achieved with MSCs in the rare skeletal disease osteogenesis imperfecta (Horwitz vd., 1999).

Osteoarthritis (OA) has become one of the most common diseases worldwide. MSCs have long been preferred for the treatment of eroded and worn articular cartilage. Studies have shown that intra-articular injection of MSCs has been shown to improve. These findings demonstrate the importance of MSCs on quality of life as a promising treatment for OA (Wang, Liu, Sytwu, Yen, & Yen, 2021). MSC treatment is effective in relieving pain and improving joint functions in patients with OA. In particular, its effect on knee OA has been thoroughly studied (Jiang vd., 2021). In the study of Emadedin et al., the study on autologous bone

marrow-derived MSC injections for patients with ankle, knee and hip OA was found to be safe (Emad edin vd., 2015).

Ischemic diseases associated with the restriction and obstruction of blood flow to certain tissues can lead to permanent disability in patients. This blood vessel related disease is usually treated with endothelial cells and endothelial progenitor cells. At the same time, MSCs have shown strong biological effects on angiogenesis in recent years. It can provide angiogenesis in ischemic regions with the help of trans-differentiation properties of MSCs (Van Nguyen, Vu, & Van Pham, 2021). Myocardial infarction (MI) occurs when the blood flow to the heart is cut off, resulting in damage to the heart muscles. This disease therapeutically involves stem cell therapy. Autologous MSCs were used for the treatment of MI in 2018 (S. H. Kim vd., 2018). Studies have shown that autologous and allogeneic MSC transplantation makes a difference in the effectiveness of treatment (Van Nguyen vd., 2021). The most common type of stroke, ischemic stroke, is a condition caused by a blockage of the artery that supplies blood to the brain. Lack of oxygen to the brain causes brain damage. MSCs used as therapeutic treatment not only provide angiogenesis but also support brain regeneration after injury. Critical limb ischemia (CLI) has been treated similarly to other ischemic diseases using MSC transplantation. MSCs from various sources have been used to treat this disease (Van Nguyen vd., 2021).

Mechanisms of MSC immunomodulation in liver disorders including cirrhosis are less well known compared to other organ systems (Wang vd., 2021). In a study, it was found that exosomes obtained from MSCs contain IL-10 or TGF-β and play a role in curing cirrhosis (Mardpour vd., 2018). Stem cell therapy is a potential therapeutic option in alcoholic liver disease (ALD). Benefits of MSCs in this disease include hepatocyte proliferation, promotion of regeneration, inhibition of liver fibrosis, modulation of inflammatory responses, and parenchymal cell trans-differentiation (Liu, Tsai, & Hsu, 2021).

Lung cell regeneration is slower compared to other adult organs. The lack of an optimal treatment for acute respiratory distress syndrome (ARDS) is an important problem in medicine. However, recovery after physiological injuries is possible thanks to the built-in stem cells and progenitor cells (Barkauskas vd., 2013). Studies have shown that therapeutic improvement is achieved with MSCs in acute lung injury (ALI) and ARDS. It is due to the anti-oxidative stress, anti-microbial, anti-fibrotic signals, regenerative, anti-inflammatory, anti-apoptotic and angiogenic properties of MSCs. At the same time, MSC secretome and especially exosomes provide therapeutic improvement in lung injury (Fernández-Francos vd., 2021).

MSCs have studies registered to treat viral infection and associated conditions (Taechangam, Kol, Arzi, & Borjesson, 2022). Recently, the most common therapeutic target of MSCs is for the treatment of respiratory problems associated with COVID-19. These trials are mostly carried out with MSCs obtained from the umbilical cord. While the symptoms were significantly reduced after the treatment, there was an increase in the number of peripheral lymphocytes. While C-reactive protein decreased, TNF-α levels showed a significant decrease. Therefore, viral injection of MSCs has a therapeutic effect on COVID-19 patients (Rafiee, Nejaddehbashi, Nasrolahi, & Khademi Moghadam, 2021).

It is a promising therapeutic method because MSCs do not have the risk of tumor initiation. However, there are concerns that MSCs may pose a risk of promoting tumor growth (Li, Fan, Wang, Wang, & Ren, 2018; Lin vd., 2018). MSCs can transform into cancer-associated fibroblasts in the tumor microenvironment. The tumor microenvironment includes immune cells, fibroblasts, endothelial cells, and cancer-associated MSCs. It is possible that MSCs capable of localizing tumor sites may be converted from their therapeutic purpose to cancer-associated MSCs. Several studies have investigated the effects of MSCs on different tumor cells and have resulted in different results (Zhuang vd., 2021). Several studies have examined the effects of MSCs on tumor cells. It has been determined that MSCs obtained from bone marrow increase the motility of prostate cancer cells through SDF-1 secreted from the damaged area (Mognetti, La Montagna, Perrelli, Pagliaro, & Penna, 2013). It has also been reported that MSCs promote in vivo glioblastoma bone metastasis by the realization of SDF-1/CXCR4 and SDF-1/CXCR7 coupling (Ma, Ye, Deng, Dee, & Chan, 2011). MSCs from human bone marrow mediate cell migration and invasion in hepatocellular carcinoma (HCC) and osteosarcoma (Fontanella vd., 2016). Human MSCs promote HCC tumor growth and metastasis. It decreases the number of natural killer (NK) cells in tumor niches by increasing the expression of IL-6 and TNF-α (Chen vd., 2019). In addition to its paracrine properties, MSCs differentiate into cancer-associated fibroblasts (CAF) and promote gastric cancer and colorectal carcinoma (CRC) progression (Shinagawa vd., 2010). CCL5 secreted by MSCs increased the motility of breast cancer cells (BCC) (Karnoub vd., 2007).

MSCs may help develop regenerative dental treatments, prevent further damage to the periodontium and regenerate lost tissue (Bartold, Gronthos, Ivanovski, Fisher, & Hutmacher, 2016). In a study, gingival mesenchymal stem cells were isolated and showed high accessibility, unlike other dental MSCs. At the same time, there is no need for tooth extraction to be obtained (Ge, Mrozik, Menicanin, Gronthos, & Bartold, 2012). Like dental MSCs, they have immunomodulatory capacities and

induce macrophage polarization. They reduce periodontal bone resorption in a mouse model by inhibiting osteoclasts (Nakao vd., 2021).

An important finding in autoimmune disease, in which the body's immune system attacks its own cells and organs, is the imbalance between Th1/Th17 and IL-10 producing Treg. In studies, autologous and allogeneic MSCs supported the healing of perianal fistulas, and mucosal inflammation was reduced by allogeneic administration of MSCs to CD and ulcerative colitis (UC) patients (Forbes vd., 2014; Hu vd., 2016).

Both autologous and allogeneic MSCs have demonstrated good tolerance for rheumatoid arthritis (RA) (Álvaro-Gracia vd., 2017; Shadmanfar vd., 2018). It is also applied for patients with Sjögren's syndrome, aplastic anemia and systemic sclerosis (van Rhijn-Brouwer vd., 2018; Zhang vd., 2017).

It is seen that MSC treatment used in several clinical kidney trial studies modulates the immune response and decreases the doses of immunosuppressive drugs used (Tan vd., 2012). MSCs cannot directly replace damaged kidney epithelial cells but can promote kidney regeneration (Bonventre, 2003). Despite promising results, it has become clear that MSC infusion may not be ideal for several reasons. Here, it was observed that MSCs were mostly kept in the microcapillary system of the lung and liver and could not reach the target organ (Burst vd., 2010). Intra-arterial delivery of MSCs directly to the kidney graft is feasible. There are studies in which MSCs were delivered to the kidney via ex-vivo machine perfusion (Brasile, Henry, Orlando, & Stubenitsky, 2019; Gregorini vd., 2017; Lohmann vd., 2021).

Because MSCs can differentiate into chondrocytes and have immunomodulatory functions, they can be used for intervertebral disc (IVD) regeneration under appropriate microenvironmental conditions. It has been determined that the IVD microenvironment has a strong effect on MSC behavior and differentiation. However, the contribution of MSCs to IVD environmental conditions is still a matter of debate. It is still unclear what factors are required for proliferation, differentiation and survival of MSCs (Esquijarosa Hechavarria & Richard, 2022). According to the results of the study, it has been shown that MSCs isolated from adipose tissue and bone marrow can differentiate into nuclei pulposus (NP)-like structures such as proteoglycan and collagen fibrils (Risbud vd., 2004; Sakai vd., 2005). Experiments of co-culture of MSCs with NP reveal proliferation of NPs and differentiation of MSCs into chondrogenic cells (Meisel vd., 2019). MSCs are capable of returning IVDD to the normal disc environment by causing the formation of extracellular matrix proteins such as aggrecan, collagen type-II and proteoglycan (Henriksson vd., 2009; Meisel vd., 2019; Sakai vd., 2005).

Acute Graft Versus Host Disease (GVHD) and Chorn are among the diseases that benefit most from MSCs due to their immunomodulatory effects in clinical practice. It has been determined that nerve conduction is improved with the administration of MSC to patients with metachromatic leukodystrophy, but its clinical reflection is not at the desired level. This is due to the fact that the administration time, mode of administration and dose of the cells could not be optimized (Voswinkel, Francois, Gorin, & Chapel, 2013).

In addition, these cells are promising in diseases such as MS and ALS due to their immunosuppressive and immunomodulatory effects (Mazzini vd., 2010).

The use of MSCs in pediatrics has several advantages. As the patients are small, the number of passages of cells in culture will be reduced. Thus, the patient is less exposed to negativities such as cell aging, cytogenetic disorders, and cancer (Nitkin & Bonfield, 2017).

Cardiovascular diseases constitute most of the studies performed with MSCs (Watt ve ark. It has been reported that the administration of MSC to a patient with acute myocardial infarction improved the left ventricle and the damage in the area decreased (Grinnemo vd., 2006).

In a different study, when haploidentic stem cell and MSC were given together to patients with acute myeloblastic leukemia, early neutrophil and thrombocyte development was observed without the development of GVHD (Lee vd., 2002).

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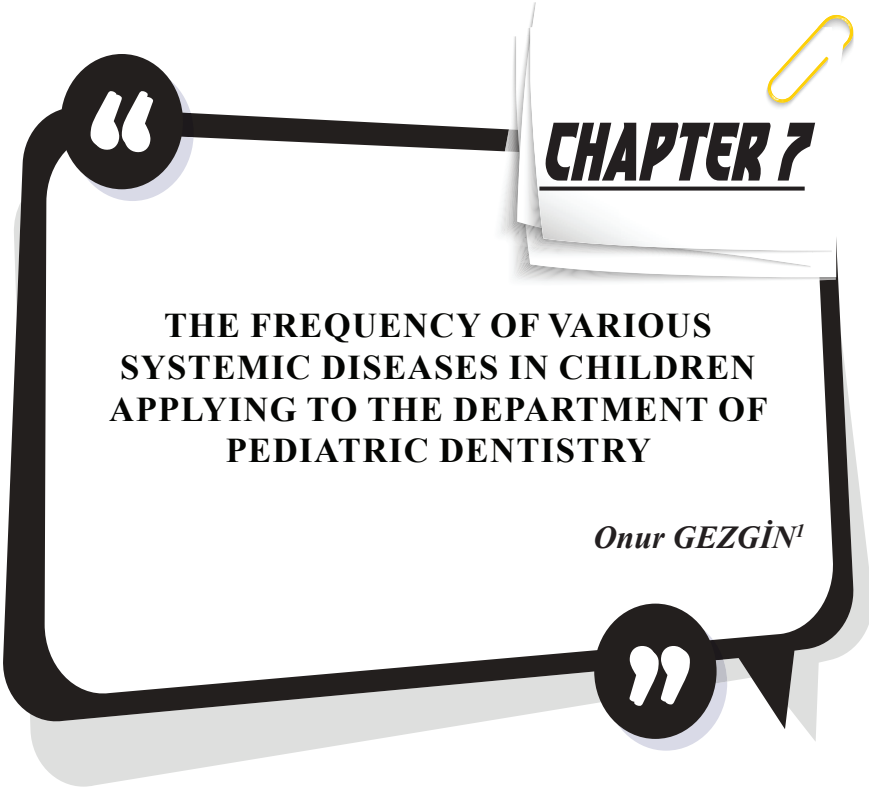

CHAPTER 7

THE FREQUENCY OF VARIOUS SYSTEMIC DISEASES IN CHILDREN APPLYING TO THE DEPARTMENT OF PEDIATRIC DENTISTRY

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1. INTRODUCTION

Dental treatments are risky practices that can affect the health of individuals with systemic disease, although these are practices that can be easily tolerated by healthy individuals. For this reason, information about the general health status, the existing systemic diseases and the drugs that patients use should be taken into consideration and necessary precautions for the complications should be taken in the process of treatment (Weichman 2002).

In addition, some diseases produce symptoms that can be observed inside the mouth. Diagnosis and planning of the treatment of systemic diseases which produce oral symptoms can be carried out in the dental clinic (Daley and Armstrong 2007).

It is also known that improvement of oral health has positive effects on the prognosis of some systemic diseases such as diabetes (Banyai et al. 2022).

Another issue to be aware of is that it is very common for patients not to report their medical history to dentists. Because patients generally do not know that it is important or do not think that this can affect their dental treatment (Cagetti et al. 2021).

For pediatric patients, detailed anamnesis taken from the parents should include medical and drug history of the patient. Children with systemic diseases should be consulted to medical doctors in appropriate specialty and dental procedures should be initiated with consideration of the response to the consultation (Doğan et al.2002).

The present study aims to determine the distribution rates of systemic diseases of the pediatric patients who applied for examination and treatment to Necmettin Erbakan University, Faculty of Dentistry, Departments of Pediatric Dentistry.

2. MATERIALS AND METHODS

In this study, oral anamneses and written consent forms that were collected from the parents of 3527 female and 4273 male, a total of 7800 patients, aged between 0-16 years, who have applied to Necmettin Erbakan University, Faculty of Dentistry, Department of Pediatric Dentistry of between September 2017 and March 2019 were examined retrospectively. The rates of the detected diseases according to age and gender were evaluated. The diseases identified in anamneses are classified under the main titles of cardiovascular, respiratory, neurological, psychiatric, endocrinological, gastrointestinal, urological, infectious, oncologic, dermatological, haematological, allergic diseases and syndromes. Diseases could not included in these groups were collected under the title of ‘other’.

3. RESULTS

It was determined that, of the 7800 children in the study group, 88.6% (6914) had no disease, 9.4% (735) had one disease, 1.7% (134) had two diseases, and 0.2% (17) had more then two diseases (Figure 1 and 2).

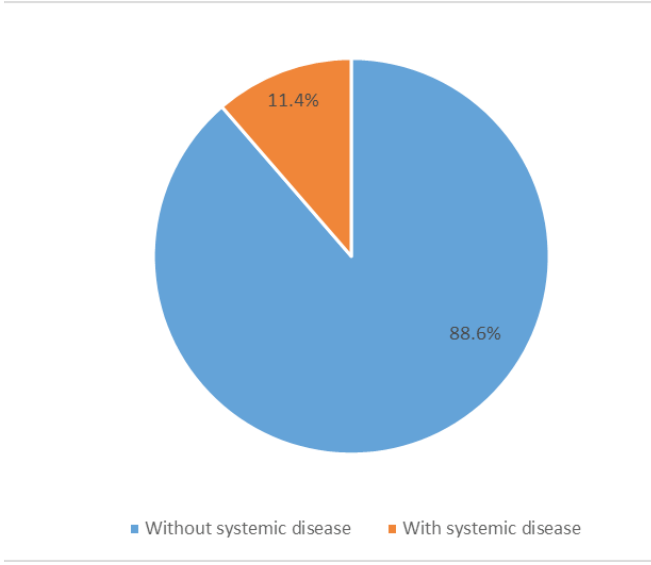

Figure 1: *Frequency of systemic disease in pediatric dentistry clinic.*

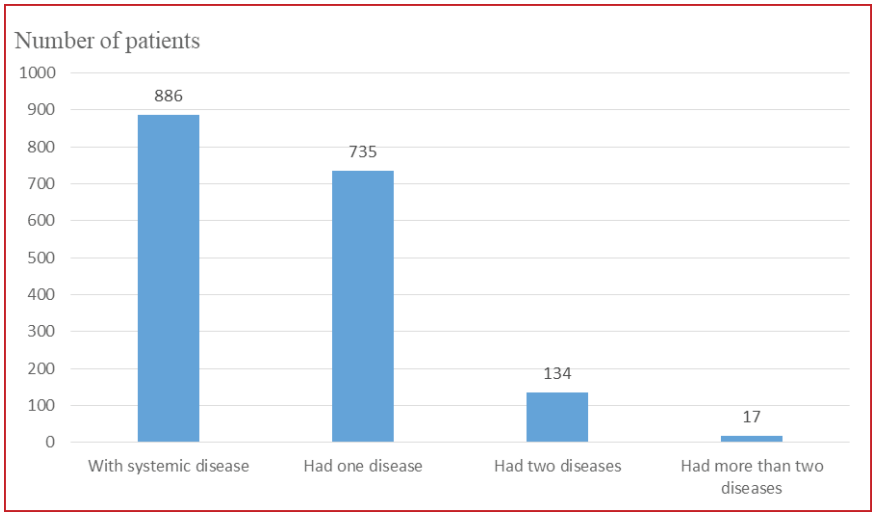

Figure 2: *The distribution of children with systemic disease who applied to pediatric dentistry clinic according to the number of diseases they have.*

Of the 886 children that have one or more diseases, 437 were female and 449 were male. The frequency of disease was found to be 12.4% in females and 10.5% in males (Figure 3).

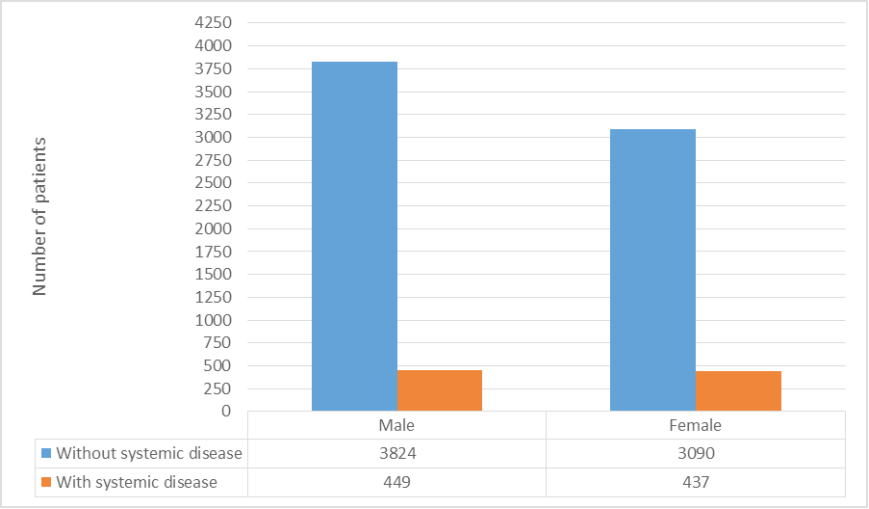

Figure 3: Number of with and without systemic diseases patients in pediatric dentistry clinic.

Cardiovascular disease is the most common disease group (2.02%). The frequencies of the other diseases that have been identified are already listed as follows; neurological, hematologic, allergic, endocrinological, respiratory, gastrointestinal, urological, syndromic, infectious, oncologic, psychological, dermatologic and others (Tablo 1).

The most prevalent disease group was cardiovascular diseases and cardiovascular diseases were present in 17.8% of children with disease and 2.02% of all children. Cardiovascular diseases seen in children in the study group include mitral insufficiency, mitral valve defects, aortic stenosis and dilatation, arythmia, myocardial infarction, cardiovascular operations and hypertension.

The second most common disease group was neurological diseases. Among the neurological diseases seen in the total of 105 children with 1.34% ratio, there were epilepsy, cerebral palsy, mental retardation, parkinsonism, microcephaly, hydrocephaly, autonomic nerve involvement, paralysis, developmental delay.

Another group with high frequency was haematological diseases. These diseases include familial mediterranean fever, thalassemia, immunologic deficiency syndrome, Glanzmann thrombasthenia, Von Willebrand disease, glucose 6-phosphate dehydrogenase deficiency,

factor VII deficiency, factor VIII deficiency, Henoch-Schönlein purpura, idiopathic thrombocytopenic purpura and leukocyte adhesion deficiency. 1.24% of the children included in the study had haematological disease detected.

Another common condition was allergies. As allergy causes; penicillin, paracetamol, pollen and several foods have been reported. The frequency of allergies in children included in the study was 1.11%.

There were 53 children with syndromic diseases. The syndromes that were found were autism, Down syndrome, Marfan syndrome, Canavan disease and Trisomy 18. The frequency of syndromes was 0.68%.

The frequency of endocrinological diseases such as diabetes, goiter, hypoparathyroidism and hyperparathyroidism was found to be 0.85% in the study.

The frequency of children with respiratory disorders was 0.79%. These include asthma and allergic asthma.

Diseases affecting the gastric, intestinal and liver were included in the group of gastrointestinal diseases and its frequency was 0.74%.

The group of urological diseases includes renal impairment and renal calculus, and the frequency of these diseases was 0.72%.

Infectious diseases group includes acute rheumatic fever, acute joint rheumatism and rheumatoid arthritis as well as hepatitis, HIV and CMV. The frequency of infectious diseases was 0,67%.

Oncologic diseases are also seen in children, albeit rarely. Acute lymphoblastic leukemia, ewing sarcoma and fibrosarcoma were detected in children included in the study. The frequency of these was 0.47%.

Diseases that affect the skin, such as epidermolysis bullosa, psoriasis and morbus behçet, were included in the dermatologic disease group and their frequency was found to be 0.14%.

There were 23 children who were treated psychologically in the study group and its frequency was measured as 0.29%.

Diseases that can not be included in all these disease groups were included under the title of ‘other’. Title of other includes patients with hearing, speech, physical or mental disabilities, children with hyperactivity, patients with attention deficit disorder, some splenic and pancreatic diseases, and situs inversus condition in which all visceral organs are reversed or mirrored from their normal positions.

Table 1: *Systemic diseases in patients who have applied to pediatric dentistry clinic, number of children with the disease and the frequency of the disease (more than one disease can be seen in one person) (table listed by frequency)*

Disease Group	Number of children with the disease	Frequency of the disease (%)
Cardiovascular	158	2.02
Neurological	105	1.34
Haematological	97	1.24
Allergic	87	1.11
Endocrinological	66	0.85
Respiratory	62	0.79
Gastrointestinal	58	0.74
Urological	56	0.72
Syndromic	53	0.68
Infectious	52	0.67
Oncological	37	0.47
Psychological	23	0.29
Dermatological	11	0.14
Other	21	0.27

4. DISCUSSION

Systemic diseases of the patient to be intervened and the dentist's approach to these diseases should be clearly known in order to prevent the complications that may arise or to minimize the damage as a result of dental treatments to be realized. The present study was designed to determine systemic diseases and the frequency of these diseases in 7800 children aged 0-16 years. Of the 7800 patients included in the study, 88.6% (6914) were found to be healthy and 11.4% (886) had at least one systemic disorder. In a study conducted for this purpose, in which 1002 adults aged between 18 and 81 years were evaluated, the rate of having systemic disease was found to be 36.5% (Aydıntuğ et al. 2010). In another study, 1400 patients aged between 12 and 82 years were examined and 39.2% of them had systemic diseases that could affect the clinical attitude of dentist in their anamnesis (Akpınar et al. 2012). It is thought that the difference in the frequency between others and this study is due to the difference in age groups. The 0-16 ages are that can be considered early age for the onset of many chronic systemic illnesses. In addition, some of the diseases may be at the beginning level, have not given symptoms and may not be diagnosed yet.

Cardiovascular diseases are the most common disease group in this study. In a study by Bodrumlu et al. (2008), cardiovascular diseases are the most frequent disease group with the rate of 41.1% (Bodrumlu et al. 2008).

When there is a disorder in the cardiovascular system, microorganisms that enter to the bloodstream due to dental treatment are likely to cause infective endocarditis by adhering to the defective area (Tomas et al. 2007). Staphylococcus and streptococcus are frequently specified as endocarditis agents, and these bacteria, which are located in the natural flora of the oral environment, can easily cause simple bacteremia even with toothbrushing. Dental procedures that require infective endocarditis prophylaxis according to the 2007 AHA (American Heart Association) recommendation include scaling and root canal treatment which involve manipulation of the gingival and periapical tissues, procedures that may involve perforation of the oral mucosa, and tooth extraction. Antibiotic prophylaxis is not recommended for local anesthetic applications to uninfected tissue, removal the suture, dental radiography, and for application or correction of removable prosthodontic or orthodontic appliances or brackets. Prophylaxis is not recommended for the exfoliation of primary teeth or after trauma of the lip and oral mucosa (Wilson et al. 2007).

Prophylaxis applications were formerly made in the form of antibiotic intake one hour before and 6 hours after the procedure. However, in accordance with the studies that were conducted, it was determined that the levels of antibiotics given before the treatment were maintained at adequate levels for 6-14 hours and the additional dose after the procedure was abandoned. Increase in resistance to antibiotic due to the additional dose is another reason to avoid this (TKD 2011).

The most common neurological disease in all populations is epilepsy (Fong et al. 2003). The incidence of dental trauma is increasing in individuals with neurological diseases due to the crises or insufficient muscle control. Inadequate motor functions for dental care also negatively affect oral hygiene. It is also known that medications used for epilepsy and cerebral palsy cause serious gingival enlargement. Angular cheilitis due to hypersalivation is frequently observed in patients with cerebral palsy (Reid et al. 2000). Patients with neurological diseases that have many dental problems who are unable to sit in a dentist's chair conformably for a sufficient period of time and who are unable to cooperate, and children whose mental state is inadequate are advised to be treated under general anesthesia if their general health status is suitable (Surabian 2001). In this study, 60 of 105 children with neurological disease were diagnosed with epilepsy.

In a similar study, the percentage of haematological diseases was found to be 1.09% (Aydıntuğ et al. 2010). In patients with haematological problems, routine dental treatment can be performed in cases with mild anemia, but treatment should be postponed until the blood levels of the patient reach the optimal levels in cases with severe anemia. It should not

be forgotten that there may be a delay in healing due to low tissue oxygen, and dental procedures should be conducted atraumatically. Oral lesions are more likely to occur in cases of leukopenia, and leukopenia is more likely to occur in the initial stages of leukemia. It should not be forgotten that in cases of thrombocytopathy, such as Glanzmann thrombasthenia or von Willebrand disease, medications which patients use may prolong bleeding times, haematological consultation should be provided to discontinue medications prior to dental procedures (Little et al. 2002). Factor infusions can be made in patients with factor deficiency according to the haematological consultation and dental procedures can be performed after the blood levels are increased to the appropriate level for the procedure. In von Willebrand disease, platelet dysfunction is present as well as factor VIII deficiency, and desmopressin, factor VIII, tranexamic acid, fresh frozen plasma (containing von Willebrand factor) and cryoprecipitates should be used before dental procedures in these patients. Patients with hematologic disease with a risk of postoperative bleeding should be monitored in the hematology clinic after procedures that involve bleeding (Coyle et al. 2013). In this study, the percentage of hematologic diseases was found to be 1.24%.

Autism is a neurodevelopmental disorder characterized by impairment in social relations, difficulties in communication, limited and repeater behaviors (Amaral et al. 2008). Complaints such as bruxism, self-injury with teeth and mouth dryness may frequently be seen in autistic individuals (Orellana et al. 2012). Acceptance of oral and dental care is more difficult than healthy individuals, and they can not perform an adequate and effective tooth brushing due to insufficiently developed manual dexterity (Jaber 2011). Because developmental impairments of autistic individuals cause great difficulty in relating to other people, and in understanding and following up information; dentists may fail to ensure patients' cooperation in the process of dental procedure, and sedation or general anesthesia may be required for dental treatment (Friedlander et al. 2006).

The typical facial appearance of patients with Down syndrome allows the dentist to easily identify the patient. It may be accompanied by heart diseases, leukopenia, neurological diseases and speech disorders. The patient may be treated in clinical conditions if cooperation can be ensured, with the assessment of accompanying conditions. General anesthesia is also an option if the mental condition does not allow and the cooperation can not be ensured (Demir ve Güler 2013).

Marfan syndrome is a genetic disorder affecting the heart, skeleton and ocular systems. Typical long bones, narrow face, high arched palate, irregularly placed teeth are observed. Heart conditions due to aortic dilatation that may lead to death are present. For this reason, consultation

is important (Lakshman et al. 2015).

Canavan disease is a rare autosomal recessive leukodystrophy that is seen with spongiform degeneration of white matter. Weak head and neck control, inability to sit unsupported, convulsions, hypotony, vision loss, macrocephaly, and mental retardation are typical findings of the disease. The life expectancy is not very long (Ellison et al. 2004).

Trisomy 18, also known as Edwards syndrome, is the most common chromosomal anomaly after Down syndrome. It includes serious psychomotor impairments and congenital defects that involve many systems (Tosun and Yanık 2004).

The syndromes identified in the study were autism, Down syndrome, Marfan syndrome, Canavan disease and Trisomy 18. Among them, autism is the most frequent. According to the information provided by the U.S. Centers for Disease Control and Prevention, the prevalence of Autism Spectrum Disorder was 1/150 in 2006, and 1/68 in 2012 (CDC 2014).

Anaphylaxis, as a result of an allergy, albeit very rare, is the most dangerous reaction. In addition to this, urticaria, angioedema, bronchospasm may also occur. Although penicillins are the most preferred, most effective and cheapest drugs among antibiotics, they are known as the most allergenic drugs among all drugs (Demirsoy 1997). Penicillin has been the most common drug that causes allergy in the study.

Oral manifestations of diabetes are overgrowth of salivary gland, hyposalivation, susceptibility to infection, delayed and abnormal wound healing, candidiasis, burning mouth, xerostomia and periodontal disease (Klokkevold et al. 2002). When patients with diabetes apply for dental treatment, their follow-up physicians should be consulted to check their blood glucose levels. All dental procedures can be performed on patients with controlled diabetes whose blood glucose level is between 120-200 mg/dL. Patients with a blood glucose level above 300 mg/dL are at high risk for ketoacidosis coma or hypoglycaemic shock in surgical procedures. Signs of hypoglycemia are tachycardia, sweating, tremor, and anxiety. Treatments should be performed after blood glucose and HbA_{1c} levels are decreased to the appropriate range (Miloro et al. 2004). The most common disease among endocrine system diseases is diabetes. According to the results of TURDEP-II study, prevalence of diabetes in adults is 13.7% in Turkey (TURDEP 2010). In the present study, diabetes was encountered at a lower rate than this. The reason is thought to be that the patient group is in the lower age range.

Although dental treatments do not carry risk in individuals with hypothyroidism and hyperthyroidism when the disease is under control,

they are at risk when the disease is not under control. Consultation to a physician is required to determine this. Patients with decompensated hypothyroidism may go into myxedema coma, and patients with decompensated hyperthyroidism may develop thyroid storm during the procedure. These require immediate intervention. In addition, children with congenital hypothyroidism may experience growth retardation, delayed tooth eruption (Rupesh et al. 2014). The rate of all these endocrinological diseases was 0.85% in the present study.

Asthma is a disease characterized by hypersensitivity of the respiratory tract mucosa and is characterized by chronic respiratory inflammation accompanied with recurrent cough, chest tightness, and dyspnea. It's a global problem that affects more than 300 million people worldwide. The incidence of dental caries, dental erosion, gingivitis and oral candida infections has increased in these patients due to the inhaler agents used as asthma treatment. For this reason, it is beneficial to take specific protective precautions before these conditions develop in these patients. In addition, an oxygen tank and bronchodilator should be available for a potential asthma attack that may develop during dental procedures. When asthma attack occurs, all operations should be stopped immediately and all materials in the mouth should be removed. The patient should be placed in a position where they can breathe easily, β_2 antagonist and oxygen should be administered if necessary, and the patient should be monitored. If there is no improvement, 0.001 mg/kg (maximum dose 0.3 mg) of epinephrine 1:1000 should be administered through subcutaneous route, and the patient should be referred to a hospital urgently (Thomes et al. 2010). In the present study, a rate of 0.79% was determined, which should not be overlooked.

Although gastrointestinal disorders do not frequently constitute a contraindication to dental procedures, drugs to be prescribed should be selected from the groups that will not trigger or exacerbate the patient's current illness (Ünsal et al. 2002).

Kidney is supports the regular functioning of the organs and systems. As a result of the renal disorders, the tendency to bleed due to dental procedures may increase and hypertension may develop. In addition, the use of drugs that are eliminated from the kidneys may cause some problems (Nowaider et al. 2003).

Dentists, dental assistants and patients that apply for dental treatment are at high risk for cross-infection. Because many pathogenic microorganisms isolated from oral fluids can be infectious. The infectious microorganisms in the mouth are hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex types I and II, HIV, staphylococcys, streptococcus and other viruses and bacteria [34]. For this reason, dentists and dental

assistants should be very careful to protect themselves and their patients from cross infection. In studies involving the general population, the frequency of hepatitis has been reported as 1.79 - 10.66% (Aydıntuğ et al. 2010). However, this rate was 0.67% in our study.

Patients with acute rheumatoid arthritis have migratoire infections in different joints. Infections do not cause permanent damage in joints outside the heart, but infections cause permanent damage when the heart valves are affected. Infective endocarditis prophylaxis must be performed before dental procedures in patients with this condition (Wilson et al. 2008).

Cancer treatments are often performed in the form of radiotherapy, chemotherapy, surgery, or combinations all of them. Before these treatments, it is very important that patients are trained in oral hygiene habits such as brushing teeth, using interdental brushes, dental floss and toothpaste containing fluoride. Products such as remineralizing solutions and toothpastes containing casein should be recommended as they ensure fluoride to penetrate into the plaque and enhance remineralization of enamel. All oral health problems of the patient should be remedied before the radiotherapy and if a tooth extraction is planned, it should be done before the procedure. In first week following chemotherapy, tooth brushing is prohibited, because even tooth brushing constitutes a risk of infection. Dental treatment should be avoided due to the defect in tissue healing after radiotherapy. During treatment, only urgent treatments can be performed with a consultation from oncologist (Kambek and Akal 2000).

The first finding in acute leukemia patients frequently is gingival enlargement; bleeding, ulcerations and infections in the mouth may also be present (Meyer et al. 2000).

Epidermolysis bullosa is a disease that involves numerous disorders characterized by recurrent bullae formation and erosion due to minor traumas as a result of increased fragility in the skin and mucous membranes. Tongue, buccal mucosa, palate, sublingual mucosa and gingiva are affected by the disease (Yogarajah et al. 2021). Dentists should perform atraumatic techniques during professional hygiene procedures. In addition, it should be remembered that individuals with this disease have a high risk of developing squamous cell carcinoma and should be under strict follow-up in this respect (Kramer et al. 2012).

Behçet's disease is a systemic inflammatory disease with recurrent oral and genital ulcers, skin and ocular involvement. It is known that poor oral hygiene increases the severity of the disease in these patients, and dentists are responsible to have a major role in improving this condition (Direskeneli 2006).

5. CONCLUSION

There is high possibility to seen systemic disease on children who consult to dental clinic for examinations and treatments. For this reason, dentists need to be careful for detection of disease and take necessary precautions while the anamnesis during the examination.

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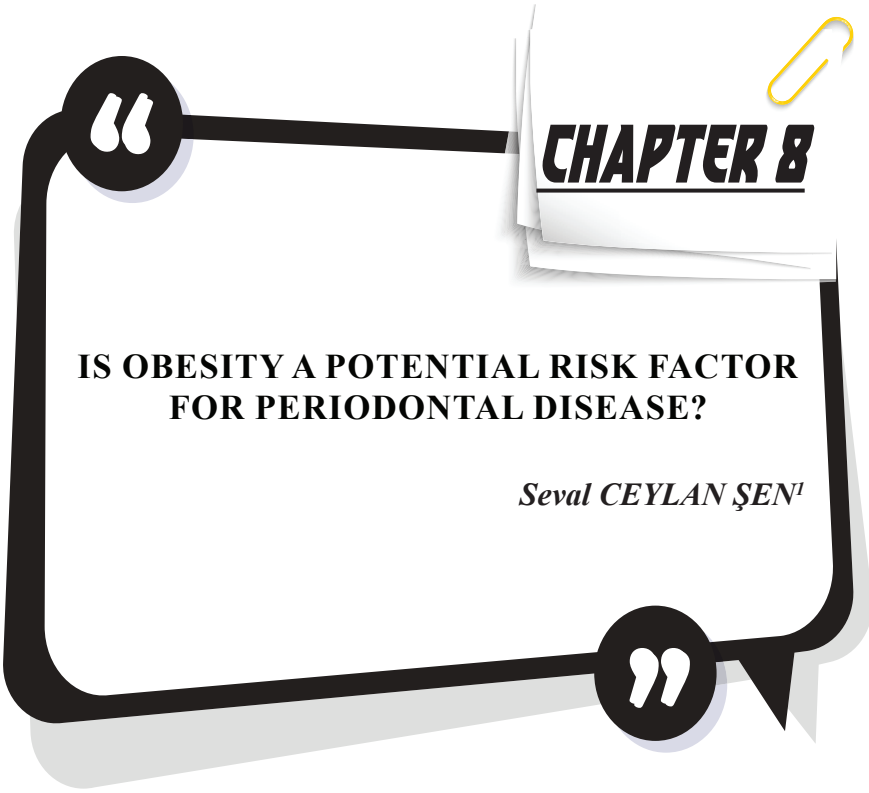

CHAPTER 8

IS OBESITY A POTENTIAL RISK FACTOR FOR PERIODONTAL DISEASE?

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Introduction

It is generally accepted that people's eating behaviors vary according to different emotions such as joy, joy, anxiety, sadness and anger. Despite the advances in technological developments, the decrease in the physical activity levels of people who complain about the lack of time, the differentiation of their eating and drinking habits due to many factors such as economic and socio-cultural reasons, cause the energy balance to be deteriorated. (Ritchie CS et al, 2002; Frıedman et al,2012). In addition, this situation can cause changes between the biological structures of people and their biological adaptation mechanisms. Disruption of this seemingly simple balance causes obesity, which is accepted as one of the most important public health problems, growing rapidly on a global scale. (Dämon et al,2016; Chourdakis et al, 2010; Landi et al, 2016).

Obesity; It is accepted as a chronic disease characterized by abnormal or excessive fat accumulation in adipose tissue, in which many factors such as social, behavioral, physiological, metabolic and cellular factors as well as molecular interactions play a role in its development. The prevalence of overweight and obesity; reached rates that should be taken into account in developed or developing countries. Increased immunological activity in adipose tissue prepares the ground for many systemic and chronic diseases; It is reported that it is especially effective in the development of insulin resistance and diabetes, and may also play an important role in the development of periodontitis (Benguigui et al, 2012; Palle et al, 2013).

Adequate and Balanced Nutrition

It is the intake of each of the energy and nutrients required for the growth and development of the body, the renewal and functioning of the tissues in sufficient and balanced amounts and their proper use in the body (Landi et a, 2016). Adequate and balanced nutrition; It is a basic requirement for individuals to reach their growth and development potential, to be protected from diseases and to lead a quality life (Steenhuis et al, 2009). For the health of the society, some basic nutritional principles should be determined and disseminated specifically to the society (O'Donovan et al, 2010).

Obesity

Obesity; in cases where the amount of energy taken from food exceeds the amount of energy consumed by metabolism and physical activity; resulting from the accumulation of excess fat in the body; It is a complex, multifactorial disease that is characterized by behavioral, endocrine and metabolic changes and requires treatment (Cancello and Clement, 2006). Obesity is also defined as a multifaceted disease that

develops depending on genetic, neurological, endocrine, nutritional, socioeconomic, psychological factors, lack of physical activity and gender (Crnobrnja et al, 2012). In obesity, an increase in the number of fat cells (hyperplasia) and/or an increase in their volume (hypertrophy) is observed. Increased Body Mass Index (BMI) is considered a risk factor for many chronic diseases, especially metabolic syndrome, and those with a BMI over 30 are considered obese (Cancello and Clement, 2006).

Lifestyle constitutes 70% and genetic factors constitute 30% of the total mortality risk in obesity. The effect of obesity on health has been shown to have the same consequences as twenty years of aging, but more harmful than the effect of smoking and alcohol (Meron et al, 2011; Adams et al, 2006). Hypertension, high cholesterol, type-2 diabetes mellitus (T2DM), periodontal disease, heart disease, heart attack and cancers are some of the health problems seen together with obesity. It has been shown that early-onset obesity or obesity in young adults continues into adulthood and can be much more harmful than obesity in middle age or old age (Cancello and Clement, 2006; Meron et al, 2011).

Obesity Prevalence and Epidemiology

Obesity is defined by the World Health Organization (WHO) as "abnormal or excessive fat accumulation in the body to the extent that it impairs health (James, 2008.). The prevalence of obesity is increasing rapidly all over the world, especially in developed countries. If the increase in the prevalence of obesity in the United States (USA) continues at this rate, it is expected that the prevalence of obesity in the USA will be 50% in 2025 (Ogden et al, 2012). According to various studies conducted on adults in Europe, the prevalence of being overweight is 32-79% in men, 28-78% in women; The prevalence of obesity varies between 5-23% in men and 7-36% in women (Ogden et al, 2012).

Etiology of Obesity

In the etiology of obesity, taking more energy than consumed is the most important factor (Meron et al, 2011). This seemingly simple situation, which requires the energy consumed for energy balance, basal metabolism and physical activity to be equal to the energy taken, becomes a complex equation as each component changes genetically (Kirchengast and Schober, 2006). Although the importance of genetic differences is known, the increase in obesity prevalence is explained by behavioral and environmental changes as a result of technological developments (Day and Loos, 2011).

In today's societies, obesity is a disease that threatens public health, reduces the quality and duration of life, with the psychiatric problems it

causes, as well as the increased risk of diabetes mellitus, ischemic heart disease, gallbladder disease, sleep apnea syndrome and certain cancer types (Adams et al, 2006.). In addition to genetic factors in the formation of obesity, environmental factors have also been of great interest to researchers (Meron et al, 2011; Day and Loos, 2011).

Genetic and Hormonal Effective Factors in Obesity Formation

Many studies have shown that many biological and genetic factors increase an individual's susceptibility to obesity (Day and Loos, 2011; Mathes et al, 2011). The BMI values of the children who were adopted at a young age were found to be much more compatible with the BMI values of their biological parents rather than their adoptive parents (Day and Loos, 2011). In studies on obesity and genetic factors, it was found that if both parents are obese, the child's chance of being obese is 80%, if only one of them is obese, the rate is 50%, and if both parents are not obese, the rate is 9% (Mathes et al, 2011).

Interest in the relationship of obesity with hormones and genes has increased in recent studies after the discovery of leptin. Leptin is a hormone that regulates long-term control of body weight (Coppari and Bjorbaek, 2012). Its most important function is known to provide weight control by reducing food intake as a result of the increase in blood level due to the increase in body fat tissue. In the absence of leptin, food consumption cannot be prevented, energy expenditure is reduced, and thus obesity occurs (Coppari and Bjorbaek, 2012; Falagas and Kompoti, 2006; Simons et al, 2005).

Although many genes associated with obesity have been discovered in animals, few genes associated with obesity have been identified in humans (Day and Loos, 2011; Fernández-Sánchez et al, 2011). It is the most frequently encountered genmelanocortin-4 receptor gene, which has been proven to be associated with obesity. This gene is found at a rate of 4% in obese people and acts as an appetite suppressant. A defect in this gene is associated with severe obesity (Day and Loos, 2011; Mathes et al, 2011; O'Rahilly et al, 2013).

Although it has been determined that a defect in a single gene causes obesity, polygenic defects have been identified in the vast majority of obesity cases in the population. Over 250 genes or chromosomal regions have been found to be associated with obesity, and the majority of them are thought to encode proteins associated with energy intake and expenditure.

Classification of Obesity

Body Mass Index

BMI value, which is the most practical and simple method in clinical applications, is calculated by dividing the body weight (kg) by the square of

the height (m^2). The classification made by the World Health Organization according to BMI is given below.

1- Underweight (BMI less than 18.5 kg/m²),

2- Normal weight (BMI between 18.5 and 24.9 kg/m²),

3- Overweight (BMI between 25.0 and 29.9 kg/m²),

4- Obese (BMI greater than 30.0 kg/m²) (Meron et al, 2011).

The obese group is also divided into 3 subgroups;

a) Class I (BMI between 30-34.9 kg/m²),

b) Class II (BMI between 35-39.9 kg/m²)

c) Class III (BMI greater than 40.0 kg/m², morbidly obese). (James, 2008; Ogden et al, 2012).

Having a BMI over 27 kg/m2 may increase the risk of some chronic diseases. (Peelman et al, 2004).

Obesity and Systemic Complications

One of the issues that preventive medicine has emphasized most recently is childhood obesity (Hancox et al, 2004). With its increasing incidence, obesity is a disease with high mortality and morbidity in childhood and adulthood (Kirchengast and Schober, 2006). A very close relationship was found between obesity in this period and various diseases in adulthood (Kirchengast and Schober, 2006). Many complications of obesity including cardiovascular, endocrinological, gastrointestinal, immunological and neurological have been reported. One of the most important and feared complications is metabolic syndrome, which affects many tissues and organs and includes many chronic diseases.

Obesity and Systemic Inflammation

Adipose tissue is a complex, active endocrine organ (Coppack, 2001). In addition to its main cells, adipocytes, it also includes connective tissue matrix, nervous system, stromavascular cells and cells of the immune system (Manios et al; 2004). Adipose tissue as an endocrine organ has two important functions, such as secreting cytokines with metabolic effects on certain cells and producing enzymes that participate in steroid hormone metabolism. Adipokines produce hormone-like structures such as leptin, adiponectin, resistin, some cytokines such as TNF-α, interleukin-1β, IL-1α and IL-6, proteins effective in vascular hemostasis, molecules that regulate blood pressure and accelerate angiogenesis and many different molecules such as acute phase protein (Coppack, 2001; Haslam, 2007). Adipokines secreted from adipose tissue contribute to low-level systemic

and vascular inflammation against gram-negative [Gr (-)] bacteria and other inflammatory mediators and affect the metabolism of the whole body (Manios et al; 2004).

In addition to the functions of storing energy and fat-soluble vitamins, physical protection and heat generation, some proteins, namely adipokines, secreted from adipocytes and connective tissue cells between adipocytes have been shown to have autocrine, paracrine and endocrine effects. (Cekmez et al, 2011). These substances are known to play a role in body balance, immune response, blood circulation and steroid metabolism (Gimble and Guilak, 2003).

Apart from its effects on food intake and energy, leptin which is one of the most important adipokines has been shown that plays a role in many physiological events such as reproductive system, angiogenesis, hematopoiesis, immune system, lipid metabolism, insulin effect, ovarian function, sympathetic activation, gastrointestinal function, brain development and bone metabolism. (Coppari and Bjorbaek, 2012; Haslam, 2007; Codoñer-Franch et al, 2011).

Pathogenesis of Periodontal Disease

Periodontal diseases are chronic diseases that affects the supporting tissues of the teeth and can cause tooth loss. The primary etiological factor of periodontal diseases is the pathogenic bacteria in the microbial dental plaque (MDP) which accumulates on the tooth surface and is in close contact with the gingival margin (Highfield, 2009; Novak et al, 2015). Many studies have revealed that the increase in TNF-α and interleukin 6 levels, which are proinflammatory cytokines, which are an important part of the host response in the development of the disease, and oxidative stress contribute to periodontal tissue destruction (Kornman, 2008; Blasco-Baque et al, 2016; Frodge et al, 2008).

Mechanisms thought to cause tissue destruction in periodontal disease are classified as;

• Bacteria and host interaction,

• Under the influence of genetic and environmental factors; proteolytic enzymes and inhibitors that play a role in the destruction and repair of cells and molecular components,

• Disruption of the balance between reactive oxygen species (ROS) and antioxidant defense system (Novak et al, 2015; Kornman, 2008; Barros et al, 2016).

In relation to this, it is thought that various systemic conditions and diseases may cause predisposition to periodontal disease by affecting the

host tissue response, and on the other hand, periodontal infection may be a risk factor for some systemic diseases (Kornman, 2008; Blasco-Baque et al, 2016).

Disruption of the balance between reactive oxygen species (ROS) and the antioxidant defense system also generates a risk for periodontal disease. (Katrın et al, 2016). If the oxidative balance is disturbed for various reasons, harmful effects of free radicals may occur (Zohrabian and Abrahams, 2015). Oxidative stress (OS); It can cause damage to important cell components such as lipids, proteins, deoxyribonucleic acid (DNA), enzymes and carbohydrate (CH) structures (Kornman, 2008; Frodge et al, 2008). OS also contributes to damage indirectly by stimulating the release of proinflammatory cytokines. (Novak et al, 2015; Barros et al, 2016). The presence of chronic inflammation may locally disrupt the oxidative balance, as well as factors such as smoking and obesity, which cause systemic OS increase, may also disrupt the oxidative balance. (Zohrabian and Abrahams, 2015; Bhat et al, 2015; Suresh et al, 2014).

The Relationship between Obesity, Periodontal Health and Disease

There are various theories that try to explain the relationship between obesity and periodontal diseases, the generally accepted view is that adipocytes (especially in the abdominal region) act as an active endocrine organ and contribute to the pathogenesis of periodontal diseases by secreting intense amounts of various proinflammatory cytokines and hormones (Suresh et al, 2014).

Another view is that obesity affects periodontal disease through the cell membrane and its structural functioning (Eun-Jin et a, 2011). The cell membrane is basically of phospholipid structure and its formation is affected by the type and quality of ingested lipids. High lipid foods or fatty acids suppress the immune function, resulting in reduced bactericidal effect in humans (Han et al, 2010). Increased body fat can induce a hyperinflammatory response in periodontal disease through mediators released from adipose tissue, and with this negative effect, it can make the individual weaker against microbial dental plaque (Han et al, 2010; Saxlin et al, 2010).

There are many studies in the literature that there may be a relationship between obesity and periodontitis (Saxlin et al, 2010; Fentoğlu et al, 2009; Shimazaki et al, 2010). The first study to investigate the possible relationship between periodontal disease and obesity was an animal study by Perlstein et al. in 1977 (Suresh and Mahendra, 2014). In this study, histopathological changes in the periodontium of hereditary obese mice were observed. Ligature-induced periodontitis was induced in obese and

normal-weight mice, and bone destruction was more severe in obese mice compared to non-obese mice during the observation period (Suresh and Mahendra, 2014; Azuma et al, 2011). In addition, it has been observed that in healthy oral conditions where plaque is eliminated, obesity does not spontaneously cause pathological periodontal changes, but periodontal inflammation and destruction in the presence of plaque is much more severe in obese animals (Suresh and Mahendra, 2014; Azuma et al, 2011; Tomofuji et al, 2009).

Bawadi et al. (Bawadi et al, 2011) reported that being at a normal weight, increasing exercise, and a high-quality diet reduce the prevalence of periodontitis. The findings suggest that a high-quality healthy diet reduces the risk of periodontal disease by 40%.

In the literature, BMI, waist-hip ratio, waist circumference measurement are recommended as risk markers that should be evaluated with known risk factors (Pataro et al, 2011; Haffajee and Socransky, 2009). A strong positive correlation was obtained between BMI and periodontal treatment requirement in studies. (Haffajee and Socransky, 2009; Han et al, 2012). In addition, it was observed that the need for periodontal treatment increased as the waist-hip ratio and body fat ratio increased (Pataro et al, 2011). It has also been reported that a wide waist circumference measurement increases the risk of periodontal disease by 127% (Haffajee and Socransky, 2009).

In a study, it was reported that the prevalence of periodontal disease increased with abdominal obesity and increased body fat ratio in young adults (Gürgan et al, 2012). In parallel with this finding, it has been reported that increased body weight and waist circumference increase the risk of periodontitis in young adolescents aged 17-21 years who do not smoke. (Zuza et al, 2011). In a study, it was reported that a 5% increase in body fat in humans may increase the risk of periodontitis by 30% (Sanders et al, 2009).

The relationship between obesity and periodontal disease can also be affected by gender. In one study, it was reported that obesity poses a greater risk for periodontal disease in non-smokers under the age of 40 (1.86 times the risk), and the risk is higher in women than in men (Khader et al, 2008). Age in systemic healthy individuals with periodontitis; while it did not affect serum IL-6 level, gender and BMI were associated with serum IL-6 concentration; it has also been reported that the serum IL-6 level is high in patients with an increased BMI value (Ylöstalo et al, 2008). In a study, it was reported that the serum IL-6 level was higher in obese women (Raunio et al, 2007.).

Many epidemiological and clinical studies draw attention to the possible relationship between obesity and periodontal tissue destruction (Gürgan et

al, 2012; Khader et al, 2008; Ylöstalo et al, 2008). However, the biological mechanisms that cause this relationship have not been fully explained. The traditional view towards this relationship is that cytokines and hormones released in excessive amounts from dense adipose tissue may play a role in periodontal tissue destruction by causing a hyperinflammatory response (Coppack, 2004). It is known that adipose tissue is not only a passive store of triglycerides, but also produces active molecules called adipocytokines or adipokines (Wisse, 2004). In addition, studies have shown that obesity can affect periodontal disease by affecting blood lipid and sugar levels (Chaffee and Weston, 2010; Sanders et al, 2009).

Barros et al. (Barros et al, 2016) reported that there is a correlation between TNF-α level in gingival groove fluid and BMI values. Studies show that the pattern of fat distribution in the body plays a very important role in the relationship with periodontitis (Bağrıaçık et al, 2009; Genco et al, 2005). It has been reported in many studies that the immunological activity in the increased adipose tissue may play an important role in the development of both insulin resistance and diabetes and periodontitis (Coppari and Bjorbaek, 2012; Genco et al, 2005; Chee et al, 2013). In a study aiming to examine the relationship of psychological state with obesity and periodontitis, it was claimed that overweight individuals, especially women, generally do not have a positive outlook on life, smoke heavily, have an anxious and depressive personality, and this situation indirectly increases the prevalence of periodontitis (Han et al, 2010.).

Recent studies have shown a low prevalence of periodontitis in normal-weight individuals who regularly do physical exercise (Teske et al, 2014; Bawadi et al, 2011; Sanders et al, 2009). In addition to studies evaluating the prevalence of periodontitis in obese individuals, there are also studies evaluating the severity of periodontitis (Bawadi et al, 2011; Sanders et al, 2009). In one study; obesity has been shown to be associated with the presence of deep periodontal pockets, independent of glucose tolerance status. In addition, a relationship has been shown between increased attachment loss and obesity (Penoni et al, 2015).

In the study performed by Pataro et al. in 594 female patients in 2010, the percentage of bleeding on probing, the rate of pocket depth >4mm, and the prevalence of periodontal disease were found to be statistically significantly higher in obese and overweight women compared to women with normal BMI values (Pataro et al,2011). In another study conducted in women, a relationship was found between BMI, waist/hip ratio and body fat ratio and a high increase in pocket depth (Suvan et al, 2011). In the study conducted by Tomofuji et al. in 2787 male and 803 female patients over a 5-year period; It has been reported that the rate of periodontal disease development is 1.30 and 1.44 times higher in individuals with a BMI of 25-

30 and ≥30 in men, compared to individuals with a BMI of ≤22, while it is 1.70 and 3.24 times higher in women. The incidence of periodontal disease was found to be higher in obese women than in men (Tomofuji et al, 2011).

On the other hand, studies conducted in recent years have shown that obesity, which is associated with increased ROS production from increased adipose tissue into the systemic circulation, and decreased enzymatic and non-enzymatic antioxidant levels; suggests that it may contribute to the oxidative destruction of periodontal tissues by also affecting local oxidative parameters (Katrın et al, 2016; Duarte et al, 2010; Li et al, 2015). Inflammation in obesity is characterized by an increase in especially IL-6, TNF-α secretion and leukocyte infiltration, indicating that obesity has a negative effect on periodontal disease through inflammatory pathways (Katrın et al, 2016; Li et al, 2015).

Recent studies on resistin, one of the adipokines, show that resistin plays an important role in the inflammatory process by causing an increase in mononuclear cells and macrophages in peripheral blood (Saito et al, 2008; Mittal et al, 2015). It has been reported that circulating resistin levels may increase as a result of subclinical inflammation of periodontal tissues (Saito et al, 2008). It was determined that resistin production increased when macrophage was stimulated in vitro with endotoxin or proinflammatory cytokines. (Mittal et al, 2015).

In many studies investigating the effects of another adipokine, leptin, on periodontal health, it has been demonstrated that the immunomodulatory role of it (Mittal et al, 2015; Shimada et al, 2010; Selvarajan et al, 2015). Studies on leptin have shown that while the leptin concentration is above a certain value in both healthy and marginal gingivitis inflammation, this concentration decreases both in the gingival tissue and in the gingival groove fluid with the progression of periodontitis (Li et al, 2015; Selvarajan et al, 2015). Considering these results, it can be concluded that leptin is more effective in maintaining periodontal health.

Adiponectin, another adipokine that acts like leptin, has been shown to be associated with modulation of the inflammatory response (Gonçalves et al,2015). It has been reported that adiponectin reduces the inflammatory response mediated by TNF-α and inhibits the phagocytic activity of macrophages and TNF-α production (Sete et al, 2015; Nokhbehsaim et al, 2014). It has also been shown that adiponectin controls the response of macrophage-like cells to toll-like receptor ligands, and inhibits osteoclast formation stimulated by lipopolysaccharides of Aggregatibacter actinomycetemcomitans (Buduneli et al, 2014; Nokhbehsaim et al, 2014.).

It has been reported that visfatin has the capacity to inhibit neutrophil apoptosis and therefore plays a role in the persistence of inflammation (Oki

et al 2007). Visfatin has also been shown to increase leukocyte activation, adhesion molecule synthesis and proinflammatory cytokine production (Kukla et al, 2011). Özcan et al. evaluated the correlations of increased visfatin level in gingival groove fluid with Porphyromonas Gingivalis (P.gingivalis) and Ebstein-Barr Virus (EBV). reported that there was a positive correlation between P.gingivalis and visfatin level and there was an increase in the visfatin level in the patient group with EBV virus (Özcan et al, 2015).

Chemerin, like visfatin, is thought to have a potential role in the immune response by acting as a chemotactic for antigen-presenting cells in inflammation or tissue damage (Zabel et al, 2005; Wittamer et al, 2003). Patnaik et al. in their study in which they examined the gingival groove fluid and chemerin level in the eye of patients with periodontitis and diabetes; It has been reported that there is an increase in the level of chemerin in the gingival groove fluid of periodontitis patients, and this increase is also positively correlated with periodontal parameters (Patnaik et al, 2015) .

Contrary to the studies mentioned above, there are also studies reporting that there is no relationship between obesity and periodontitis. Suvan et al. reported that the measurement of BMI and waist circumference was not associated with the severity of periodontitis in their 2005 study on the Thai population (Suvan et al, 2015). In some studies conducted in Denmark, it has been suggested that there is no significant relationship between obesity and periodontal disease (Linden et al, 2007; Ylöstalo et al, 2008). Saxlin et al. reported that there was no statistically significant relationship between overweight (26<BMI<30) and obesity and the number of teeth with increased periodontal pocket depth in their 4-year longitudinal study conducted in 396 individuals aged 30-59 years (Saxlin et al, 2010).

Conclusion

In order to create a healthy society, it is necessary to protect and improve the health of all individuals, to increase the quality of life and to raise awareness of the society on this issue. In this respect, it is of great importance to solve nutritional problems, create a lifestyle for the prevention of chronic diseases related to nutrition, improve and improve environmental conditions, and ensure access to and consumption of healthy food.

Obesity is one of the most important health problems of our age, the prevalence of which is increasing all over the world and especially in developing societies, and together with the diseases it causes, it has the most important place in the national economy in health expenditures. There

are studies reporting that obesity is a risk factor for many chronic diseases as well as oral diseases and especially periodontitis, and is even the second most important acquired risk factor for periodontal diseases after smoking. However, although the relationship between obesity and periodontal diseases has been investigated for many years, the role of obesity in the pathogenesis of periodontal diseases is still controversial. Studies focus on inflammation in the relationship between periodontal disease and obesity, based on the hypothesis of the presence of inflammatory components in both diseases. Although the mechanism of the increase in the incidence of infection and weak antibody response in obesity is not known exactly, it is thought that cytokines and hormones secreted from adipose tissue and involved in the inflammatory process, as well as local factors such as low oxygen pressure, may be associated with deterioration in the host response and therefore with tissue destruction and periodontitis.

Although many studies have been conducted on nutrition, obesity and oral health, new literature on the patho-physiological mechanism is published every day. A full understanding and correct analysis of the role of nutrition in the deterioration of the balance between health and disease is very important for the development of new treatment strategies in the future.

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CHAPTER 9

INVESTIGATION OF AGENTS CONTAINED IN TOOTH WHITENING PRODUCTS: REVIEW

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1. Introduction

The inorganic content of teeth consists of calcium phosphate in the form of hydroxyapatite " $Ca_5(PO4)_3(OH)$ " (Epple, Meyer, & Enax, 2019). Enamel tissue is a highly mineralized texture with an organized microstructure consisting of % 97 hydroxyapatites (P. W. Brown & Constantz, 1994; Legeros & characterization, 1981). The hardness and breaking strength of enamel derives from the structure formed by connecting hydroxyapatites to each other through organic matrix. The hardness and breaking strength of enamel derives from the structure formed by connecting hydroxyapatites to each other through organic matrix. Additionally, the enamel surface is coated with pellicle, which contains saliva proteins, carbohydrates, and lipids (Hannig, Hannig, & Kinderzahnheilkunde, 2007; Jowett, Marlow, & Rawlinson, 2013). The dentine tissue located at the under of the enamel layer is a bone-like tissue, which is approximately % 70 hydroxyapatite by volume and is rich in protein, mostly collagen, and the remaining part is water (Forien et al., 2015; Forien et al., 2016).

Pure hydroxyapatite is colorless / white. In later ages, natural enamel is eroded, thinned and its transparency decreases due to erosion etc. In addition, the tooth color begins to darken (Algarni et al., 2018). Seperately, the natural white color of the teeth may be lost due to factors such as wine, tea, coffee, smoking etc. (A. J. J. o. d. Joiner, 2010). Whitening products for home use (eg toothpastes containing bleach, household whiteners etc.) and professional applications in dentistry (eg office whiteners or professional teeth cleaning) try to solve this problem. In this context, whitening applications are one of the frequently applied treatments to increase the visual whiteness of teeth.

The purpose of production and use of modern oral care products is to prevent and treat dental caries and problems in surrounding tissues, which are common problems of all societies in the world (Fejerskov & Kidd, 2009; Kassebaum et al., 2015; Meyer & Enax, 2018). Modern toothpastes contain calcium phosphates and surfactants such as fluoride (sodium fluoride, aminfluoride, etc.), chlorhexidine, tin, zinc salts, hydroxyapatite or amorphous calcium phosphates for the prevention of caries and periodontitis; it also has formulations containing different abrasives for an effective plaque removal (Epple et al., 2019).

Many substances have been used in whitening products from past to present (Table 1). Nowadays, studies to increase the effectiveness and permanence of whitening products continue rapidly today.

Table 1: Classification of whitening agents

CLASSIFICATION OF WHITENING AGENTS						
1.Abrasives	1.1.Hydrated Silica	1.2.Perlite	1.3.Alumina	1.4.Calcium Carbonate	1.5.Dicalcium Phosphatedihydrate	1.6.Calcium Pyrophosphate
2.Preventing Redeposition Agents	2.1.Polyphosphate	2.2.Sodium Citrate				
3.Calcium Phosphates	3.1. Hydroxyapatite					
4.Colorants	4.1. Blue Covarine					
5.Enzyme/ Proteases	5.1.Papain	5.2.Bromelain				
6.Peroxides	6.1.Hydrogen Peroxide	6.2.Calcium Peroxide	6.3.Carbamide Peroxide			
7. Polyaspartate	7.1.Sodium Polyaspartate					
8. Surfactants	8.1. Sodium Lauryl Sulfate					
9. Borates	9.1.Sodium Perborate					

The purpose of this review is; to give information about the structure of active ingredients in commonly used teeth whitening products, their effectiveness in whitening and potential risks.

CLASSIFICATION AND REVIEW

1.Abrasives

Insoluble chemicals called abrasives are added to toothpaste to assist remove stains, plaque, and food debris physically. Abrasives have been used in toothpaste for over 2000 years, when preparations were tested using bones and other structural skeletons. (Forward, 1991). An important stage in determining the whitening effectiveness of toothpaste is to determine its clinical effectiveness in terms of removing stains on the tooth surface(A. J. I. d. j. Joiner, 2006).

Tooth brushing is the most common oral hygiene practice to prevent carious lesions. Whitening toothpastes on the market contain abrasives that remove stains on the enamel surface. In general, the cleaning efficiency of the teeth is directly related to the hardness, size, shape, concentration of the particles in the toothpaste, the properties of the toothbrush and the pressure applied during brushing the teeth. Materials used in dentistry to clean teeth effectively should provide a degree of abrasivity (Hefferren, 1998; A Joiner et al., 2002). The International Organization for Standardization (ISO) determined the value of dentin abrasiveness in the toothpastes used so that the Relative Dentin Abrasivity (RDA) does not exceed 250 (Pickles, Joiner, Weader, Cooper, & Cox, 2005; Stookey, Burkhard, &

Schemehorn, 1982). The abrasiveness of whitening toothpastes is often medium (60-100 RDA) or high (RDA> 100) (Maldupa, Brinkmane, Rendeniece, & Mihailova, 2012). Abrasives in toothpaste come in a variety of formulas, with some formulations being more abrasive than others. As a result, today's commercially available toothpastes contain a wide range of abrasive systems, including precipitated silicas of various particle sizes, alumina, dicalcium phosphate dihydrate, insoluble metaphosphate, calcium carbonate, and other polishing agents. Toothpaste can damage the tooth structure significantly in order to accomplish good cleansing and stain removal (Hirschfeld, 1939). Therefore, these considerations should not be overlooked when choosing an abrasive or blend containing abrasive for inclusion in products (Bull, Callender, Pugh, & Wood, 1968).

The most commonly used abrasives in tooth whitening products are;

1.Hydrate Silica (H_4SiO_4)

2.Perlite

3.Alumina (Al_2O_3)

4. Calcium Carbonate ($CaCO_3$)

5. Dicalcium Phosphate Dihydrate ($CaHP0_4.2H_20$)

6. Calcium Pyrophosphate ($Ca_2P_2O_7$)

1.1.Hydrate Silica (H_4SiO_4)

A silica (silicon dioxide) derivative, hydrated silica is a substance that makes up around 12% of the earth's surface. H4SiO4 is the most widely used formula (Iler, 1964). Silica is found in a variety of forms, including sand and obsidian. This component is an odorless, tasteless, white gelatinous material that is chemically inert in aqueous form. Amorphous silicon dioxide, silicic acid, and silica gel are all terms for the same thing (Belsito, Klaassen, Liebler, Marks Jr, & Shank). Silica is found in a variety of forms, including sand and obsidian. This component is an odorless, tasteless, white gelatinous material that is chemically inert in aqueous form. Amorphous silicon dioxide, silicic acid, and silica gel are all terms for the same thing. (Go).

1.2.Perlite

Perlite is a chemically inert, amorphous, glassy silicate with volcanic origins and neutral pH (A. J. I. d. j. Joiner, 2006). It contains 2-6% water in its structure and when heated to 870^{o}C, it creates a foam-like structure. This structure is ground to produce fine flat particles (L. Collins et al., 2005). Perlite; it is routinely added as abrasive for relatively low abrasiveness in dental cleaning products with stain removal and polishing

properties (Lutz, Sener, Imfeld, Barbakow, & Schüpbach, 1993). Joiner et al. (A Joiner et al., 2002), it was shown that silica-based toothpastes increase their stain removal properties.

1.3.Alumina (Al_2O_3)

Alumina is a white / colorless, crystalline substance produced from bauxite, a naturally occurring metal containing variable amounts of aqueous aluminum oxide (Ramkumar & Sugumaran, 2016; SARIDEDE & BİROL, 2005). Alumina is used as a raw material for various ceramic products and as an active ingredient in chemical processing (SARIDEDE & BİROL, 2005; Willhite, Ball, & McLellan, 2012). Alumina obtained from bauxite can be used in toothpaste production as an abrasive % 7. (Teşkilatı & İhtisas, 2000). The term alumina is sometimes used in a general way to refer to a series of aluminum oxide, oxide hydroxide, and trihydroxide compounds. "Corundum", a special name of Al_2O_3, is produced by calcination of aluminum trihydroxides and oxide hydroxides. This material can be abrasively added into toothpastes with particle sizes between 1.0 - 3.5 μm and 6 m2 / g surface area(Wills & Duncan, 2004).

1.4.Calcium Carbonate ($CaCO_3$)

The chemical molecule "CaCO3" stands for calcium carbonate (Tai & Chen, 1998Calcite and aragonite minerals (found notably in limestone, a form of sedimentary rock made entirely of calcite) are a prevalent substance in the shells of marine animals, snails, and eggs in rocks and as the principal component of pearls (Al Omari, Rashid, Qinna, Jaber, & Badwan, 2016). Calcium carbonate is the active component of agricultural lime and occurs when calcium ions in water react with carbonate ions to form lime (Abd & Technology, 2014). It is used medically as a calcium supplement or antacid, but its excessive consumption can cause digestive upset. It is a dangerous substance (Maton & Burton, 1999). In the 1850s, abrasive calcium carbonate was first included to toothpaste formulations (Lynch & Ten Cate, 2005). Calcium carbonate has the ability to neutralize plaque acids, and it has been demonstrated by scanning electron microscopy that it is maintained within the plaque when used as an abrasive in toothpaste (Davis & Fellowes, 1981; Lynch & Ten Cate, 2005). Mainwaring et al. (Mainwaring & Duke, 1980) showed that long-term use of a calcium carbonate-based toothpaste can provide a significant increase in plaque pH compared to the use of a non-buffering control toothpaste. The size of calcium carbonate particles in toothpastes produced for whitening changes. While some vary in the range of 1-12 μm, the size of calcium carbonate particles reduced to nanoscale is smaller. Anisja et al. (Anisja, Indrani, & Herda, 2017), in their study on the effect of calcium carbonate particle sizes on the surface roughness of restorative materials, they found

that paste contents with a large particle size created more roughness on the surface compared to nanoparticle toothpastes.

1.5. Dicalcium Phosphate Dihydrate (DPCD)

Dicalcium phosphate dihydrate ($CaHPO_4.2H_20$) is a precipitate produced by reacting calcium chloride with dibasic sodium phosphate. In the form of anhydrous calcium phosphate, abrasive in toothpaste, chewing gums, snacks etc. and has been reported to be effective in preventing tooth decay in combination with fluorine compounds. The addition of DCPD to snacks and chewing gum reduces the incidence of caries in children (Finn & Jamison, 1967; Stralfors, 1964). Toothpastes containing DCPD as abrasive are available in the market with a whitening effect. These toothpastes often contain sodium monofluorophosphate as the main fluoride source and have been clinically proven effective in preventing caries (Gaffar, Blake-Haskins, & Mellberg, 1993). Tooth whitening agents with low pH values may cause demineralization on the tooth surface, lower surface hardness and morphological changes in teeth. Adding DCPD to bleaching materials containing 35% hydrogen peroxide increased the pH (4.7-4.8) and reduced the negative properties of whitening agents (Jeoung, Oh, & Shim, 2010).

1.6. Calcium Pyrophosphate ($Ca_2P_2O_7$)

By heating the dicalcium orthophosphate in the range of 100-300 ^oC, the water causing hydration is removed and anhydrous dicalcium orthophosphate is obtained. Calcium pyrophosphate ($Ca_2P_2O_7$) salts are obtained by heating in anhydrous dicalcium orthophosphate above about 300 ^oC (Broge & Grabenstetter, 1959). Calcium pyrophosphate is a chemical compound that contains the pyrophosphate anion and is an insoluble calcium salt (Pritzker, 1998). Pyrophosphate has attentioned attention because of its role in the inhibition of crystal growth formed in the plaque (Fleisch & Bisaz, 1962). However, it is unlikely that pyrophosphate in toothpastes will remain in the mouth for a long time, due to the presence of significant amounts of pyrophosphatase enzyme in saliva and oral bacteria (Draus, Tarbet, & Miklos, 1968; Wöltgens, Bervoets, & De Vries, 1977). When pyrophosphate is introduced into a simulated oral environment, it is hydrolyzed by various phosphatases for calcification in vitro, with its effect disappearing over time (Gaffar, Polefka, Afflitto, Esposito, & Smith, 1987).

Calcium pyrophosphate is an abrasive material that, when added to toothpastes, produces a Relative Enamel Abrasivity (REA) value of about 450 or higher. An abrasive system containing at least about % 0.0065 zinc compound by weight is formulated together with calcium pyrophosphate to reduce the abrasive effect on enamel even if the REA value of a calcium pyrophosphate containing toothpaste is less than 450 (Cordon & Norfleet, 1976).

2.Antiredeposition Agents

With the new formulations created in recent years, toothpastes that prevent caries, gingivitis, tartar formation and whitening effects are put on the market. The anti-calculus and whitening effects of toothpastes are to some extent based on the effectiveness of high affinity active compounds on the mineralized part of the tooth. Due to this affinity, the accumulation of substances that cause coloration and calculus formation on the tooth surface can be prevented and can be replaced with chromophores that cause coloration. New formulations that can both prevent gingivitis and whiten teeth can be developed. These active compounds can be successfully incorporated in fluoride toothpastes to produce considerable reductions in deposition and stain formation, as well as to aid their removal, according to clinical research (van Loveren & Duckworth, 2013). Some of the chemicals have also been included in toothpastes with anti-plaque and anti-gingivitis compounds, and it has been demonstrated that toothpastes containing fluoride improve whitening effectiveness.

To a degree, toothpastes' anti-calculus and anti-stain properties are based on the same active components. As a result, toothpastes with similar formulas that are marketed as anti-calculus or whitening are available (Duckworth, 2013). Polyphosphate and sodium citrate are the whitening materials added to toothpastes that provide a whitening effect by preventing sedimentation.

2.1.Polyphosphate

Polyphosphates are salts or esters of polymeric oxides consisting of tetrahedral phosphate (PO4) molecules linked together by distribution of oxygen atoms (Corbridge, 1971). Preventive properties of polyphosphates from calculus formation, their ability to convert into crystallized calcium phosphate and inhibit the formation of redeposition have been reported in previous studies. Polyphosphates can be among the active ingredients of whitening toothpastes. Some of the whitening toothpastes also contain low peroxide concentrations. However, evidence to date suggests that the primary stain removal component in these toothpastes is abrasives (De Menezes, Turssi, Hara, Messias, & Serra, 2004; Demarco, Meireles, & Masotti, 2009). Shiba et al. (Shiba, 2016) in their studies, they concluded that polyphosphates increase the whitening effect of hydrogen peroxide and carbamide peroxide; they used colored hydroxyapatite powders, % 1 gelatin solution, concentrated tea and coffee solution to evaluate the effect of polyphosphate with peroxides. As a result, they found that when used together with polyphosphates, peroxides increased approximately 14.8, 7.1, 5.1 and 18.5 times the whitening effect, respectively, compared to their use alone.

SHMP (sodium hexametaphosphate) is a large polyphosphate molecule with many calcium binding sites in a single molecule. It's a powerful anti-deposition agent. It's also referred to as a surface active calcium builder because it only works on the surface. SHMP is sensitive to hydrolysis and must be formulated in a toothpaste with a small amount of water to be stable (D. White, Cox, Suszcynskymeister, & Baig, 2002; D. J. T. J. o. c. d. White, 2002). SHMP particles are insoluble in formulations with little water, and show durability in these situations. However, as the water content of these particles increases, their solubility increases and they begin to dissolve immediately after brushing without abrasive effects (Koenigs & Faller, 2013).

2.2 Sodium Citrate ($C_6H_5Na_3O_7$)

Sodium citrate is explained by the formula "$C_6H_5Na_3O_7$" and represents any of the three sodium salts of citric acid. It has a salty, slightly sour taste.

It is slightly basic and is used with citric acid to have biocompatible effects(Lussi, 2006). It has been shown in vitro that the sodium citrate containing gel significantly reduces the hydrodynamic conductivity of dentin. According to the American Dental Association (ADA), the use of a toothpaste containing % 2 dibasic sodium citrate gel is a safe and effective treatment for the control of dentin hypersensitivity(Lussi, 2006). Joiner(A. J. J. o. d. Joiner, 2010)stated that citrate whitening toothpastes contain additional agents that increase abrasive effectiveness by helping to remove and / or prevent external stains. Sharma et al.(Sharma & Edelstein, 2007) showed in their studies that metal-citrate compounds such as sodium citrate were present in dental cleaning materials that whiten teeth but do not contain peroxide compounds.

3.Calcium Phosphates ($Ca_3(PO_4)_2$)

Calcium phosphate is a mineral that contains calcium ions (Ca^{2+}) along with inorganic phosphate anions. It is explained with the formula "$Ca_3(PO_4)_2$". Some calcium phosphates may also contain oxides and hydroxides and are defined as white solids with nutritional value (Schrödter et al., 2000). Calcium phosphates are found in many living organisms (bone and tooth enamel). In milk, magnesium, called colloidal calcium phosphate (CCP), is found in colloidal form in micelles bound to the casein protein with zinc and citrate (Tamime, Thomas, & Wiley, 2005).

Recently, calcium phosphates have been added to toothpastes instead of oxidizing chemicals and have been suggested for whitening treatments (Jin, Xu, Lai, & Kunzelmann, 2013). Hydroxyapatite, a compound of calcium phosphate, is a proven material with the purpose of whitening, as well as its use in the prevention of caries, the treatment of hypersensitivity and periodontal diseases (Kay, Young, & Posner, 1964).

3.1.Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

In recent studies, hydroxyapatite (HAP) has been suggested as a whitening agent instead of oxidizing chemicals. Onuma et al. (Onuma, Yamagishi, & Oyane, 2005) used an acidic solution with fluoride reinforced HAP to create a new HAP layer on the natural enamel layer. According to the results of the study, the HAP layer was formed and showed sufficient adhesion to the dental tissue. The phosphoric acid solution served two purposes: dissolving the enamel surface and separating calcium phosphate clumps from the surface. It has been shown that it can make the enamel surface clean and rough, and that dissociated HAP crystals can contribute to faster ion growth than calcium phosphate clusters. In addition, it has been observed that the artificially created crystallographic harmonization is compatible with the elongation of the original apatite crystals found on natural enamel. Jin et al. (Jin et al., 2013) stated that the white appearance of the teeth is effective not only by the white HAP layer, but also by the fact that the HAP layer, which has a spread like the original apatite, reflects the light more smoothly.

Kim et al. (Kim et al., 2006) conducted a study to examine the effectiveness of nanohydroxyapatite (n-HAP) toothpastes. The whitening effects of n-HAP-containing pastes, commercially available silicate-phosphate-containing pastes, and silica-micronized HAP-containing pastes on tooth specimens were investigated in this study. As a result, pastes containing n-HAP have a whitening effect that is comparable to that of other pastes. In another study, Niwa et al. (Niwa et al., 2001) evaluated the brightening and whitening properties of hydroxyapatite-containing toothpastes in an experimental clinical study. While polishing properties of artificial teeth were evaluated by polishing with different toothpastes, polishing and whitening properties were examined using two specially produced fibrous colorimeters on volunteer participants. The results of this study revealed that the addition of hydroxyapatite to the toothpaste did not alter the polishing properties of the tooth surface, but produced a significant increase in tooth whitening. It was observed that as the amount of hydroxyapatite in toothpaste increased, its whitening feature increased.

4.Colorants

Most whitening toothpastes use abrasives to physically remove stains on the surface of the teeth. Oxidizing agents such as peroxide have also been used in toothpaste to whiten stains, a process that removes the color-absorbing properties of a chromophore (Abdallah, 2013). In addition, studies have also been carried out on staining the teeth to improve the whiteness of the teeth. Oliveira et al. (Fondriest & Dentistry, 2003) stated that a thin colored layer can also be formed on the surface of the teeth, like

lipstick that temporarily changes lip color, and stated that a blue colorant may be ideal for the teeth to appear whiter(Oliveira et al., 2016). The reason an object looks white is because it reflects all wavelengths of light back to the eye. The reason for the yellow and brown appearance of the teeth is that the stains and other chromophores on the teeth absorb blue light and reflect red and yellow light.

A blue colorant can make teeth appear whiter by reflecting blue light back into the eye before it is absorbed by the stains. It may be ideal to combine the temporary whitening effectiveness provided by the blue colorant with another permanent whitening solution. There are products on the market that use a combination of blue colorants and silica abrasives to mask stains and remove surface stains permanently. Blue covarine is used as a colorant in most whitening toothpastes on the market that are used to mask stains. Pigments have very low water solubility, which facilitates their adhesion to the tooth surface when in water (Strotman, Gronlund, Smolsky, & Chopra, 2010).

4.1 Blue Covarine

Because of its capacity to alter the optical effects of the tooth surface and hence boost the measurement and perception of tooth whiteness, silica-based blue covarine has been optimized and added to several modern toothpastes as a novel optical approach to dental whiteness. (Luisa Z Collins, Naeeni, & Platten, 2008; Andrew Joiner, Philpotts, Alonso, Ashcroft, & Sygrove, 2008). Considering the importance of reducing yellowness in tooth color, it is assumed that the accumulation of blue-colored agents on the tooth surface will cause changes in the optical properties of the tooth and shift the yellow appearance to blue (A. J. I. d. j. Joiner, 2009).

Joiner et al. (A. J. I. d. j. Joiner, 2009) treated human extracted teeth with % 0.2 blue covarine in deionized water for 30 seconds and then rinsed in deionized water. The color of the teeth was measured with a colorimeter before blue covarine application, at different time intervals after application and after rinsing. In terms of its ability to reduce jaundice on the tooth surface, blue covarine has been identified as an effective material immediately after application and 1, 2, 5 hours. Tao et al. (Tao et al., 2017) stated that toothpastes containing blue covarine provided a statistically significant difference in terms of reduction in tooth jaundice and improvement in whiteness immediately after brushing in both in vitro and clinical studies. In addition, they reported that toothpaste with a higher concentration of blue covarine provided a statistically significant level of whiteness compared to toothpaste with a lower concentration of blue covarine.

5.Enzyme / Proteases

Enzymes are usually added to toothpastes to decompose plaques on the tooth surface and to normalize the impaired oral flora by creating hydrogen peroxide. For this purpose, enzymes such as glucose oxidase, trypsin and chymotrypsin have been added to toothpastes (Hoogendoorn, Matthijsen, & Moelker, 1979). Enzymes are generally classified according to the type of reaction, such as hydrolysis, oxidation or reduction. Hydrolytic enzymes are known as hydrolases and allow the degradation of a substrate by the addition of water (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010). With the use of enzymes in toothpaste, it also aims to contribute to the breakdown of proteins, carbohydrates and lipids (Ratnaningsih). Recently, natural enzyme extracts obtained from plants have been added to toothpastes to whiten the teeth by breaking down the protein-like pellicle on the tooth surface. Papain and bromelain are two enzymes obtained in this way (Patil, Ankola, Hebbal, & Patil, 2015).

5.1.Papain

Papain is a proteolytic enzyme derived from the Carica Papaya plant's uncooked fruit. Proteolytic enzymes aid in the breakdown of proteins into peptides and amino acids, which are smaller protein fragments (Kimmel & Smith, 1957). Papain is a proteolytic enzyme that has a whitening effect on tooth surfaces and can clean the protein layer formed by saliva (Hernandez, 2002). This enzyme works best at temperatures between 40 and 65 degrees Celsius (Foegeding & Larick, 1986). Papain can operate on a variety of protein products in a pH range of 3 to 9 due to its poor substrate specificity. The enzyme is inactive outside of these ranges (Aspmo, Horn, & Eijsink, 2005). In general, toothpaste containing papain must have a pH close to neutral in order for its enzymatic activity on enamel minerals to be sustained (Hernandez, 2002).

Papain enzyme, which can be used as an active ingredient in some toothpastes, also has anti-plaque properties. In addition, it achieves the whitening effect by breaking the glycoprotein and lipoprotein connections in saliva and connecting calcium salts that form the basis of coloring. Papain is found at 6000 U / mg and % 0.1-1 levels in whitening toothpastes on the market as an enzyme(Ratnaningsih). However, the age of toothpaste containing papain is shorter due to the proteolytic properties of papain (85).

5.2.Bromelain

Bromelain is a protease derived from the root and fruit of pineapple fruit (ananas comosus) (Arshad et al., 2014). Bromelain enzyme has been used in various studies by being added to toothpaste or mouthwash as an

antiplaque agent. Harmely et al. (Harmely, Lucida, & Mukhtar, 2015) stated in their study that the antiplaque effect of bromelain added to toothpaste as % 5 was strong. Although there are not enough studies in the literature on the side effects of enzymes in toothpastes, both papain and bromelain enzymes are proteases that can be divided into peptide subunits in high concentrations that may cause side effects. Although theoretically these enzymes can cause tissue breakdown and irritation, these effects are unlikely to occur in toothpastes at the concentrations allowed. Chakravarthy et al. (Chakravarthy & Acharya, 2012) found that the application of bromelain extracts in toothpastes increased the whitening effect compared to control groups. However, due to the proteolytic properties of bromelain, pastes containing bromelain have a shorter shelf life than normal toothpastes.

6.Peroxides

Peroxide is a chemical compound in which two oxygen atoms are connected together by a single covalent connection. Many organic and inorganic peroxides are used in the preparation of hydrogen peroxide and other oxygen compounds as whitening agents and as initiators of polymerization reactions (Sanchez & Myers, 2000). Peroxide and similar chemicals have been used for many years to whiten teeth (V. B. Haywood & Heymann, 1991). Bleaching treatments with peroxides can be applied professionally, and home use forms are often preferred. Home product forms include peroxide gels applied to transparent plates, gel-impregnated strips, and paint gels applied with a brush applicator (Luisa Zoe Collins et al., 2004). Unlike other vital bleaching methods, whitening with peroxides does not involve processes such as abrasion of teeth and treatment with acid (V. B. Haywood, Leech, Heymann, Crumpler, & Bruggers, 1990).

Peroxide releasing compounds such as hydrogen peroxide and carbamide peroxide are the most common peroxides used for teeth whitening. When we look at the bleaching mechanism of peroxide containing bleach; When it comes into contact with the enamel surface, it disperses through the enamel, separates into water and oxygen and causes the oxidation of organic pigments in dentin. This allows the color change to be reduced or completely eliminated (Meireles, Heckmann, Santos, Della Bona, & Demarco, 2008). Different concentration, formulation, activation mode, exposure time and application methods are available for peroxides used for bleaching vital teeth (Auschill, Hellwig, Schmidale, Sculean, & Arweiler, 2005; A. J. J. o. d. Joiner, 2006; Karpinia, Magnusson, Barker, & Gerlach, 2003). These properties are effective in determining whether the whitening procedure will be applied by the dentist in the clinic using higher concentration gels or by the patient at home with lower concentrations (V. B. J. C. Haywood, 2003; Matis, Mousa, Cochran, & Eckert, 2000).

6.1 Hydrogen Peroxide (H_2O_2)

With superoxide (O2-), hydroxyl (HO), peroxyl (ROO), and alkoxyl, hydrogen peroxide is a reactive oxygen species (RO) (Walsh, 2000). These products whiten the tooth color by whitening the chromogens in the dentin. It's typically utilized in conjunction with an activation agent like heat or light. These substances can be used for both vital and non-vital whitening (Tredwin, Naik, Lewis, & Scully, 2006). Organelles (particularly mitochondria), salivary cells, microbes, and the lungs are inherent sources of H2O2 in human tissue. The generation of reactive oxygen species can be followed by the production of hydrogen peroxide, which is mainly caused by spontaneous redox reactions of enzymes that use metals like iron or copper as cofactors. Catalase, glutathione peroxidase, and superoxide dismutase are enzymes that catalyze the breakdown of H2O2 into water and oxygen (Marshall, Gragg, Packman, Wright, & Cancro, 2001).

When % 30 H_2O_2 comes into contact with the skin or eyes, it can cause severe irritation or burns (Walsh, 2000). Rotstein et al. (Rotstein, Wesselink, & Bab, 1993), in their study on rats, reported that they observed changes such as edema, intraepithelial and subepithelial vesiculation following the application of % 30 H2O2 in four applications to the tip of the tongue at 15-minute intervals. In addition, they reported that these were preventable changes with the prior application of catalase. In this study, a cellular response similar to acute inflammation occurred, with severe edema, multiple acute inflammatory cells, increased vascular permeability due to the presence of hemoconcentration in blood vessels and fibrin strands. However, it was observed that there was no more cellular response to the chronic reaction after 48 hours.

Contact of hydrogen peroxide with oral tissues may cause objective and subjective changes such as soft tissue irritation, dry mouth, loss of taste, elongation of filiform papillae and diffuse mucosal whitening (Tombes & Gallucci, 1993). Alterations in epithelial proliferation rate, epithelial thickening, and morphological changes may also occur. The proliferating cell nuclear antigen (PCNA) index, which is a measure of cell proliferation, may rise in the epithelium's basal and parabasal layers (Da Costa Filho, Da Costa, Sória, Taga, & medicine, 2002). Chng et al. (Chng, Palamara, & Messer, 2002), in their study on non-vital teeth, stated that intracoronal whitening with 30% hydrogen peroxide reduced the microhardness of dentin and enamel and mechanically weakened dentine.

6.2 Calcium Peroxide (CaO_2)

Calcium peroxide, also known as calcium dioxide, is an inorganic compound with the formula "CaO_2". Its pure compound form is white, as its commercial samples may be yellowish in color. It is almost insoluble in

water. When it comes into contact with water, it hydrolyzes by releasing oxygen, and if it encounters acid (if it comes into contact with H^+ ions), hydrogen peroxide is formed (Jakob, Leininger, Lehmann, Jacobi, & Gutewort, 2000).

The effectiveness and safety of whitening, which is very popular in dentistry, is discussed due to the decrease in enamel surface hardness and the calcium loss under the surface. Raofi et al. (Raoufi & Birkhed, 2010) observed the whitening effect of CaO_2 gel as well as its effect on the remineralization of enamel and pre-existing initial lesions in enamel in a 12-week study. According to the results of the study, it has been shown that CaO2 gel provides initial lesion healing as well as whitening teeth. CaO_2 gel-activated peroxides and activated calcium gel release large amounts of calcium ions in the cavities in enamel and dentin. This combination promotes the precipitation of apatite crystals within the lesion and dentin pores during the tooth whitening process (Schemehorn & Novak, 2007).

6.3.Carbamide Peroxide (CP)

Carbamide peroxide (CP), consisting of urea and hydrogen peroxide; It is a colorless, odorless, tasteless compound with a pH of 6.3 at 20 ^oC. First of all, Dr. Haywood and Heymann (V. B. J. Q. i. Haywood, 1989) tried to whiten the teeth with overnight use in plaques created specifically for teeth in 1989 and were successful. To change the color of the tooth, free peroxide radicals react with chromophores and create a whitening effect. Heyman et al. stated that whitening with using 10% CP is a simple and effective method to improve the color of teeth with mild coloration.

Worschech et al. (Worschech, Rodrigues, Martins, & Ambrosano, 2006) stated that the use of toothpaste containing abrasive agent together with 10% CP treatment caused a decrease in enamel hardness and significant increases in surface roughness. Apart from the abrasive toothpastes available in the market, there are also pastes containing low concentrations of peroxide and showing the whitening effect by releasing the free oxygen it contains(Ritter & Dentistry... 2002). Therefore, it is important to determine which type of toothpaste is suitable for whitening, bearing in mind that there is a wide variety of whitening toothpastes on the market and that patients can use these products to augment their whitening treatment (de Medeiros Melo, Manfroi, & Spohr, 2014).

7.Poliaspartate

Polyaspartate prevents the inhibition of sedimentation on the tooth surface, stabilization of the solid-liquid balance and the organization of microorganisms. It is an anionic polymer that has a number of uses, including corrosion inhibition. Sikes(Little & Sikes, 1991), suggested that

polyaspartate may reduce calculus (112). Epple et al. (Epple et al., 2019) stated that sodium polyaspartate can be used in whitening toothpastes.

7.1. Sodium Polyaspartate

Sodium polyaspartate; it is a whitening substance that has been used relatively recently in toothpastes. It contains sodium polyaspartate carboxyl groups. These may cause a different color absorption and reflection by causing interaction with the tooth surface or pellicle (Epple et al., 2019). Toothpaste formulations used in in vitro studies have been shown to be effective in controlling bacterial biofilm formation. In a 3-month clinical study, a whitening effect was observed with the use of a toothpaste containing 2% polyaspartate and 0.45% zinc citrate (van Loveren & Duckworth, 2013). Jowett et al. (Jowett et al., 2013). tested sodium polyaspartate toothpastes on volunteer participants during a 3-6 month trial period and stated that these pastes showed better anti-stain properties than the control group.

8. Surfactants

Surfactants are substances that lower surface tension between two liquids, a gas and a liquid, or a liquid and a solid. In detergents, moist agents, and additives (emulsifiers) used in solid-liquid combinations, it acts as a foaming agent and dispersion (Rosen & Kunjappu, 2012). Surfactants are one of the essential ingredients in toothpastes. It is responsible for the foaming effect, intraoral distribution, taste formation and micelization of water-insoluble ingredients. Surfactants can also affect the remineralization balance on the tooth surface, as they can change the surface tension of hydroxyapatite and alter the binding activity of fluoride (Almohefer, Levon, Gregory, Eckert, & Lippert, 2018).

8.1 Sodium Lauryl Sulphate (SLS)

A common surfactant is sodium lauryl sulfate (SLS), sometimes known as sodium dodecyl sulfate. Surfactants make toothpastes more effective at removing dental plaque. In addition to being in toothpaste content, SLS can be used in many commercial areas. It can be used as an oil solvent, as an active ingredient in washing detergents, shampoos, bath foams, and facial cleansing soaps (R. S. Brown, Smith, Glascoe, & radiology, 2018). In addition, they are known for their allergic and toxic potential. There are reports that SLS plays a role in both irritation and sensitivity reactions (Ersoy, Tanalp, Ozel, Cengizlier, & Soyman, 2008; Rietschel, Fowler, & Fisher, 2008). SLS consists of a hydrophobic hydrocarbon chain containing 12 carbons, a sulfate group (SO_{4^-}) as the polar group, and a sodium that separates from the molecule when dissolved in water. Twelve carbon chains, while dispersing in solution with an aqueous environment,

also provide the hydrophobic effect that micelles and bubble films can form (J. A. Reynolds & Tanford, 1970).

The purpose of SLS in toothpaste is to cover the tooth surfaces by producing foam. This foam ensures that there is always a layer of toothpaste solution between the teeth / gums and the bristles of the toothbrush. Toothpaste foam is formed by brush movements and the addition of water. This foam is different from typical foams because it is formed when the solid and liquid components in the paste become a slurry. Foam reduces surface tensions and allows cleaning agents to penetrate small spaces between and on the teeth (E. C. Reynolds, 1994).

9.Borates (BO_3)

Borates are boron-oxygen compounds that form boron oxyanions. They can be in trigonal or tetrahedral structure. Boron element is found in nature as borate minerals and borosilicates (Smith & McBroom, 2000). Borate minerals are minerals that contain a borate anion group. These minerals can be polymerized similarly to the SiO_4 compound of the silicate mineral class. Borate minerals such as borax, colemanite and ulexite are also found in a soft, easily soluble evaporite structure (Klein & Cornelius).

9.1 Sodium Perborate ($NaBO_3$)

Under normal conditions, pure sodium perborate (NaBO3) is a white, odorless solid. A NaBO3 molecule, on the other hand, usually crystallizes with 1, 2, or 4 water molecules. Commercial uses for sodium perborate monohydrate (NaBO3.H2O) and sodium perboratetetrahydrate (NaBO3.4H2O) include detergent and bleach. Both types of sodium perborate have a more stable structure than comparable chemicals like sodium percarbonate and sodium perphosphate, making them safer to use (Boulos, 2008). Sodium perborate monohydrate is also referred to as sodium peroxyborate, sodium peroxoborate in some sources. Sodium perborate monohydrate is similar to sodium percarbonate as an oxygen whitening agent. Sodium perborate's oxidative power is also employed in powder detergent formulas, denture cleansers, automated dishwashing detergents, and a variety of institutional and industrial laundry products to increase cleansing, whitening, stain removal, and deodorization (Reinhardt & Borchers, 2008).

Sodium perborate is used in dentistry for intracoronal bleaching of teeth that have been treated endodontically but darkened over time with the "walking bleach" technique. Hydrogen peroxide and sodium perborate are among the commonly used intracoronal bleaching materials in the walking bleach technique, first introduced by Spasser and later modified by Nutting and Poe (Nutting, 1963; Spasser, 1961). Although

sodium perborate is effective in whitening tooth color, their use has been associated with undesirable complications. Possible complications include cervical root resorption, increased dentin permeability, and changes in the chemical structure of dentin (Chng et al., 2002).

CONCLUSIONS

The purpose of the products used for whitening treatment in the office environment, the products that the patient can transfer into the plaque used by the patient or the whitening substances contained in toothpastes that have whitening properties are to remove the discoloration in the tooth tissue. Researchers and manufacturers have aimed to mechanically erode the way of chemical interactions in whitening teeth, dissolve them with enzymes, paint the surface or change the optical properties of the surface. The agents in the whitening materials that remove discoloration by mechanically cleaning the tooth surfaces should be hard enough to remove stains, but should not cause abrasion on the enamel. In recent years, significant progress has been made in this direction with toothpaste formulations containing silica. Efforts are underway to provide a balance between whitening and unwanted enamel abrasion with other existing formulations. In toothpastes containing anti-redeposition agents such as polyphosphates, anti-calculus and anti-staining effects are provided with the same active ingredients. There are also toothpastes with similar formulations for preventing tartar formation or whitening. Toothpastes for the desired purpose can be formulated in this context. Fluorine can also be added to toothpaste formulations to protect and strengthen the tooth structure.

Considering the commonly used whitening products, it is seen that the use of peroxides in the office environment is more preferred today. Successful results can be obtained in office bleaching treatments with high peroxide concentrations. It is also seen that whitening toothpastes, which are the most preferred products, come to the fore with their abrasive properties. Although products aiming to change the optical properties of the surface or to obtain whiteness by painting the surface are launched, there is not enough work related to these products as abrasive toothpastes and peroxide-based materials. It should be the first goal of the whitening agents used to remove only the factors that cause discoloration without damaging the tooth tissue.

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CHAPTER 10

REIKI PRACTICES IN WOMEN HEALTH

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Bu çalışma, 27-28 Mayıs 2022 tarihinde düzenlenen "Kadın Sağlığında Geleneksel ve Tamamlayıcı Tıp Uygulamaları Sempozyumu II: Üreme Sağlığı ve Sorunları" sempozyumunda sözel bildiri olarak sunulmuştur.

A woman's life consists of various stages like childhood, teenage, sexual maturity, menopause, and senility. In each of these periods, hormonal, physical and psychological changes specific to that period occur (Taşkın, 2020). To cope with these changes, women might use pharmacological and/or nonpharmacological methods. Some of these nonpharmacological methods are as follows; biological-based methods (herbal supplements such as evening primrose, angelica, soybean, flax seed, vitamin E), body-based methods (e.g., acupuncture, acupressure, massage, osteopathy), mind-body based methods (e.g., yoga, meditation, hypnosis, biofeedback, Neurolinguistic Therapy-NLP, music, imagery and pray) and energy-based methods (e.g., bioenergy practices, Reiki, bioelectromagnetic) (Akarsu & Kuş, 2017; Yüce & Taşçı, 2020; Soldamlı & Arslanoğlu, 2019).

Energy-Based Therapies

The history of energy-based therapies dates back to ancient times. The human organism consists of energy systems that surround and penetrate the physical body. Energy-based therapies consider an individual a holistic mind-body and depend on healing with pure energy. Voice, measurable wavelengths, and specific frequencies are the practice methods. Reiki and bioenergy practices are the main energy therapies (Özcan Yüce & Taşçı, 2020).

An electromagnetic field is created around the body with the movement of electrons, protons, and ions (Movaffaghi & Farsi, 2009). Energy therapies affect these electromagnetic fields surrounding the human body (Mills & Jain, 2010). There are some evidence-based studies suggesting that energy therapy is effective in general health, and well-being, supporting immune system functions, accelerating wound healing, regulating vital signs, and decreasing pain and fatigue (Baldwin et al., 2017; Dyer et al., 2019; Engebretson & Wardell, 2012; Nield-Anderson & Ameling, 2001). Briefly, an energy therapy is a supplementary method focused on restoring an individual's energy by eliciting his existing one.

Supplementary and integrated treatment methods such as energy therapies are the methods that have been frequently used by health care professionals and individuals recently. Because of increased interest in supplementary and integrated medicine, nurses should take an active role in this type of practice to meet health needs. Among energy therapies, Reiki is an independent nursing practice. It could be used in all nursing care areas from preventive health services to rehabilitation services for the diagnosis of "Disturbance of Energy Field" included in the Nursing Intervention Classification (NIC). In Nursing Regulations published in the year 2011 in Turkey, it was stated that it is effective in helping nurses use relaxation techniques, massage, therapeutic touch, moral support to reduce anxiety and apply calming techniques (https://dosyaism.saglik.gov.

tr). American Holistic Nurses Association (AHNA) approved it as a valid nursing intervention to use energy therapies in patient care (AHNA, 2017). Energy therapies including therapeutic touch, Reiki, and healing touch, are non-drug curative methods that help improve well-being and general health. Any obstruction or disruption in energy flow around an individual causes disorder in the interaction between body, mind, and soul that should be in balance, and this state is defined as a nursing diagnosis of "Disorder in Energy Field" (Natale, 2010).

In the model of Rogers, nursing theoretician who defends Integrative Human Theory, Reiki touch is included in therapies. Rogers stating that environment and human should not be considered separately as they are always in interaction, suggests that this interaction is provided by energy fields. Rogers who defined energy fields as a basic unit states that energy fields are suitable for mutual exchange and expansion (Velioğlu, 2012).

In a study reviewing 12 nonpharmacological clinical practices (distraction, humor, massage, music, aroma therapy, imagination/ daydreaming, meditation, Reiki, positioning, TENS, reflexology, and hot-cold practices) evidence levels of the studies conducted in the last 20 years were evaluated. Of the 7 Reiki articles, 6 were evaluated as D and 1 as C (Williams, 2009). As a result, it is thought that Reiki practice should be a part of nursing care and defined as an independent function of nurses.

Nurses and other health care professionals have been using Reiki in nursing homes, hospitals, operation rooms, palliative care fields, emergency, and psychiatry clinics, obstetrics and gynecology clinics, neonatal units and different areas for symptom management (Sağkal & Eser, 2015). In studies, psychological effects of Reiki (e.g. anxiety, stress, apprehension) have been studied more, while physiological effects less (Vergo, 2018; Baldwin, 2017; Kurebayashi,2016; Sağkal & Eser, 2015).

Effects of Reiki on Body

Reiki which is considered an energy-based healing technique was first systemized in Japan and accepted as a supplementary health source. Mikao Usui stated that Reiki came to light towards the end of the 19th century, its rebirth was ensured and the foundations for its becoming widespread were laid. Preexistent Reiki knowledge was rearranged by Mikao Usui (Usui, 2003). Reiki restores harmony between body, mind, and soul and provides a channel for universal energy by easily integrating into the recipient's body through the practitioner's hand touching the energy centers in the recipient's body (Bondi, Morgan & Fowler, 2021). It increases immunoglobulin A levels and decreases the secretion of stress hormones like cortisol by increasing parasympathetic system activity and functions as a relaxation provider. Thus, Reiki is known to reduce stress,

anxiety, fear, fatigue, and acute and chronic pain; stabilize vital signs such as blood pressure, pulse, and respiratory rate; ease wound healing, increase care satisfaction, quality of sleep, appetite, quality of life and self-esteem (Aydemir, 2021; Anus Topdemir, 2019; Dyer et al., 2019). Effects of Reiki on the control of the chemical structures of body fluids, function of endocrine glands, and circulation system are also known. (Ergin, 2019). Thus, it is suggested that Reiki strengthens the ability to heal the body, increases systemic resistance to stress, and provides relaxation with reduced stress by rebalancing the biological field (Koçoğlu, 2021).

Reiki Training

Reiki is applied by trained individuals. Reiki training consists of three stages: first level, second level, and third level (Ergin, 2019). The *first level;* is the level known as an initiation where information about the history of Reiki, its sense of values, and the structure are given and synchronization is made. Information about how to use the hands in Reiki practices and how to purify aura is given (Anus, 2014). The *second level;* is where symbols are used to facilitate the flow of Reiki. At this level, information about the symbols to be used and synchronization is present. Symbols are used to increase the power of energy and solve the mental and emotional problems. Second level practitioners could send Reiki to recipients living in distant places (Ergin, 2019). The *third level;* is the master level of Reiki training. At this level, the symbol of mastery is taught. Long-term practices are needed. Following the third level, an individual could train new practitioners. Trained individuals practice Reiki by touching the body chakras (Ergin, 2019; Kocoglu, 2021).

Chakras

There are energy centers "chakras" in our bodies. Chakra is placed over aura, and it means circle or wheel that spins clockwise. Aura is the energy that circulates the physical body and it exists in layers (Ergin, 2019). Every chakra is evaluated as entry and exit gate for the energy of different frequencies. Chakras are connected to endocrine glands, endocrine system, and nervous system. Although there are 7 chakras, they correspond to various points along the medulla spinalis from the coccyx to very top of the head. The presence of an obstruction in any energy entry gate causes problems and diseases by hindering energy uptake of the body (Ergin, 2019; Özer, Boz, Teskereci ve Kavradım, 2016).

First Chakra (Root Chakra): The root chakra is over the coccyx. It is connected to adrenal glands. It affects the function of the nervous and circulatory systems and controls the chemical structures of body fluids. The color of the root chakra is red (Ergin, 2019).

Second Chakra (Sacral Chakra): Sacral chakra is below umbilicus

in the abdomen. It affects the function of digestive organs, kidneys, reproductive organs, blood and bladder. The color of the sacral chakra is orange (Ergin, 2019).

Third Chakra (Solar plexus): Solar plexus is also known as the stomach chakra. It is located below the chest and above the umbilicus / between lower ribs. It affects digestive system organs and their problems. The color of the solar plexus is yellow (Ergin, 2019).

Fourth Chakra (Heart Chakra): The heart chakra is below the sternum. It is the energy center of connections and love. It affects the function of the heart, lung, and thymus gland. The color of the heart chakra is green or pink (Ergin, 2019).

Fifth Chakra (Throat Chakra): The throat chakra is on the throat. It is the energy center for communication and expression. It is the chakra related to our ability to express ourselves. It is the center of the thyroid, and parathyroid glands, vocal cords, pharynx, neck, nape, and esophagus. The color of throat chakra is light blue (Ergin, 2019).

Sixth Chakra (Third Eye Chakra): Third eye chakra between the eyes is also known as brow chakra. This chakra is related to the brain and nervous system. It is the energy center that affects intuitive and mental processes. This chakra is connected directly with vision both physically and spiritually and affects the pituitary gland. The color of the third eye chakra is blue (indigo) (Ergin, 2019).

Seventh Chakra (Crown Chakra): The crown chakra is located on the very top of the head. This chakra is related to the pituitary and epiphyseal secretory gland. The crown chakra is the energy center for spiritual connections. It affects the central nervous system, serotonin, and melatonin hormones. The color of the crown chakra is white, golden, or violet (Ergin, 2019).

Reiki Practices

Reiki is the orientation of the energy to the recipient for self-healing by assisting the recipient's innate healing energy. Reiki practice is a supplementary health approach in which practitioners lay their hands on the recipient's chakras (NCCAM, 2018). Reiki applied with hands is practiced on areas such as the face, cheeks, back of the head, throat, heart, below chest, abdomen, back, lower back, knee cap, or foot. Reiki is either sent by touching directly to these regions or from 10-15 cm away. To give energy by touching with hands, Reiki could also be applied to the clothes without always having to touch the body of the person. Reiki practices could last 30-90 min (Ergin, 2019; Kocoglu, 2021). Each practice lasting an average of 30 min could be applied to give energy to each region from

head to toes for 3-5 min. Energy could be applied longer to the area of discomfort (Ergin, 2019; Sagkal & Eser, 2015; Reikiinmedicine.org). Reiki-applied individuals feel a state of deep relaxation, heat, tingling, and the tendency to sleep or rejuvenation. Reiki is considered generally safe and has no real side effects.

Reiki in Health Practices

In the last period, Reiki has also been used in woman health practices. When the literature is reviewed, it is seen that it has often been used for episiotomy recovery after vaginal delivery, for pain management and wound healing after the cesarean section, for symptom management during the menopausal period which is one of the phases of a woman's life, for coping with dysmenorrhea a which is a problem of reproductive age, for anxiety before surgical interventions like hysterectomy and for pain control and stability of vital signs after intervention (Bondi, Morgan, & Fowler, 2021; Aydemir, 2021; Thrane & Cohen, 2014; Doğan, 2018; Das et al., 2017; Koçoglu,2021). In this context, it might be concluded that Reiki has positive and permanent effects on women.

When studies conducted in the obstetric field are examined; when Reiki's effects on episiotomy healing and pain in women who had vaginal delivery were evaluated, it was found that it has a positive effect on healing and it reduces perineal pain. It was determined that Reiki practice has effects on episiotomy, redness, ecchymosis, and edema, which is considered wound healing due to its effect on the functioning of the circulatory system and endocrine glands by touching the chakras (Aydemir, 2021). In a study, Sağkal (2012) examined the effects of Reiki therapy on pain, and anxiety after cesarean section; it was determined that it reduces pain severity (66.80%), level of anxiety, respiratory rate and the need for analgesics. In a similar study in which the effects of Reiki were applied to the incision area on pain and vital signs after cesarean section, it was shown that it reduces pain severity (76.06%), respiratory rate, and need for analgesics (Sağkal & Çıray, 2016).

There are various studies in the gynecological field. In a study that was conducted to evaluate the effects of Reiki practices on menopausal symptoms, it was shown that it decreases somatic, psychological, and urogenital symptoms in women and this effect is long term (Yeşil, 2021). In addition, there is some evidence that Reiki practice has positive effects on pain (Thrane & Cohen, 2014; Dogan, 2018). According to gate control theory, elimination of physiological (like increase in pulse, blood pressure, and respiratory rate) and psychological (like attention deficit, fear, restlessness, and anxiety) problems caused by pain with Reiki is based on intense sensory inputs. Sensory inputs are stimuli from the environment that we perceive. Perception and identification of the energy field are made

by a sense of touch (Uzelli,2014). When any of the seven main chakras are touched with Reiki, sensory input is provided by energy conduction, thus oxytocin release and parasympathetic vagal regulation take place. Following Reiki, activation of the parasympathetic nervous system decreases heart rate, constricts pulmonary bronchioles, and increases secretion (like saliva, cortisol, Ig A). Thus, opposite effects are seen with the activation of the sympathetic nervous system when sensory input problems like pain impair the natural energy flow of the body (Richeson,2010). With touch therapies like Reiki, the patient's energy is regulated, increased, and balanced by giving sensory inputs and a holistic approach to relieve pain or symptoms is applied. In a meta-analysis of the randomized placebo-controlled clinical studies that were conducted to evaluate the effects of Reiki to relieve pain; in Reiki and placebo control groups that VAS pain scores were evaluated, Reiki was found to statistically and significantly decrease VAS scores (Dogan, 2018). Dysmenorrhea is a painful experience that could interrupt daily life activities in women's lives. When the dysmenorrhea is perceived as severe, this could create obstacles in reaching educational goals as a result of changes in body perception, deterioration in life activities, decrease in productivity at function and function quality, decrease in self-confidence, negative impact on social relations and attendance at classes. In addition to medical treatment, supplementary and integrated methods also have been used for pain management. In a study conducted with 746 female students between the ages of 18-30 years to examine the effects of Reiki on dysmenorrhea, 580 students were found to have dysmenorrhea. 30 students with dysmenorrhea were assigned to intervention group and 30 students to the control group. Reiki therapy was applied to the students of the intervention group for 30 minutes every day for 3 weeks. In this in which the frequency of dysmenorrhea was found to be 77.8%, dysmenorrhea pain scores were found to be significantly decreased ($p < 0.001$) with pretest-posttest measurements of the intervention group at the end of Reiki Therapy (Das et al., 2017). In another study conducted with adolescents with dysmenorrhea, Reiki practice was found to affect pain, fatigue and quality of life. The study was randomized, single-blind, Reiki, and placebo controlled. In the first stage of the study, cycle and dysmenorrhea features of students (n=217) were determined, in the second stage students with a diagnosis of primary dysmenorrhea (n=75) were assigned to Reiki (n=38) and placebo (n=37) groups with simple randomization. At the end of the study, Reiki practice was found to decrease pain and fatigue scores but did not affect the quality of life of adolescents with dysmenorrhea (Koçoglu, 2021). In still another study evaluating the effect of Reiki on pain after abdominal hysterectomy, it was found to reduce pain, pulse, respiratory rate, systolic, and diastolic blood pressure and the amount of analgesic use (Utli, 2021).

Conclusion

When the studies in the literature were reviewed, it could be concluded that Reiki has positive effects on female health problems in reducing pain, anxiety, fear, and fatigue, increasing the quality of sleep and life, accelerating wound healing and regulating vital signs. From this point of view, it can be suggested that Reiki practice could be integrated into health care services because it is cheap, safe, effective, and easy to use for the issues related to Female Health by nurses. In addition, evidence-based studies should be conducted with different patient groups and larger sample sizes to promote Reiki practice training for health professionals and to evaluate the effectiveness of Reiki practice. For this purpose, Reiki energy Therapy might be included in nursing education and curriculum and in-service training in health institutions.

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CHAPTER 11

MICRORNA REGULATION OF LIPID AND LIPOPROTEIN METABOLISM

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MicroRNA Regulation of Lipid and Lipoprotein Metabolism

1. MicroRNA

1.2. Introduction

Since the discovery of the structure of DNA by Watson and Crick, rapid and important advances have been made in the field of molecular biology. The majority of studies have focused on elucidating the DNA sequence and identifying the proteins encoded by this DNA sequence. Although most of the DNA is transcribed into RNA, about 2% of the genome is used to synthesize functional proteins. (*Wijnhoven et al., 2007*). While the remainder of the genome was thought to be less important until recently, the fact that non-protein-coding small RNAs play an important role in the post-transcriptional regulation of gene expression has dispelled this view (*Mattick et al., 2005*).

Studies over the past fifteen years have identified numerous classes of noncoding RNA molecules as critical regulators of gene expression. MicroRNA (miRNA) is a single-stranded RNA molecule with a length of approximately 20-23 nucleotides that plays a role in the regulation of gene expression. MiRNAs regulate the function of many cellular pathways and mechanisms and are important regulators of gene expression controlling physiological and pathological processes. (*Bartel, 2004, Winter et al., 2009*).

MiRNAs constitute approximately 3% of the human genome is stated (*Mattick et al., 2005*). Genomic sequences encoding miRNAs are known to be conserved in many living species (*Wijnhoven et al., 2007*). In addition, miRNAs regulate approximately 50% of messenger RNAs (mRNAs) in vertebrates is estimated.

1.3. History of MicroRNA

In 1993 by the Lee et al. Ambros and Ruvkun groups discovered the first miRNA, Lin-4, during their work on Caenorhabditis elegans (C. elegans), a worm from the nematode group (*Lee et al., 1993*, *Almeida et al., 2011*). In these organisms, the regulation of Lin-14 protein for the transition from L1 to L2, which is the first larval stage, was determined to be regulated by Lin-4. The let-7 miRNA was one of the first miRNAs, was discovered 22 nucleotide-long miRNA in C. elegans (Reinhart et al. In 2000). The biological function of Let-7 is highly conserved across humans and some species in sequence and function. These miRNAs are important regulators that control the expression of genes associated with numerous biological processes. (*Mattick et al., 2005, Wijnhoven et al., 2007*).

1.4. Pathways of miRNA Biogenesis

MicroRNAs are a class of non-protein-coding RNAs that play an important role in regulating gene expression and are estimated to regulate

approximately 50% of all mRNAs in vertebrates. MiRNAs are transcribed from DNA sequences into primary miRNAs. The copied primary miRNAs are then transformed into precursor miRNAs, and mature miRNAs are then formed. Generally, miRNAs cause degradation or translational repression of mRNA. It does this by binding to the 3'untranslated region (3'UTR) of the target mRNAs. Under certain conditions, miRNAs can activate translation or regulate transcription. MiRNAs are in constant interaction with their target genes. It has been determined that how they are located in the cell depends on many factors such as the affinity of miRNA-mRNA interactions. It is also known that it can be secreted into extracellular fluids and/or transported to target cells via vesicles such as exosomes, by binding to proteins such as Argonaute (Ago) (*O'Brien et al., 2018*).

MiRNA biogenesis begins with posttranscriptional processing of RNA polymerase II/III transcripts. About half of all currently identified miRNAs are intragenic and are processed from relatively few protein-coding gene exons, mostly from introns, while the other half are intergenic. It is transcribed independently of a host gene and regulated by its own promoters. Sometimes miRNAs are also transcribed as a long transcript that may have similar regions (*Ha et al., 2014*).

MiRNAs are transcribed in hairpin genomic sequences and these sequences are recognized by Dicer and Drosha enzymes. The formation process of miRNAs takes place in 3 steps. The first step is transcription in the nucleus by RNA polymerase II. In the second step, Drosha and its cofactor Di George Syndrome Critical Region 8 (DGCR8) pri-miRNA, which is also located in the nucleus, are cleaved from certain regions to form a 70 nucleotide pre-miRNA. Drosha nuclease and Pasha, a double-stranded RNA-binding protein, form a complex. Pre-miRNA is transported to the cytoplasm via Exportin 5 and RAN-GTP. Pre-miRNAs are cleaved by Dicer in the cytoplasm to form mature double-stranded miRNA. Firstly, Dicer cleaves the stem loop structure of the pre-miRNA. The Ago2 protein in the RNA-induced silencing complex (RISC) complex selects the more stable miRNA and incorporates it into the RISC complex. The stem loop structure of mature miRNA is named miRNA-5p or miRNA-3p depending on its origin. Incorporation of this mature miRNA structure into the RISC molecule directs the complex structure to the mRNA to which it is complementary and inhibits the function of the mRNA either by inhibiting translation or facilitating degradation of the mRNA. In addition, the 2-7 nucleotides located at the 5' end of the miRNA, also called the nucleus, are extremely important in terms of matching with the relevant target (*Bartel et al., 2004, Bhaskaran et al., 2014, Liu et al., 2012*).

1.5. Function and Molecular Mechanism of Action of MicroRNAs

The main function of miRNAs is to bind to sequences in the 3′-UTR of target mRNAs and inhibit protein synthesis of protein-coding genes by suppressing translation or causing degradation of mRNA. MiRNAs have the ability to recognize target genes that are complementary to their nucleotide sequences. The active RISC complex containing single-stranded miRNA binds to mRNA with base-pairing property, causing inhibition of its translation and/or destruction of mRNA (*Hussain et al., 2012*). In target selection, the region of miRNA (a region of 2-8 nucleotides at its 5 end) called the core-seed is the part that provides mRNA binding. The miRNA binds to the untranslated region (UTR) at the 3' end of the target mRNA or the ORF (open reading frame) region of the target mRNA. If there is a high degree of complementarity between the miRNA and the 3' UTR region of the mRNA, mRNA degradation occurs. However, if the complementarity is low, it results in suppression of translation. Binding within the ORF region demonstrates complete complementarity and results in degradation of mRNA by Ago2. The decrease in the amount of mRNA is important for the evaluation of miRNA activity. In addition, each miRNA can regulate the expression of multiple mRNAs, and each mRNA can be targeted by more than one miRNA (*O'Brien et al., 2018*).

1.6. Circulating MicroRNAs

Most miRNAs are found in the cellular environment, but they have also been found in different biological fluids. Recent studies have shown that miRNAs can be transported between cells and tissues, and not only in serum or plasma, but also in saliva, tears, urine, breast milk, colostrum, peritoneal fluid, cerebrospinal fluid, bronchial lavage, seminal fluid, and follicular fluid. found to be present in different extracellular biological fluids. Extracellular miRNAs (ECmiRNA), unlike intracellular miRNAs and other types of RNA that degrade within a few seconds in the extracellular environment, are highly stable and can remain undegraded for long periods under adverse conditions. Apart from being very stable against RNAases, it is stated that they remain stable even when exposed to harsh conditions such as boiling, high or low pH, long-term storage and freeze-thaw cycles. Although various studies have confirmed the presence of ECmiRNAs in various biological fluids, the mechanisms responsible for the release of these miRNAs in the extracellular environment have not been fully elucidated. Studies have shown that miRNAs are released out of the cell via exosomes and microvesicles. In addition, it has been shown that ECmiRNAs can be secreted extracellularly via apoptotic bodies, high density lipoprotein (HDL) and AGO protein complexes (*Sohel., 2016*). Extracellular miRNAs in biological fluids are stable. Due to their stable structures, these molecules are thought to be an important biomarker in

the diagnosis and prognosis of diseases. The mechanisms related to the release of these miRNAs into the circulation, targeting and sensing of the relevant tissue attract the attention of researchers and there is a great interest in studies in this area today (*Sohel., 2016*). Remarkably, miRNAs have recently been revealed to be critical regulators of circulating lipids and have important roles in regulating lipoprotein metabolism.

2. The Role of MicroRNAs in Lipid Metabolism

While the role of miRNAs in regulating multiple physiological processes, including apoptosis, cell differentiation, and cancer, has been understood, the importance of these small RNAs in regulating lipid metabolism has only recently been revealed. Studies in the last decade have revealed the importance of miRNAs that regulate lipoprotein metabolism in diseases such as metabolic syndrome, obesity, and atherosclerosis. MiRNAs have been found to play an important role in the control of energy metabolism in recent studies. MiRNAs are important regulators of cholesterol metabolism and have emerged as therapeutic targets for the treatment of cardiometabolic disorders, including atherosclerosis. In addition, miRNAs have an important role in lipid metabolism, as they also act on triglyceride (TG) and cholesterol (*Aryal et al., 2017*). In diseases in which miRNAs function, miRNA antisense oligonucleotides (ASO) are used. Thus, miRNA function is suppressed. Antisense oligonucleotides are similar in base sequence to miRNA targets. Nucleic acid lockers (LNA), anti-miRNA oligonucleotides (AMO) and antagomirs are named according to their effects and durability (*Elmén et al., 2008, Krützfeldt et al., 2005*). Recent findings in the field are discussed in this section, highlighting the mechanisms of miRNAs controlling lipid metabolism and atherogenesis, and new miRNAs regulating HDL and low-density lipoprotein (LDL) metabolism. Finally, recent findings of important miRNAs controlling cardiovascular homeostasis are summarized.

2.1. MicroRNA-30c

The miRNA-30 family is one of the earliest identified miRNA families and has been discovered in short RNA sequences in different organisms. The miRNA-30 family has the sequence GUAAACA and consists of five members, miRNA30 (a-e). MiRNA-30c is produced from two genes, miRNA-30c-1 and miRNA-30c-2. Although the reason for the existence of the two genes is not known exactly, it is thought that it probably provides more convenience for tissue-specific expression and better response to physiological, pathological and environmental changes (*Irani et al., 2015*).

MiRNA-30c, which has an important role in lipid metabolism, shows its effect by binding to the 3' untranslated region of mRNA of microsomal triglyceride transfer protein (MTTP) (*Soh et al., 2013a*). Through MTTP,

TGs are taken into the structure of lipoproteins containing Apo B. The binding site of MTTP mRNA is highly conserved for the miR-30c sequence. Studies have shown that miR-30c acts on the mRNA of this protein and induces its destruction. In particular, miRNA-30c has been shown to reduce the activity of MTTP by degrading the mRNA of post-transcriptional MTTP. For this reason, a decrease is observed in MTTP activity and apolipoprotein (Apo) B secretion (*Zhou et al., 2014*). A study in Western diet-fed Apo E -/- mice overexpressing miRNA-30c showed significant reductions in plasma cholesterol, fasting plasma triglycerides, and plasma apo B levels. Moreover, it was observed that atherosclerotic lesions were less common in Apo E -/- mice expressing miRNA-30c and even these lesions were reduced. It has also been shown that miR-30c expression levels may play a critical role in increased and prolonged postprandial lipemia by regulating MTTP activity (*Yaman et al., 2021*). These studies demonstrated that miRNA-30c reduces lipoprotein production, plasma lipids, and lipid synthesis. Thus, miRNA-30c coordinately regulates hepatic and plasma lipid levels by reducing lipid biosynthesis and lipoprotein secretion (*Soh et al., 2013a, Soh et al., 2013b*).

2.2. MicroRNA-122

Studies have shown that miRNA-122, which constitutes 70% of the total miRNAs in the liver, has an effect on lipid metabolism (*Fernández-Hernando et al., 2011*). In order to elucidate the physiological function of miRNA-122, ASOs and LNAs targeting miRNA-122 have been developed and its mechanism of action by suppressing the function of miRNA-122 has been elucidated. It has been determined that plasma cholesterol and TG levels decrease with the suppression of miRNA-122, which is involved in cholesterol and lipid metabolism (*Fernández-Hernando et al., 2011, Yang et al., 2015*). It has been found that the effect of miRNA-122 on cholesterol metabolism is through 3-Hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) steps, and its effect on TG metabolism is through MTTP. Studies have shown that the expression of these genes is decreased by using anti-miRNA-122 therapies (*Yang et al., 2015*). In addition, using ASOs targeting miRNA-122, an increase in AMP-activating protein kinase (AMPK) expression was observed in the liver, and accordingly, activation of fatty acid β-oxidation was detected (*Alrob et al., 2017*).

When the circulating (plasma) expression levels of microRNA-122 were examined, it was observed that miRNA-122 expression was more than three times higher in obese individuals compared to normal subjects (*Pirola et al., 2015*). It has also been shown that increased circulating miR-122 expression in individuals with postprandial lipemia may be associated with increased MTTP activation, resulting in increased CM particle size and TG levels (*Yaman et al., 2021*).

It has been observed that plasma triglyceride levels decrease significantly as a result of inhibition of miRNA-122, which plays an important role in lipid metabolism, with antagomir (*Alrob et al., 2017*).

2.3. MicroRNA-33a/b

miRNA-33, one of the miRNAs involved in cholesterol metabolism, is an intronic miRNA localized within the Sterol regulatory element-binding protein (SREBP) genes. In the mature form of miRNA-33, only a and b differ in two nucleotides and have the same target. SREBPs, which are key transcriptional regulators of lipid metabolism, control many genes involved in cholesterol and fatty acid biosynthesis and uptake, as well as TG and phospholipid production (*Yang et al., 2015*). SREBP-1 and SREBP-2 have different functions. In particular, SREBP-1 activates genes involved in fatty acid metabolism and de-novo lipogenesis. However, SREBP-2 is involved in the activation of genes in cholesterol homeostasis (*Fernández-Hernando et al., 2011*). MiRNA-33a is a miRNA located in the intron of human SREBP-2 with a key role in controlling genes involved in cholesterol uptake and synthesis. Among the gene targets on which miRNA-33a is most effective is the expression of ATP-binding cassette transporter-A1 (ABCA1), which is responsible for the transport of cholesterol out of the cell. ABCA1 is an important molecule for HDL biogenesis and reverse cholesterol transport. ABCA1 mRNA and protein have short half-lives (1-2 hours), further increasing the importance of controlling de-novo transcription and translation. With the increase in miRNA-33a expression, ABCA1 expression decreases and cholesterol flow to HDL via Apo A1 is impaired. Thus, a decrease in HDL-C levels is observed. With the suppression of miRNA-33a, an increase in ABCA1 expression and therefore an increase in the amount of HDL-C occur. Thus, it has been determined that the increase in miRNA-33a expression inhibits the expression of ABCA1 protein and reduces cholesterol transport to HDL. Studies have shown increased ABCA1 and ABCG1 expression in the livers of mice treated with antisense oligonucleotides or antimiRNA-33a lentivirus. In addition, inhibition of endogenous miRNA-33a increases the flow of ABCA1 and ATP binding cassette transporter G1 (ABCG1) protein and cholesterol to Apo AI and HDL-C, indicating the physiological role of this miRNA in the regulation of ABCA1 (*Ono, 2016, Rayner et al., 2010*). In mice treated with anti-miRNA-33a experimental atherosclerosis model, plasma HDL levels were increased by 35-50% without affecting other lipoproteins (*Rayner et al., 2010*). In miRNA-33a knockout mice, it was observed that there was an increase in ABCA1 and ABCG1 protein expression, and accordingly an increase in serum HDL-C by 25-40% (*Horie et al., 2010*). In postprandial lipemia, MiRNA-33a may play a role in reducing reverse cholesterol metabolism by acting on ABCA1 on

HDL-C. (*Yaman et al., 2021*). Studies have shown that miRNA-33a/b acts as a post-transcriptional regulator of lipid metabolism and its inhibition increases HDL levels (*Ono, 2016*).

2.4. MicroRNA -27a/b

The miRNA-27 family consists of two members, miRNA-27a and miRNA-27b. Expression of miRNA-27 occurs mostly in endothelial cells, smooth muscle cells and macrophages. In the study, it was found that miRNA-27b expression was induced in the livers of mice fed a high-fat diet. With the increase in miRNA-27b expression, it was determined that the expression of critical regulators of lipid metabolism such as peroxisome proliferator- activated receptor gamma (PPARγ), Angiopoietin-like 3 (ANGPTL3), HMGCR, SREBP-1, SREBP-2 and ApoB, are suppressed (*Chen et al., 2012, Vickers et al., 2013*). Studies have shown that ABCA1 is a conserved direct target of miRNA-27 in both hepatocyte and macrophage cell lines (*Zhang et al., 2014, Goedeke et al., 2015a, Shirasaki et al.*), as well as in mouse liver (*Goedeke et al., 2015a*). Accordingly, transfection of hepatoma cells with miRNA-mimics or miRNA-inhibitors resulted in a change of ABCA1-dependent cholesterol efflux to ApoA1 (*Alvarez et al., 2015, Zhang et al., 2014, Goedeke et al., 2015a).* These data suggested that a therapeutic intervention aimed at reducing miRNA-27 could increase hepatic and vascular ABCA1-dependent cholesterol efflux and reverse cholesterol transport (*Musunuru et al., 2010*). Murine models show that loss of ANGPTL3 (another target of miRNA-27) activity is associated with decreased HDL-C, LDL-C and very-low-density lipoprotein (VLDL)-triglycerides in plasma, further suggesting that miRNA-27 The ANGPTL3/ ABCA1/low density lipoprotein receptor (LDLR) axis may be of interest to reduce cardiovascular risk (*Shimamura et al., 2007, Jin et al, 2015*). Thus, Anti-miRNA-27 ASOs can increase both LDL-C clearance (via LDLR) and HDL biogenesis (via ABCA1), resulting in a better therapeutic effect for hypercholesterolemic patients.

2.5. MicroRNA 148-a

In vitro and in vivo studies have shown that miRNA-148 is an important regulator of hepatic LDLR expression and lipoprotein metabolism (*Goedeke et al., 2015b*, *Wagschal et al., 2015)*). As a result of high-throughput genome analysis studies, miRNA-148a was determined to be a negative regulator of LDLR expression and activity in human liver cells. miRNA-148a binds to the 3'UTR region of LDLR. Thus, it has been shown to directly target many genes involved in lipid metabolism, including ABCA1, salt-inducible kinase 1 (SIK1), PPAR-γ coactivator-1α (PGC1α), AMPK and insulin induced gene 1 (INSIG1) (*Goedeke et al., 2015c*, *Wagschal et al., 2015).* Interestingly, overexpression and inhibition

of miRNA-148a in mice significantly reduce and increase hepatic LDLR expression, respectively. This demonstrates the physiological importance of miRNA-148a in controlling LDLR activity in vivo. Studies Inhibition of miRNA-148a has been observed to significantly lower circulating LDL-C levels in two different mouse models of hypercholesterolemic (*Goedeke et al., 2015c).*

2.5. MicroRNA-128-1

MiRNA-128-1 exerts its efficacy by binding to the 3'UTR of LDLR and ABCA1, thereby directly targeting lipoprotein metabolism. In human hepatoma cells and mouse macrophages, miRNA-128-1 regulates cellular cholesterol efflux to ApoA1 by acting on ABCA1 and adapting to its function. (*Wagschal et al., 2015).* Furthermore, overexpression of miRNA-128-1 was found to reduce hepatic ABCA1 expression and circulating HDL-C levels in C57BL/6 J mice. Similar to miRNA-148a, miRNA-128-1 binds to the 3'UTR region of LDLR and ABCA1, resulting in a reduction in circulating TG and cholesterol levels (*Wagschal et al., 2015).* Moreover, in vivo inhibition of miRNA-128-1 improved glucose tolerance and insulin sensitivity. Importantly, miRNA-128 ASO potentiated the expression of insulin receptor (INSR) and insulin receptor substrate 1 (IRS-1), thereby increasing insulin sensitivity (*Wagschal et al., 2015).* Furthermore, miRNA-128-1 controls the expression of genes in fatty acid synthesis (fatty acid synthase (FASN) and Sirtuin 1 (SIRT1) (Price et al., 2012, *Wagschal et al., 2015*).

3. Conclusions

In recent years, contributions from many research groups have clearly demonstrated the important role of miRNAs in the regulation of lipid metabolism and CVD. Studies have shown that miRNAs are central and very important regulators of lipoprotein metabolism and cholesterol homeostasis. On the other hand, it has been shown that HDLs carry miRNAs functionally and metabolically and deliver them to the relevant tissues and have an important place in regulation. For this reason, the role of lipoprotein-transported miRNAs as functional regulators and/or useful biomarkers is emerging, and they are implicated as therapeutic targets for therapy.

Lipoprotein-mediated miRNAs play an important role as regulators and are emerging as a new therapeutic target for diseases. However, larger studies are needed to fully explore the biomarker and therapeutic potential of these miRNAs in diseases. In conclusion, the transport of miRNAs via lipoproteins appears to be a promising candidate in the prevention and treatment of cardio-metabolic diseases. Due to this functionality of lipoproteins, the ability to perform miRNA-based clinical applications once again emphasizes the importance of this field.

Table 1. miRNAs involved in the regulation of lipid and lipoprotein metabolism.

miRNA	Target genes	Function
miRNA-1	LXRα	Transcriptional regulation of lipid metabolism genes
miRNA-9	ACAT1	Intracellular cholesterol homeostasis
miRNA-10b	ABCA1 ABCG1	HDL biogenesis and cholesterol efflux
	PPAR-α	Transcriptional regulation of lipid metabolism genes
miRNA-19b	ABCA1	Cholesterol efflux (reverse cholesterol transport)
miRNA-21	HMGCR	Cholesterol biosynthesis
miRNA-26	ABCA1 ARL7	Cholesterol efflux (reverse cholesterol transport)
miRNA-27a/b	ABCA1	Cholesterol efflux (reverse cholesterol transport)
	OSBPL6	Cholesterol trafficking
	ACAT1	homeostasis of intracellular cholesterol
	SR-BI	HDL-C uptake
	LDLR LDLRAP1	ApoB100-containing lipoproteins clearance such as VLDL, IDL, LDL
	RXRα PPARγ	Transcriptional regulation of lipid metabolism genes
	GPAM NDST1 ANGPTL3 LPL	Regulation of triglyceride-rich lipoprotein metabolism
miRNA-30c	MTP LPGAT1	ApoB100-containing lipoprotein assembly and secretion
miRNA-33a/b	ABCA1 ABCG1a NCP1 OSBPL6	HDL biogenesis, cholesterol trafficking and efflux (reverse cholesterol transport)
	CROT CPT1A HADHB	Fatty acid oxidation
	NSF	VLDL trafficking and apolipoprotein secretion
	SRC3 SREBP1	Transcriptional regulation of lipid metabolism genes
	SIRT6	Post-translational regulation of associated genes
miRNA-34a	SIRT1 HNF4α	Post-translational regulation of associated genes
		Lipid storage and secretion
miRNA-96	SR-BI	HDL-C uptake
	INSIG-2	Regulation of SREBPs activity
miRNA-101	ABCA1	Cholesterol efflux (reverse cholesterol transport)
miRNA-107	FASN	Triglyceride biosynthesis and lipid storage
miRNA-122	AGPAT1 CIDEC	Triglyceride biosynthesis and lipid storage
	PPARβ/δ	Transcriptional regulation of lipid metabolism genes
miRNA-125a	SR-BI	HDL-C uptake, steroidogenesis
miRNA-128	ABCA1 ABCG1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)
	RXRα SREBP2	Transcriptional regulation of lipid metabolism genes
	LDLR	ApoB100-containing lipoproteins clearance such as VLDL, IDL, LDL
	SIRT1	Post-translational regulation of metabolism-associated genes
miRNA-130b	ABCA1	Cholesterol efflux (reverse cholesterol transport)
	LDLR	ApoB100-containing lipoproteins (VLDL, IDL, LDL) clearance
	PPARγ PPARGC1A	Transcriptional regulation of lipid metabolism genes, adipogenesis
miRNA-144	ABCA1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)

miRNA-145	ABCA1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)
miRNA-148a	ABCA1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)
	LDLR	ApoB100-containing lipoproteins clearance such as VLDL, IDL, LDL
	SIK1	Effect of lipogenesis genes (Post-translational regulation)
	CPT1A	Fatty acid oxidation
miRNA-181a	IDH1	Regulation of lipid biosynthesis and β-oxidation
miRNA-182	FBXW7	Regulation of SREBPs activity
miRNA-185	SR-BI	HDL-Ch uptake
	SREBP2	Transcriptional regulation of lipid metabolism genes
	LDLR	ApoB100-containing lipoproteins clearance such as VLDL, IDL, LDL
miRNA-206	LXRα	Transcriptional regulation of lipid metabolism genes
miRNA-223	SR-BI	HDL-C uptake
	SP3	HDL biogenesis and cholesterol efflux
	HMGCS1 SC4MOL	Cholesterol biosynthesis
miRNA-301b	ABCA1	Cholesterol efflux (reverse cholesterol transport)
	LDLR	ApoB100-containing lipoprotein clearance such as VLDL, IDL, LDL
miRNA-302a	ABCA1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)
miRNA-378	ABCG1	Cholesterol efflux (reverse cholesterol transport)
miRNA-455	SR-BI	HDL-C uptake, steroidogenesis
miRNA-613	ABCA1	Cholesterol efflux (reverse cholesterol transport)
	LXRα	Transcriptional regulation of lipid metabolism genes
miRNA-758	ABCA1	HDL biogenesis and cholesterol efflux (reverse cholesterol transport)

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CHAPTER 12

PRACTICE OF FIDUCIAL MARKER IN PROSTATE CANCER: A SINGLE-CENTER EXPERIENCE WITH UP TO 6 YEARS' FOLLOW-UP RESULTS

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Introduction:

It is well known that there is a significant correlation between dose increase and disease control in prostate cancer radiotherapy [1-4]. However, the radiotherapy technique to be chosen gains importance since an increase in adverse effects may occur due to an increase in dose. It has been demonstrated that in treatments performed with conformal radiotherapy techniques, normal tissues are protected better and late adverse effects associated with radiotherapy are reduced [5]. Apart from the importance of the treatment technique, another effective method in reducing the adverse effect profile is fiducial marker (FM) applications [6-9]. The most remarkable advantages of this technique are the ability to reach high doses safely, higher disease control, and lesser adverse effect profile. Fiducial marker applications in prostate cancer are an effective and exclusive method for accurately identifying the anatomy of the prostate, which is subject to changes due to bladder and rectal contents and constitutes a target, reducing the side effect profile and allowing high rates of disease control. Several published patient series have reported low side effect profiles [10]. Transperineal approach is safe and recommended [11]. There are various types of it, which are made up of gold, Polyether ether ketone (PEEK), and carbon-containing materials. Although the most used type is gold FM, PEEK FM usage experiences have also been revealed [12]. FM applications require a multidisciplinary approach with Urology or Radiology Clinics. As the experience increases, the application duration of the technique decreases, and its quality increases. It is vital that FMs are placed in the prostate on xyz coordinates in a way that allows 3-dimensional assessment spatially (Figure 1-2). Patient tolerability and adverse effects of FM application technique have been reported commonly [13,14]. Knowing particularly late-term adverse effects together with acute adverse effects and assessing their effects on the patient's quality of life would enable us to make better decisions about the future of these applications. Assessment of adverse effects can be performed by subjective criteria based on the statements of the patient or by using objective criteria. Pre-prepared, validated, measurable, easy-to-apply, and repeatable tools should be used in the assessment with objective criteria. The first of the modules selected in this study is the European Organization for Research and Treatment of Cancer, Quality of Life Group-Prostate 25 (EORTC QLQ-PR25). It consists of 25 items that assess urinary and intestinal symptoms, sexual activity, the functioning and adverse effects of the treatment, and it has been translated into 14 different languages [15]. As the second evaluation module, National Cancer Institute, Common Terminology Criteria for Adverse Events-Version 5.0 (NCI CTCAE-V 5.0) was used [16]. With the use of these modules, it is aimed to assess the late period adverse effects

of patients who underwent radiotherapy with FM application for prostate cancer in our clinic, based on the objective criteria.

Material-Method:

In the study, 40 patients who were diagnosed with prostate cancer, treated with FM, and received radiotherapy between 2012 and 2017 were assessed retrospectively (Table 1). All patients treated with Varian Trilogy model linear accelerator were evaluated using the Volumetric Modulated Arc Therapy (VMAT) planning technique and Image-Guided Radiotherapy (IGRT) in the Eclipse (ver.13.6) treatment planning system. Informed consent from the patient/patient‘s family and institutional approval was obtained for this study. Patients who accepted the fiducial marker application, completed the treatment and were followed up regularly were included in this study. Patients who received radiotherapy without fiducial marker application, who were treated with fiducial marker, but who did not want to participate in the study and were not followed up were not included in this study. Six patients who were treated with FM-guided radiotherapy but died due to non-illness causes during follow-up, and 14 patients who were treated after 2017 - since late-term complications would be assessed - were not included in the study. Before radiotherapy, 35 patients received gold FM and 5 patients received PEEK FM. The first of the NCI CTCAE-V 5.0 module was applied immediately after the FM procedure, and the second was repeated at the median 6.5th year (4 years-9 years). The module was performed immediately after the FM procedure. Hence, the effects of radiation were excluded, and only the adverse effects of the FM implant procedure were recorded. EORTC QLQ-PR25 Turkish Symptom Module was performed once following radiotherapy and it was applied with NCI CTCAE-V 5.0 module in median 6.5th years. Forty patients were included in the study, the number of patients was the same in both the first measurement and the second measurement.

SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program was used to evaluate the data. Variables are expressed using mean ± standard deviation, percentage and frequency values (Table 2). Variables were evaluated after checking the preconditions for normality and homogeneity of variances (Shapiro Wilk and Levene Test). While analyzing the data, Independent 2 group t test (Student’s t test) was used for the comparison of two groups, and Mann Whitney-U test was used if the prerequisites were not met (Table 3). A value of $p < 0.05$ was accepted for the significance level of the tests. The meaning of the p values in Table 3; it can be said that the differences in the sample size were not statistically different and the randomization was successful.

Results:

The mean symptom value for all patients was 17,33%. The lowest symptom value was 0% and the highest symptom value was 62,5%. The mean symptom value for patients implanted with Gold FM was 16% and for patients implanted PEEK FM was 17,33%. No significant correlation was found between Gold FM and PEEK FM in terms of symptom value.

Symptoms such as fever, rectal bleeding, dysuria, hematuria, hematospermia, and frequency of urination were questioned for the assessment of genitourinary and gastrointestinal system adverse effects associated with FM procedure. In the presence of symptoms in patients, the degree of symptom was labeled as "yes," "a little," or "no". The same assessment was repeated and compared after a median of 6.5 years following the FM procedure (Table 4). NCI CTCAE-V 5.0 was used to rate the severity of responses. Severity grades for each adverse event were categorized as follows; grade 1: mild adverse event, grade 2: moderate adverse event, grade 3: severe adverse event, grade 4: life-threatening adverse event, and grade 5: death related to the adverse event. In the first assessment, 6 (15%) patients described adverse effects, 34 (85%) patients had no adverse effects. In the second assessment, this rate was 3 (7.5%) and 37 (92.5%) patients, respectively. In the first assessment, rectal bleeding was present in 2 (4%), dysuria 3 (6%), hematuria 5 (11%), and frequency of urination in 5 (11%) patients. In the second assessment, there was no rectal bleeding and hematuria, and dysuria was determined to be 2 (5%) and frequency of urination was 4 (10%). In the comparison, it was found that no new symptoms were added and there was a decrease in the overall symptom rate. In the first assessment, it was determined that the adverse effects of 9 patients (20%) who answered "yes" and "a little" to the questions about genitourinary and gastrointestinal system adverse effects were grade 1, corresponding to mild side effects, and none of the patients experienced grade 2 or higher adverse events. On the other hand, in the second assessment, this rate was at grade 1 in only 6 patients (15%). Fever and hematospermia were not detected in any patient, both in the first measurement and in the second measurement.

Discussion:

The advancement of radiotherapy techniques has contributed to the reduction of gastrointestinal and specifically genitourinary system adverse effects [17-20]. Thanks to this situation, it is possible to observe the adverse effect profile of FM applications without being masked by the adverse effects of radiotherapy. For FM applications, it is very crucial to know particularly the late complications [21-24]. Because FM implant is not a necessity for radiotherapy application, and late complications might cause

negligence of the application.

It has been revealed that the questionnaire questions of the EORTC QLQ-PR25 Symptom Module were answered by patients with high compliance [25]. We also observed this patient compliance in our study. The use of modern radiotherapy techniques can be shown as the reason for fewer observed gastrointestinal adverse effects compared to previous studies. Developing radiotherapy techniques allows a lesser intestinal toxicity. Some side effects such as frequent urination might be taken for granted by elderly patients due to their age and they might not consider these complaints as severe. However, it is not possible to make a differential diagnosis of this condition.

Conclusion:

The important limitation of this single-center study is the small number of patients. However, the length of the follow-up period can be considered an advantage. In the study, objective evaluation criteria were preferred as much as possible. Subjective evaluations were supported by objective evaluation criteria. As a result of this study, it can be suggested that ultrasound-guided fiducial marker applications used in prostate radiotherapy are a well-tolerated and safe technique that does not lead to adverse effects and loss of comfort in patients with long-term follow-up.

Table 1: Patient characteristics

Age; median,range	71 (55-81)
TNM T category ; n(%)	T1-2; 39 (%97,5) T3; 1 (%2,5)
TNM N Category; n(%)	N0; 38 (%95) N1; 2 (%5)
PSA, before treatment; median, range	8,7 (1.23-75)
Gleason score; median, range	7 (6-10)
Radiotherapy dose	7800 cGy
Radiotherapy technique	IGRT
Fiducial markers; n (%)	Gold marker; 35 (87,5) PEEK marker; 5 (12.5)

Table 2: The EORTC QOL-PR 25 QOL descriptive statistics

		Functional scales	Symptom scales	Urinary symptoms	Incontinence aid	Bowel symptoms	Hormonal treatment-related symptoms
Gold marker	N	35	35	35	35	35	35
	Mean	82,78	17,22	15,12	1,90	4,05	3,33
	Std. Deviation	5,33	5,33	13,14	7,85	6,51	4,09
	Median	84,00	16,00	12,50	0,00	0,00	0,00
	Minimum	62,67	10,67	0,00	0,00	0,00	0,00
	Maximum	89,33	37,33	62,50	33,33	25,00	11,11
Peek marker	N	5	5	5	5	5	5
	Mean	81,87	18,13	17,50	0,00	3,33	3,33
	Std. Deviation	3,07	3,07	9,04	0,00	4,56	4,97
	Median	82,67	17,33	12,50	0,00	0,00	0,00
	Minimum	78,67	14,67	8,33	0,00	0,00	0,00
	Maximum	85,33	21,33	29,17	0,00	8,33	11,11
Total	N	40	40	40	40	40	40
	Mean	82,67	17,33	15,42	1,67	3,96	3,33
	Std. Deviation	5,08	5,08	12,63	7,36	6,26	4,13
	Median	84,00	16,00	12,50	0,00	0,00	0,00
	Minimum	62,67	10,67	0,00	0,00	0,00	0,00
	Maximum	89,33	37,33	62,50	33,33	25,00	11,11

Table 3: The EORTC QOL-PR 25 QOL results at the groups

	Gold marker n=35	**Peek marker** n=5	p
Functional scales	82,78±5,33	81,87±3,07	0,710¥
Symptom scales	17,22±5,33	18,13±3,07	0,710¥
Urinary symptoms	15,12±13,14	17,5±9,04	0,700ψ
Incontinence aid	1,9±7,85	0±0	0,590¥
Bowel symptoms	4,05±6,51	3,33±4,56	0,810ψ
Hormonal treatment-related symptoms	3,33±4,09	3,33±4,97	0,999ψ

ψ Mann Whitne-U test; ¥ Student's t test;

Table 4: Evaluation and comparison of genitourinary and gastrointestinal system side effects.

	measurement	**yes, n (%)**	**a little, n (%)**	**no, n (%)**
fever	first	0/40 (0)	0/40 (0)	0/40 (0)
	second	0/40 (0)	0/40 (0)	0/40 (0)

rectal bleeding	first	0/40 (0)	2/40 (4)	0/40 (0)
	second	0/40 (0)	0/40 (0)	0/40 (0)
dysuri	first	0/40 (0)	3/40 (6)	0/40 0)
	second	0/40 (0)	2/40 (5)	0/40 (0)
hematuria	first	0/40 (0)	5/40 (11)	0/40 (0)
	second	0/40 (0)	0/40 (0)	0/40 (0)
hematospermia	first	0/40(0)	0/40 (0)	0/40 (0)
	second	0/40 (0)	0/40 (0)	0/40 (0)
frequency of urination	first	1/40 (2)	4/40 (9)	0/40 (0)
	second	0/40 (0)	4/40 (10)	0/40 (0)

Figure 1: Treatment planning system. Axial, coronal and sagittal views of the markers

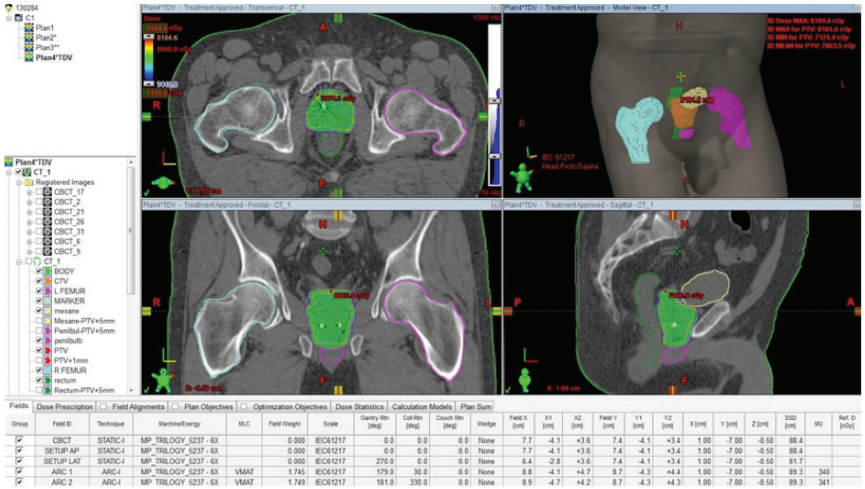
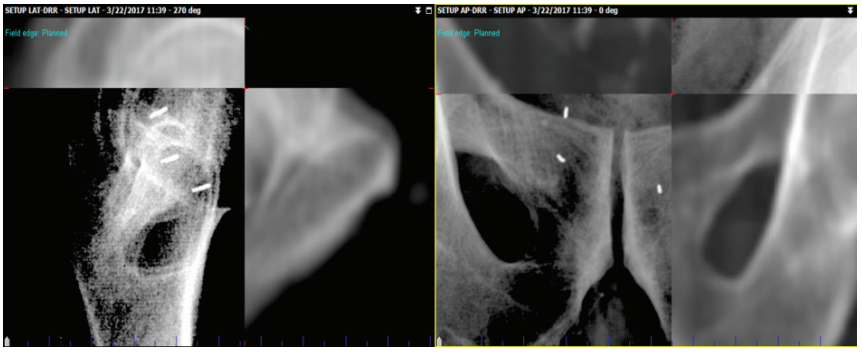


Figure 2: Portal imaging system. Lateral and anteroposterior views of the markers



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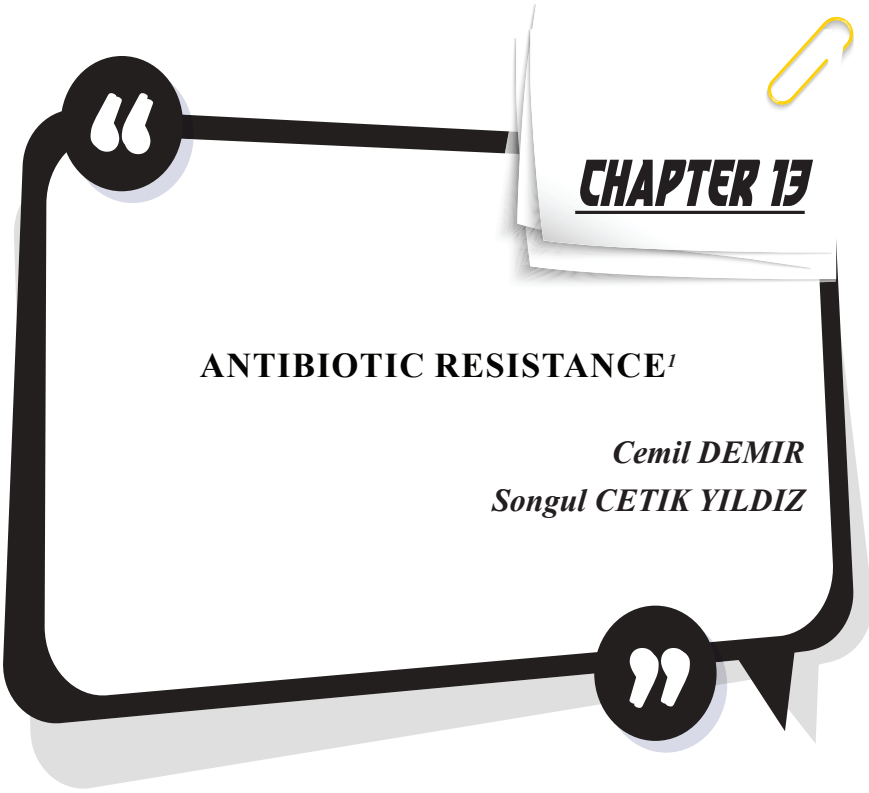

CHAPTER 13

ANTIBIOTIC RESISTANCE[1]

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1. Introduction

Antibiotics can be defined as synthetic, semi-synthetic or naturally prepared substances that can be produced by molds, bacteria and plants and have an inhibitory effect on microorganisms such as bacteria, viruses, fungi, actinomycetes, and are used to protect against infectious agents and stop the growth of microorganisms or kill them even at very low concentrations. Antibiotics have become indispensable in the treatment of many diseases that were known to be deadly, by providing great benefits in the treatment of infectious diseases. However, antibiotics are not always used for treatment.

It is among the most important issues in the world due to its effects on public health and economic burden. Especially recently, the rapidly increasing antibiotic resistance rates in the world; It has an impact on global health, sustainable development, the global economy, trade and the stability of countries, and the predictions that it will have very important effects in the coming years are increasing day by day. While most bacterial diseases could be treated with antimicrobial agents in the past, today the target microorganisms are exposed to these substances at low levels for a long time as a result of incorrect and non-therapeutic use of antibiotics. Therefore, resistance develops in a short time against every antibiotic found and an increase in resistant bacterial populations is observed. As a result of resistance, bacterial agents that do not respond to classical antibiotic treatment in the community cause infections with high morbidity and mortality. Thus, antibiotic resistance has become an important health problem all over the world, threatening not only the present but also the future. The rapid development of resistance to antimicrobial agents has made it necessary to investigate new agents as well as different mechanisms of action in the treatment of different bacterial infections.

2. Antibiotics

Antibiotics can be classified in different ways according to their mechanism of action, potency on microorganisms, spectrum of action, pharmacokinetic properties and chemical structures.

They are classified in two ways as bactericides (which kill bacteria directly) and bacteriostatics (which stop the growth of bacteria) according to their spectrum of action on bacteria at the concentrations they form in different body fluids (1).

According to the mechanism of action of antibiotics; antibiotics that inhibit cell wall synthesis, antibiotics that inhibit protein, DNA and RNA synthesis, and antibiotics that prevent membrane permeability.

I. Antibiotics that inhibit protein, DNA, RNA synthesis: Antibiotics such as tetracycline, aminoglycoside, macrolide, quinolone and fluoroquinolone are used to inhibit or stop the protein, DNA or RNA synthesis of microorganisms. Antibiotics, which stop the synthesis of protein, DNA or RNA, negatively affect the synthesis of DNA and RNA, together with the proteins needed for the bacteria in the reproduction process (2).

II. Antibiotics that inhibit membrane permeability: The effects of antibiotics on membrane permeability and mechanism cause the microorganisms to lose their viability by showing a bacteriostatic effect. Antibiotics cause changes in the structure of lipids in the membrane. In addition, they negatively affect membrane permeability and structure by providing displacement of some proteins (3).

III. Antibiotics that inhibit cell membrane synthesis: Substances that cause cell membrane disruption and inhibit the synthesis of membrane polymers inhibit the developing bacteria by sensitizing them to osmotic effects. Antibiotics that act on cell membrane biosynthesis include β-lactam group antibiotics (penicillin, cephalosporins) and vancomycin. B-lactam group antibiotics prevent cell membrane biosynthesis and cause significant changes in the functions of the structures (peptidoglycogan) there. Antibiotics in this group increase their negative activities on microorganisms by inhibiting the enzymes involved in the synthesis of the cell membrane, as well as by containing compounds or molecules resistant to β-lactamase enzyme activity in the side chains added to their chemical structures (4).

3. Antibiotic Resistance

Since the invention of antibiotics, antimicrobial agents have been used for the treatment of bacterial infections. However, the use of antibiotics in the treatment of bacterial infections and the development of antibiotic resistance in pathogenic bacteria are a major threat. It has been observed that antibiotic resistance was rarely seen before the use of antibiotics, but with the widespread use of antibiotics, microorganisms have become resistant to each new antibiotic (5). Resistance is known as the ability of a microorganism to resist the lethal and growth-inhibiting effect of an antimicrobial agent. When the microorganism becomes resistant to different antibiotics, it is called antibiotic resistance. Cross-resistance is also the case when a group of microorganisms that become resistant to a drug type can acquire resistance to another drug with a similar mechanism of action in a different structure.

The widespread use of antibiotics in both clinical and agricultural fields has led to the development of antibiotic resistance in pathogenic

bacteria (6-8). Microorganisms acquire resistance to antibiotics in various ways. In some cases, an increase in resistance is observed due to the ability of microorganisms to damage antibiotics. Another development of resistance is observed as a result of preventing the entry of the antibiotic into the cell due to changes in the permeability of the microbial cell wall and membrane. In addition, drug resistance can occur with the alteration of metabolic pathways in microorganisms (9,10).

Mutation and selection, together with genetic change mechanisms, facilitate the rapid adaptation of many bacterial species as a result of the introduction of antibacterial agents into bacterial environments. In some cases, mutation alone is sufficient to produce clinically high levels of resistance in organisms (11). Resistance can be achieved by changing the target area where the antibiotic acts. By changing the amino acid in the target region, the affinity of the antibiotic is reduced. For example; Penicillin binds irreversibly to penicillin binding proteins (PBP), thus preventing peptidoglycan synthesis. As a result of the change in this target area where penicillin binds, the antibiotic cannot be bound and resistance is provided. By preventing the entry of the antibiotic into the cell, resistance is provided against its toxic effect. The cell membrane acts as a barrier preventing the entry of the antibiotic into the cell. Changes in the number of proteins and the amount of lipopolysaccharides in the outer membrane affect the permeability of the barrier membrane and can provide resistance to antibiotics (12). Two different antibiotic resistances can be seen in microorganisms: natural and acquired resistance. Due to the hereditary structures of the microorganism, resistance to antibiotics is defined as natural resistance.

3.1. Intrinsic (natural) resistance

Resistance to antibiotics in the natural structure of microorganisms is called natural resistance. It occurs when there is no target molecule to which the antimicrobial agent binds in the cell or there is a difference that will prevent the binding (13). Natural resistance means that the microorganism is resistant due to its structure. It is not expressed as a hereditary resistance. The absence of the target molecule that binds the antimicrobial agent to which it is effective creates natural resistance. No origin of the strain naturally resistant to an antimicrobial agent is affected by that antibiotic (13). Chromosomal coding of enzymes that inactivate antibiotics, such as enzymes that inactivate β-lactamases and aminoglycosides, the multi-drug resistance efflux pump, and target protective factors contribute to natural resistance by counteracting the effect of the antibiotic (14).

3.2. Acquired resistance (genotypic, hereditary)

Acquired resistance is known as acquired resistance later. It occurs when bacteria gain resistance due to overuse and misuse of antibiotics.

It occurs as a result of mutations in chromosome, transposon or plasmid DNA, or by the acquisition of resistance genes from other bacteria by transformation, transduction or conjugation. When the bacterial population with this resistance comes into contact with the antimicrobial agent for the first time, the antibiotic becomes effective on the microorganism. However, resistance to antibiotics is observed in the microorganism population during contact or repeated treatments. Genetic (chromosomal) resistance mechanisms create permanent resistance as a result of changes in the genetic structures of microorganisms (13).

4. Antibiotic Groups and Resistance

4.1. β-lactam group antibiotics (Cefalosporins, monobactams, carbapenems**):**

β-lactam group antibiotics inhibit or inhibit cell growth by affecting the cell wall biosynthesis of bacteria (15). β-lactamase enzymes react with bacterial penicillin binding proteins (PBP) and act by hydrolyzing the β-lactam ring, inactivating cell wall synthesis. Antibiotic activity is lost when this ring is opened (16). The first penicillin-resistant bacteria appeared to do this by producing inducible beta-lactamase. This enzyme inactivates the beta-lactam ring by breaking down. Beta-lactamase is carried on the beta-lactamase gene (*blaZ*), which is carried in *Tn552*-like transposons and residues (17).

The β-lactamase resistance gene, *blazA*, is part of a transpositive construct on the plasmid containing resistance genes to other antibiotics. In 1961, methicillin, a semi-synthetic penicillin, was introduced. The penicillin G resistance problem was briefly resolved. The formation of resistance is formed by the production of a new penicillin binding protein called *PBP2a*. *PBP2a*, encoded by the *mecA* gene, is resistant to all β-lactam antibiotics (18).

4.2. Glycopeptides/ Lipoglycopeptides group antibiotics (vancomycin and teicoplanin**)** Glycopeptide antibiotics such as vancomycin and teicoplanin are antibiotics that are composed of glycosylated non-ribosomal peptides and inhibit cell wall synthesis (19). Vancomycin and teicoplanin are the first generation glycopeptide antibiotics to come into clinical use. These antibiotics are tricyclic glycopeptides naturally derived from bacteria and composed of a seven-membered peptide chain. Especially in the clinic, it is used in the treatment of serious infections caused by Gram-positive bacteria sensitive to antibiotics, although there are bacteria resistant to antibiotic groups such as β-lactam and methicillin. These agents act on both aerobic and anaerobic Gram-positive microorganisms. However, the irrational use of these antibiotics has led to the development of resistance in bacterial agents. In addition, the low

lipophilicity of these antibiotics leads to their inability to reach some tissues in sufficient concentration and failure in treatment. Due to the rapid increase in Staphylococcus and Enterococcus resistant to β-lactam antibiotics, glycopeptide/lipoglycopeptides antibiotics have been widely used in the clinic (20).

4.3. Aminoglycoside group Antibiotics (streptomycin, neomycin, kanamycin**)**

Aminoglycosides prevent peptidyl tRNA translocation by binding to the 30S ribosome. But the actual mechanism leading to bacterial death is unknown (21). One of the antibiotic groups frequently used in the treatment of infections caused by gram-positive pathogenic bacteria is aminoglycosides. Aminoglycosides have a bactericidal effect and also create a synergistic effect with other antibiotics. There are two mechanisms of resistance to these antibiotics. The first of these is aminoglycoside-modifying enzyme (AME)-mediated or AME-dependent resistance formation. The second is resistance formation independent of AME (22). Resistance occurs by changing the ribosome structure or changing the membrane energy. Thus, the aminoglycoside cannot be bound or taken up into the cell. (23). However, the most common mechanism is the alteration of the antibiotic structure by enzyme. Aminoglycoside acetyltransferase replaces amino groups or hydroxyl groups are replaced by adenyl/nucleotidyl transferase or aminoglycoside phosphotransferase. Thus, it cannot bind to ribosomes and prevent protein synthesis (24). The basic mechanism of aminoglycoside resistance is drug inactivation by aminoglycoside-modifying enzymes encoded in mobile genetic elements.

4.4. Tetracyclines group antibiotics (tetracycline, oxytetracycline, metacycline**)**

It has been observed that bacteria begin to gain resistance in a very short time after the use of tetracyclines. Resistance to tetracycline is determined by too many genes (25). The four main genes associated with tetracycline resistance among Gram-positive bacteria; tetK, tetM, tetO and tetL (21). Three of these resistance genes, tetK, tetL, and tetM, are found in staphylococci. The clinically important tetM gene encodes chromosomal resistance to minocycline and doxycycline (26).

4.5. Macrolide group antibiotics (erythromycin, clarithromycin, azithromycin**)**

They have a wide range of action against gram positive and negative bacteria, spirochetes, anaerobes and obligate intracellular pathogens. The most important drug of this group is erythromycin (27).

4.6. Quinolones group antibiotics (nalidixic acid, oxolinic acid, sinoxacin**)**

Although quinolone antibiotics are effective against Gram-positive bacteria, intracellular pathogens and some anaerobes, they are mainly effective against Gram-negative bacteria (27).

4.7. Sulfonamides and trimethoprim group antibiotics (sulfadiazine, sulfamerazine, sulfamethazine**)**

Sulfonamides, which are widely used antibiotics in the world, are used in the treatment of bacterial infections in humans and animals. In addition to being low cost, they have low toxicity. It has been observed that bacteria gain resistance to sulfonamides due to misuse of drugs (28). Trimethoprim-sulfamethoxazole (TMP-SMX), also known as co-trimoxazole, is a combination of two antimicrobial agents that act synergistically against a wide range of bacteria (29). Bacterial resistance to trimethoprim and sulfonamides is explained by 5 different mechanisms. These are identified as permeability barrier and efflux pumps, naturally insensitive target enzymes, regulation changes in target enzymes, mutation or recombinational changes in target enzymes, and acquired resistance by drug-resistant target enzymes (30).

4.8. Cephalosporin group antibiotics

Cephalosporins, which have a bacteriocidal effect, are one of the most widely used antimicrobial agents (27). In cephalosporins, the β-lactam ring is fused with a 6-carbon dihydrothiazine ring. In this way, resistance formation is observed. In penicillins, on the other hand, resistance occurs by binding to a 5-carbon dihydrothiazine ring (26).

4.9. Oxazolidinone group antibiotics (tedizolid, linezolid, pozolid**)**

Linezolid is a clinically important oxazolidinone antibiotic. This antibiotic binds to the ribosomal subunit of many Gram-positive bacteria such as MRSA and VRSA and inhibits protein synthesis in these bacteria by targeting the center of ribosomal peptidyl transferases. The mechanism of resistance to linezolid is through mutations in the rRNA of its ribosomal subunit (31).

4.10. Phenicol group antibiotics (chloramphenicol, thiamphenicol**)**

Chloramphenicol acts on most Gram-positive bacteria (26). It is known that resistance of S.aureus strains to chloramphenicol is caused by antibiotic inactivation. Antibiotic inactivation occurs in the presence of acetyl coenzyme A (CoA). (24).

4.11. Ansamycin group antibiotics (geldanamycin, herbimycin-a, rifamycin**)**

Rifampin, a member of the ansamycin group, can be used in Gram-positive and Gram-negative bacterial infections (26). Rifampin inhibits transcription by binding to the β subunit of RNA polymerase and thus exerts a bactericidal effect (21). Rifampin is specifically encoded by the rpoB gene. It is known that there are conserved domains in the predicted amino acid sequence of the RNA polymerase β subunit in *S.aureus* strains. Resistance to rifampin is observed due to changes in target (32).

4.12. Antibiotics of the streptogramins group (pristinamycin, quinupristin, dalfopristin**)**

Streptogramins are widely used against staphylococcal infections. Streptogramin consists of two components with synergistic activity, type A and type B.

5. Commonly Used Antibiotics and Resistance

5.1. Penicillin

Penicillins, which are among the β-lactam group antibiotics, are obtained from various mold species, especially Penicillium notatum and P. chrysogenum. It inhibits the synthesis of the cell wall and forms cross protein bonds by binding to enzymes. The enzymes formed are known as penicillin-binding proteins, and together with autolytic enzymes, they weaken the cell wall structure in susceptible bacteria and ultimately cause the cells to break down. (33). β-lactamase enzymes are known as enzymes that hydrolyze and neutralize the β-lactam ring in antibiotics in the penicillin group. As a result, bacteria become resistant to β-lactam group antibiotics that inhibit cell wall synthesis (34). Resistance to β-lactam group antibiotics that are not hydrolyzed by β-lactamase enzyme, methicillin, oxacillin, nafcillin, cloxacillin and dicloxacillin is defined as methicillin resistance (35). Natural penicillins are more effective on aerobic and Gram-positive bacteria (Emrerococcus, Sireptococcus). They are also known to be effective against Sraphvlococeys species that cannot produce some β-lactamase enzymes. It is known that synthetic penicillins were first developed with the emergence of microorganisms capable of producing penicillinase enzyme. Penicillins in this group contain large side chains attached to the molecule in their structures and prevent the ability of Staphylococcus to produce the penicillinase enzyme and to change the β-lactam ring (8).

5.2. Methicillin

It is important in the penicillins group, which is included in the synthetic penicillins and is resistant to the penicillinase enzyme. Its use was short-lived due to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA).

5.3. Vancomycin

This antibiotic, a glycopeptide with activity against a wide variety of gram-positive organisms, was discovered in 1952 (36). It is the first glycopeptide antibiotic developed for clinical use. It has a bactericidal effect against many Gram-positive microorganisms. vancomycin; It is widely used in the treatment of infections caused by susceptible Gram-positive microorganisms that cannot be treated with less toxic drugs such as penicillin and other antibiotics in the cephalosporin group. Vancomycin is an agent to which almost all Staphylococcus strains are sensitive (37). Many bacteria have developed resistance genes against vancomycin. For this reason, there was a need to develop new generation glycopeptides.

5.4. Daptomisin

Daptomycin is an antibiotic obtained from *Streptomyces roseosporus*. Daptomycin is a lipopeptide antibiotic with a unique mechanism of action involving insertion and insertion into the bacterial cytoplasmic membrane in the presence of a physiological concentration of calcium ions. Daptomycin resistance has been associated with several point mutations in at least three different proteins. Resistance mechanisms include increased voltage difference across the cytoplasmic membrane and less binding of the drug to its target area (38).

5.5. Streptomycin

Streptomycin is an inhibitor of bacterial protein synthesis, and streptomycin-resistant mutants obtained in vitro have abnormal ribosomes. The gene that determines this type of resistance has a chromosomal location (39).

5.6. Teicoplanin

It is similar to vancomycin in terms of both its chemical structure and antimicrobial activity (Marcone et al., 2018). Teicoplanin; It has advantages such as less side effects, ease of intramuscular use and once-daily use. In addition to its positive pharmacokinetic and pharmacodynamic properties, it has taken its place in the health field in a short time as an alternative agent to vancomycin due to less side effects compared to vancomycin. It is used to treat infections caused by susceptible Gram-positive bacteria, including those resistant to antibiotics (such as methicillin and cephalosporins). It is an effective antibiotic against both aerobic and anaerobic Gram-positive microorganisms (37).

5.7. Tetracycline

Bacterial resistance to tetracycline is common. Five classes of tetracycline resistance (Tc') genes have been distinguished in Gram-

positive cocci (Staphylococcus, Streptococcus and Enterococcus spp.), tet (K), tet (L), tet (M), tet (N), and tet (O). The tet (M), tet (N), and tet (O) genes confer resistance to tetracycline veminocycline, a lipophilic analogue of tetracycline, while tet (K) and tet (L) confer resistance only to tetracycline (40).

5.8. Rifampicin

Rifampicin is an antibiotic that acts by binding to the β-subunit of RNA polymerase in Gram-positive bacteria (41). Mutations in the rpoB gene region that encodes the RNA polymerase enzyme lead to resistance. It is a hydrophobic compound and is naturally resistant to Gram-negative bacteria as it cannot pass through the outer membrane (42).

Conclusion

Microorganisms show sensitivity to the drug at the first contact with the antibiotic; however, during the contact period or during repeated contacts, the bacterial population develops resistance to the antibacterial effect of the drug. An important point to emphasize is that almost every bacterial population has the potential to develop resistance to any antibiotic sooner or later. For this reason, a microbial drug that is initially effective against certain bacteria at low concentrations may be ineffective against the bacterial species at these concentrations or even at thousands of times higher concentrations. Antibiotic resistance, together with the discovery of antibiotics, is that bacteria can develop resistance as they are used against these pharmacological agents and if the desired precautions are not taken; It is estimated that the effect of the antibiotics we currently use in the treatment of infectious/infectious diseases will disappear, so in other words, humanity may be faced with the pre-antibiotic era again. As a result, multi-antimicrobial drug resistance has become a major health problem worldwide. In addition, this situation causes important problems such as unresponsiveness and cost increase in treatment.

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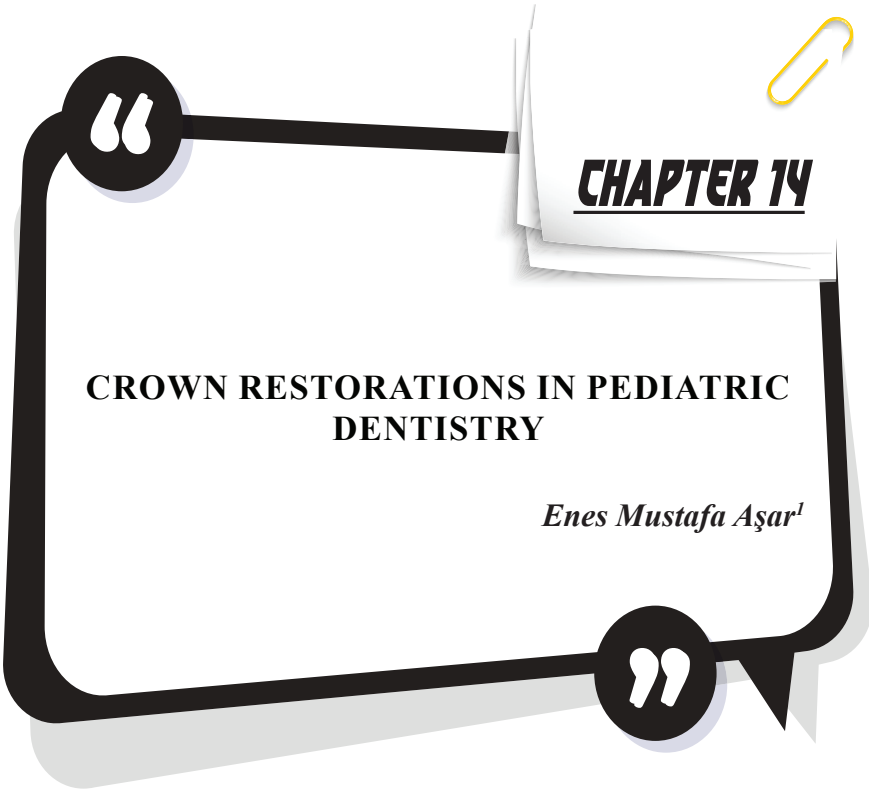

CHAPTER 14

CROWN RESTORATIONS IN PEDIATRIC DENTISTRY

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INTRODUCTION

Dental caries cause loss of enamel and dentin tissues following acid production as a result of the fermentation of carbohydrates accumulated on the surface by bacteria (Margolis & Moreno, 1994). It is a common health problem in developing countries and among individuals with low socioeconomic status (Petersen & Lennon, 2004). Primary teeth are affected by caries much faster than permanent teeth, and this may result in severe tissue loss over time (Üstün & Koruyucu, 2021). Material losses in the teeth for various reasons, especially dental caries, cause unpleasant tooth appearance. It can also lead to rejection or negative effects, especially among peer groups at school age (Qureshi, Vashistha, Khan, & Krishna, 2021). In a study, it was shown that the appearance of teeth in school-aged children can affect mobbing and this may have long- or short-term psychological consequences (Seehra, Newton, & DiBiase, 2011). The primary treatment is to preserve the structural integrity of primary teeth with a lot of tissue loss, to preserve the mesio-distal dimension, and to make a long-term biocompatible restoration. For this reason, crown restorations are preferred primarily in the treatment of primary teeth with high tissue loss (Mulder, Medhat, & Mohamed, 2018).

Crown restorations were first introduced in 1947 by the Rocky Mountain Company. Stainless steel crowns (SSC) were introduced by Engel in 1950, but it was thanks to William Humphrey that they began to be used routinely. In the following years, the use of SSC as space maintainers was discussed. Strip crowns were introduced by Webber in 1979. In 1983, Hartman developed aesthetic anterior crowns by veneering SSC. Randy et al. used band loop space maintainers by adding loops to SSC in the early 1990s. In the 1990s, Norna Hall developed a technique known as the Hall technique, in which teeth are restored with stainless steel crowns without removing caries. The American Academy of Pediatric Dentistry (AAPD) recommends SSC for the restoration of teeth with multiple surface caries (Subcommittee & Affairs, 2016). Although various modifications were made with stainless steel crowns, aesthetic results could not be obtained for anterior teeth. For this reason, pediatric zirconium crowns have been developed to obtain an aesthetic appearance, especially in the anterior region (Fellagh, 2016).

Diverse types of crowns are used as a full-coverage restoration in primary teeth with excessive tissue loss. The most commonly used crowns in pediatric dentistry are SSC, strip crowns, and polycarbonate crowns (Garg, Panda, Shah, & Panchal, 2016).

PRE-FORMED METAL CROWN

Pre-formed metal crowns were defined in 1950 by Engel (1950) and followed by Dr. William Humphrey (1950). These crowns are composed

of a combination of iron, carbon, chromium, nickel, manganese, and other metals (Mathewson & Primosch, 1995). They are called SSC because they are made of stainless steel. These crowns are produced as prefabricated by the natural tooth anatomy. Adaptation of SSCs to teeth is limited as they are prepared prefabricated. However, due to its circular shrinkage from the margins and its flexible structure, it is an advantageous material in terms of marginal edge compliance (Croll, 1987; Radcliffe & Cullen, 1991). SSCs are frequently used in both primary and permanent teeth (Garg et al., 2016).

Treatments with SSC are an alternative treatment to traditional restorative treatments because of their longevity and good prognosis (Nicola P Innes, Evans, & Stirrups, 2007). In a systematic analysis study, Innes et al. showed that SSCs are the most appropriate restoration option compared to traditional restorative treatments (Nicola PT Innes et al., 2015). Although longevity, durability, and prevention of caries formation are the biggest advantages of SSCs, it has been shown in many studies that it does not meet the aesthetic expectations of patients and parents (Pani et al., 2016; Ram, Fuks, & Eidelman, 2003; Townsend et al., 2014).

Stainless steel crowns are classified into three different types according to their shape (Marwah, 2018).

Untrimmed stainless steel crown

These types of crowns are not trimmed or contoured. It is difficult and time consuming to adapt (Qureshi et al., 2021). Nevertheless, due to its completely cylindrical structure, it is still preferred for closing long crown margins, especially in teeth with deep approximal caries.

Pre-trimmed stainless steel crown

These crowns are pre-trimmed but not contoured. Therefore, they need pre-contouring and cervical crimping.

Pre-contoured stainless steel crown

These crowns are prefabricated and trimmed from all side surfaces and cervical margins. It is the most frequently preferred SSC type today (Marwah, 2018; Qureshi et al., 2021).

There may be many reasons why SSCs are preferred in clinical applications in primary and permanent teeth.

SSC indications for primary teeth (Subramaniam, Kondae, & Gupta, 2010);

1. Extensive caries in primary teeth that cannot be restored

2. The need for a sealed restoration after endodontic treatments

3. As a preventive and preventive treatment option

4. Having developmental problems affecting one or more teeth

5. As an abutment for a space maintainer or denture

6. As a long-lasting and leak-proof restoration in children treated under general anesthesia

7. Restoration of children with severe bruxism

SSC indications in permanent teeth (Dean, 2021);

1. As a temporary restoration until the age when a permanent restoration of a fractured or traumatized tooth can be achieved

2. As a medium-term restoration when clinically appropriate for socio-economic reasons

3. SSCs may be preferred in permanent teeth with developmental enamel or dentin problems, especially in tooth sensitivity caused by dysplasia

4. Restoration of permanent teeth with excess material loss and requiring veneer

Pre-Veneered Stainless Steel Crown

An aesthetic appearance has been tried to be obtained by using various facing materials such as composite resin or thermoplastic resin in SSC. Thus, the durability of traditional SSCs and the aesthetic advantages of composite resins are combined. Aesthetic veneers are maintained in SSCs with various mechanical and chemical bonding approaches (Randall, 2002). Although it was produced for anterior primary teeth at first, it started to be preferred in primary molars later. These crowns differ in terms of the shades available, method of facing attachment to the SSC, crown length, and clinician's ability to crimp the crown (Evans, Southwick, Foley, Innes, & Pavitt, 2000).

Open Faced Stainless Steel Crown

Open faced SSC has been developed to be more aesthetically acceptable and to take advantage of the strength and durability of SSC. Open faced SSC can be used in both anterior and posterior teeth (Garg et al., 2016). While preparing the open faced SSC, the SSC is first cemented to the tooth. The labial surface of the cemented crown is opened and the cement residue is removed. After the tooth surface is made visible, the composite is placed by opening the retaining grooves on the tooth (Hartmann, 1983; Weinberger, 1989).

POLYCARBONATE CROWN

Linear polyesters of carbonic acids form the structure of polycarbonates. They show high rigidity and impact strength and are called thermoplastic resins because they are molded as solids by heat and pressure into the desired form. They start to degrade at 270°F (Myers, 1976). They are acrylic resin shells that are adapted to the tooth by putting acrylic resin inside polycarbonate crowns (Qureshi et al., 2021). Although they are more successful than SSC in terms of aesthetics, polycarbonate has disadvantages such as the inability to resist abrasion forces and being decimated frequently. Although they were popular in the 1970s, their use decreased with the advent of composite strip crowns (Garg et al., 2016).

STRIP CROWN

In pediatric dentistry, SSCs are frequently used as durable and long-lasting restorative materials. But aesthetic failure is the biggest disadvantage of SSCs. Strip crowns are among the most aesthetic crowns in the treatment of decayed primary teeth. Composite resin strip crowns have been used in dentistry for many years (Champagne, Waggoner, Ditmyer, & Casamassimo, 2007). It is often preferred because it can be easily removed from the tooth after application and is easy to repair. Compatibility with gingival tissue after the application is more successful than SSC (Qureshi et al., 2021).

OTHER NEWER CROWN

Pedo Jacket Crown

The composite resin strip is in the form of a crown. Unlike the strip crown, it is made of copolyester material. It is filled with resin and cemented to the tooth, and unlike strip crowns, it is not removed from the tooth (Evans et al., 2000). They are aesthetic crowns that look like natural teeth. Because they are flexible, they can be cut and shaped with scissors. It has a short setting time in the mouth and can be preferred in uncooperative patients. After the tooth is prepared, acid and bonding agents are applied. Resin modified glass ionomer cement in areas with isolation difficulties, in areas where isolation can be achieved, composite resin cement is adapted to the tooth. Because it is made of copolyester material, high-speed finishing burs can melt the crown, so cannot be trimmed or reshaped with a high-speed finishing bur. Some of its disadvantages are that discoloration can be seen over time and its wear resistance is weak against occlusal forces. The most common cause of failure is the separation of the crown from the cement. However, even if the crown is separated, the adhesive material, composite resin or resin-modified glass ionomer cement, can remain on the tooth and act as a crown (Daniels, Sim, & Simon Jr, 1966; Üstün & Koruyucu, 2021).

New Millennium Crown

This type of crown was developed in the laboratory from composite resin materials. It's like a pedo jacket crown and strip crown. These crowns, like strip and jacket crowns, are filled with resin and bonded to the tooth. Alternatively, they can be trimmed and shaped with high-speed burs. They have some disadvantages such as not being bent, being expensive, and being very fragile (Sahana, Vasa, Sekhar, & Prasad, 2010).

Pedo Pearl Crown

It is a type of crown that is still under development and testing. It is a type of SSC-like crown that is completely veneered with tooth-coloured epoxy. But it is made of aluminum instead of stainless steel. This is because the epoxy coating adheres much better to aluminum (Mittal, Verma, Pahuja, Agarwal, & Tomar, 2016). It has advantages such as being cut, easy to cut and crimp without chipping, and being able to add composites later. It is relatively soft and has poor durability (Sahana et al., 2010).

Artglass Crown (Glass Tech Crowns)

It is produced from artglass, a polymer glass for use in the restoration of anterior primary teeth. Due to its micro glass and silica content, it provides more aesthetics and durability compared to strip crowns (Mittal et al., 2016). It has a bond ability similar to composite and has a long-lasting and aesthetic appearance similar to porcelain. It is resistant to plaque as it does not contain a composite interface. Since the abrasion of polymer glass is similar to enamel tissue, it is suitable for opposing tooth structures (Lee, 2002).

CAD/CAM CROWN

In pediatric dentistry, many crown restorations have been used from past to present. Since most of these crowns are prefabricated, it takes time to adapt to the tooth. The lack of ideal crowns that can meet both durable and aesthetic expectations and some disadvantages of crowns in the market have revealed the need for an ideal crown. With the introduction of technology into a dental practice, tooth-specific crown designs can be produced on computers by taking digital measurements (Mourouzis, Arhakis, & Tolidis, 2019). Systems with computer aided shaping and production are called Computer Assisted Design/Computer Assisted Manufacture (CAD/CAM). This technology was introduced in the 1980s and has been continuously developed and made more successful until today. The CAD/CAM system consists of collecting the data scanned in the mouth or on the models in the computer, creating the three-dimensional model on the screen, planning the design digitally, and then producing the planned design (Oguz, Bezgin, Orhan, & Orhan, 2021). Today, many restorations such as inlays, onlays,

laminate veneers, partial crowns, full crowns, and bridges can be made using this technology. Despite its widespread use in dentistry, it is not widely used in the restoration of primary teeth (Mete, 2014). In this system, since the restoration can be designed on the computer, unnecessary excess tissue removal can be avoided and more minimal tooth preparations can be made. At the same time, since they are designed specifically for the tooth, they can be finished in a single session during the day, much more compatible than prefabricated crowns (Tsitrou & Van Noort, 2008). The biggest disadvantage is the high cost of both the CAD/CAM system and the production cost with this system. In addition, difficulties may occur in scanning and transferring subgingival deep teeth to a computer. Finally, experienced personnel may be needed to obtain CAD/CAM restorations. For these reasons, its use has not become widespread today (Liu, 2005).

PEDIATRIC ZIRCONIA CROWN

Zirconium was discovered in Sri Lanka in the 18th century. As a chemical element, it has an atomic weight of 40 and is denoted by the symbol Zr. They are not found in nature in pure form, they can usually be found in different compounds. The most well-known compounds of zirconium are zirconium silicate (Zircon) (ZrSiO4) and zirconium oxide (Zirconia) (ZrO2) (Hisbergues, Vendeville, & Vendeville, 2009). The mechanical properties of zirconium are good, and the structure of the metal is resistant to corrosion, wear, and heat. Physically, it is much more successful when compared to ceramic materials. The surface of zirconium, which is a reactive metal, creates an oxide layer when it encounters the air, and this layer increases its corrosion resistance. Hip prostheses made by Helmer and Driskell in 1969 pioneered their use as a biomaterial (Helmer & Driskell, 1969).

In recent years, the continuity of the tooth structure, the durability of the restoration, and especially the parental satisfaction in terms of aesthetics have gained importance in the treatment of primary teeth with excessive tissue loss. The advancement of technology and the development of dental materials have revealed new treatment alternatives (Taran & Kaya, 2018). Zirconia is currently the strongest dental ceramic and produces aesthetic results (Garg et al., 2016). Zirconia restorations have been used in adult dentistry for over 10 years. Its aesthetically successful results have led to the adaptation of zirconia crowns to pediatric dentistry (Holsinger, Wells, Scarbecz, & Donaldson, 2016). *In vivo* and *in vitro* studies, although short-term, have demonstrated the successful results of zirconium crowns in primary teeth. As a result of the studies, zirconia crowns are acceptable as a restorative material in pediatric dentistry (Ashima, Sarabjot, Gauba, & Mittal, 2014; Townsend et al., 2014; Walia, Salami, Bashiri, Hamoodi, & Rashid, 2014).

The first commercially marketed pediatric zirconia crown was EZ-Pedo. In the following years, the use of pediatric zirconia crowns of different brands has become more widespread. Zirconia crowns show excellent aesthetic results. They require less precision for cementation than strip crowns and do not have components that can be separated after cementation like strip crowns. Although it provides aesthetic results, the main disadvantages are the lack of color options, the inability to change the shape of the crown and trimming it, and the need for more preparation than traditional crowns (Clark, Wells, Harris, & Lou, 2016).

In one study, zirconia crown restoration was performed on the first primary molar treated with a pulpotomy, and it was followed for 29 months until the tooth exfoliated. It was observed that the gingiva regained its healthy appearance within 3 days after cementation. It was observed that there was no wear on the opposing tooth of the crown and exfoliated at the same time as its symmetry (Cazaux, Hyon, Prud'Homme, & Trutaud, 2017). In another study, parental satisfaction and clinical success were evaluated in zirconia crowns in anterior primary teeth. No loss of retention was observed in the mean follow-up of 21 months, and it was observed that almost all of them remained healthy in the mouth. Again, in this study, wear was not observed in the opposing teeth. It was stated that the marginal integrity of the crowns was not impaired and no inflammatory reaction was observed in the gingiva. In addition, the majority of children and parents who participated in the study reported that the crowns had a natural tooth appearance and were visually satisfied (Holsinger et al., 2016).

FIGARO CROWNS

One of the aesthetic crown restorations that have come to the market in recent years is Figaro crowns. There are five different sizes available. This crown consists of either fiberglass or quartz fibers/filaments embedded with an outer cosmetic composite resin material. It is claimed to be the strongest of all crowns. Easily placeable, biocompatible and autoclavable. Like SSC, Figaro crowns require less tooth preparation, thus reducing the operating time at the patient's side (Amrutha, 2019; Chakraborty et al., 2019). Some of the disadvantages are that they are not seen clearly in radiographic evaluations and they are that they cannot be crimped (Amrutha, 2019; Chakraborty et al., 2019).

CONCLUSION

In summary, Tooth decay, which can cause tissue loss in different amounts, is one of the most common health problems. Sometimes, the loss of dental tissues can be a situation that cannot be restored with traditional treatments. In such cases, full crown restorations are the first choice to keep

the primary teeth healthy in the mouth until the age of exfoliation. There is a wide variety of restorative treatment options in pediatric dentistry, from SSC to strip and zirconia crowns. SSC has been preferred in pediatric dentistry for many years. However, today's increasing aesthetic expectations have led to various alternatives to SSC. Especially zirconia crowns are very successful in terms of patient and parent aesthetic expectations. Many types of crowns have been used successfully restoratively over the years. Many factors such as the child's behavior and compliance, the preferences of the dentist, aesthetic expectations, humidity, and bleeding control can affect the material selection. Therefore, there is a need for long-term studies on the search for the ideal crown material.

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CHAPTER 15

THE ROLE OF STORE OPERATED CALCIUM ENTRY (SOCE) IN THE FORMATION OF VASCULAR RESISTANCE CAUSED BY IMMUNOSUPPRESSIVE DRUGS, CYCLOSPORINE (CSA) AND TACROLIMUS (TAC: FK506), THAT ARE CALCINEURIN INHIBITORS

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INTRODUCTION

Immunosuppressive drugs, Cyclosporin-A (CsA) and Tacrolimus (Tac: FK506) that are calcineurin inhibitors, cause serious negative effects (Pennigna et al., 2010; Liu, 2007; Hathavay et al., 2003) and histopathological changes (Uz et al., 2012; Liptak et al., 2006). The formation of vascular resistance via the contraction of smooth muscle cells in the vascular wall can be an important factor for these results (Kihm et al., 2012; Albert et al., 2007). This study aimed to examine, in smooth muscle cell contraction, the role of store-operated calcium (Ca^{+2}) entry (SOCE) that was discovered in recent years, associated with the signal pathways of plasma membran and endoplasmic reticulum providing calcium regulation.

CALCINEURIN

Calcineurin is a molecule that activates T cells (Li et al.,2011). It is characterized by calmodulin and calcium dependent serine / threonine phosphatase molecules. Its activities dephosphorylation, protein expression, kinase and phosphatase activities (Sugimoto et al., 1997). Calcineurin is involved in the transmission of signal molecules received by T-cell antigen receptors to the cell nucleus. In the normal process, an increase in intracellular calcium level develops with the binding of the antigen to the T-cell receptor. Increased Ca^{+2} ions bind to calmodulin, a cytoplasmic protein that activates calcineurin (Feske et al., 2003). Calcineurin, which is activated by calmodulin, activates the nuclear factor of the activated T cell (NFAT), a cytoplasmic transcription factor, and enables the transition from the cytoplasm to the nucleus. Thus, expression of IL-2 and cytokine genes that are effective in T-cell proliferation occurs (Hogan et al., 2003).

CALCINEURIN INHIBITORS

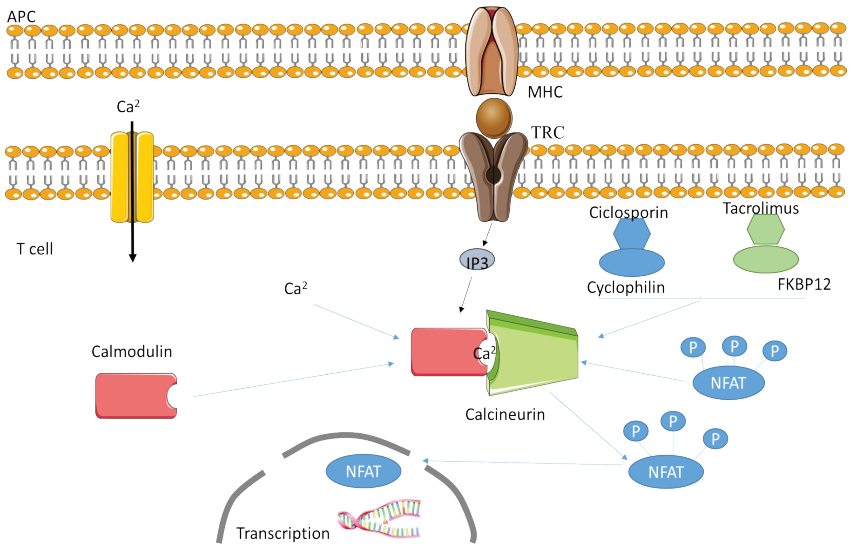
Calcineurin inhibitors are immunosuppressive drugs that inhibit T-cell functions and proliferation by inhibiting calcineurin molecules. Immunosuppressive drugs suppress the immune system (Karaduman, 2011). These drugs are used in the prevention of autoimmune diseases such as Behçet's disease, systemic lupus erythematosus, in the treatment of skin diseases such as atopic dermatitis, psoriasis (Karaduman, 2011) and in the prevention of tissue rejection after transplantation of organs such as heart, liver and kidney. Immunosuppressive drugs constitute the mechanism of action on the functioning of the immunoregulatory system (Kansu, 2002).

CSA AND TAC

CsA is a 1203 kd molecular weight, 11 amino acid chain and highly hydrophobic cyclic endecapeptide isolated from the fungus Tolypocladium inflatum (Shah, 2016). Tac is a macrolide group antibiotic with a weight of 822 daltons, produced by Streptomyces tsukubaensis, that prevents

T cell activation (Rie & Bos, 2003). CsA binds to cytoplasmic proteins cyclophilin, which is a peptidyl-propyl isomerases that plays an important role in protein folding and cell activation, and Tac binds to FK-binding protein-12 (FKBP-12) is an immunophilin (Shah, 2016). After this binding, cyclophilin and FKBP-12 also bind to target molecules, calcineurins. Thus, CsA-cyclophilin-calcineurin and Tac-FKBP-12-calcineurin complexes are formed and provide dephosphorylation of NFAT and NFATc target molecules respectively. Due to the dephosphorylation of NFAT and NFATc target molecules, the inhibition of genes of interleukin-2 (IL-2) and other cytokines is occurred. Thereby the synthesis and proliferation signals of T-cells are inhibited (Figür 1) (Borlongan et al., 1999; Olsen, 2014; Mcshane et al., 2010).

Although there is no structural and biological similarity, there is similarity in terms of negative effects of between the two drugs. These two drugs are metabolized in the liver by the p-450 system. Several side effects and complications can develop due to these drugs including hemodynamic instability, hyperlipidemia, hepatotoxicity, nephrotoxicity, neurotoxicity, infection, malignancy, obesity, hypertension, diabetes, hirsutism, gingival hyperplasia, and renal dysfunction (Pennigna et al., 2010; Liu, 2007).



Figür 1: *CSA AND TAC mechanism of action (Quoted from Fantini et al., 2006).*

HISTOPATHOLOGICAL CHANGES CAUSED BY CSA AND TAC

Uz et al. (2012), reported that oxidative stress marker levels increased in kidney tissues of Wistar albino rats treated with CsA and findings of arteriopathy, renal tubular damage and interstitial fibrosis were observed histopathologically (Uz et al., 2012). Acar et al. (2015), reported that bilateral ulcerated lesions were observed in the areas of the lower and upper premolars of a kidney transplant patient who presented with gum enlargement two months after the use of Tac. Liptak and Ivanyi reported that in the histological evaluation of kidney biopsies taken from CsA and Tac treated patients, acute toxicity findings such as necrosis of the smooth muscle cells of afferent arterioles and early hyalinization and isometric vacuolization in the proximal straight tubules were observed (Liptak et al., 2006). Andia et al. (2008), reported that there was an increase in maxillary alveolar bone formation in rats treated with Tac, and that most of the osteoclasts were separated from the bone surface and observed inactive in electron microscopy (Andia et al., 2008). Hwang et al. (2012), investigated the effects of CsA used in humans on umbilical vein endothelial cells. They identified the apoptotic cells under fluorescent microscope with cells stained with 4,6-diamino- 2-phenylidole (DAPI). As a result of the research, they reported that endothelial damage can be triggered through the activation of proapoptotic proteins (Hwang et al., 2012). Sagiroglu et al. (2014), reported that CsA caused decrease in tissue antioxidant levels. The number of endothelial nitric oxide synthase positive cells and the number of apoptotic cells increased with immunohistochemical staining and the tunnel method, respectively.

It is significant that most of histopathological changes have vascular damage origin (Liptak et al., 2006; Khim et al., 2012; Rie & Bos, 2003; Hwang et al., 2012; Tapperman et al., 2010). It has been observed that CsA and TAC cause pathologies related to vascular resistance (Khim et al., 2012; Tapperman et al., 2010). in many organ, but the cause of vascular resistance has not been fully elucidated yet. Studies show that the contraction of vascular smooth muscle cells is one of the most important factors involved in vascular resistance (Khim et al., 2012; Albert et al., 2007; Yıldız et al., 2011; Kalman, 2011).

THE MECHANISM OF CONTRACTION OF SMOOTH MUSCLE

The regulation of calcium, which plays an important and effective role in contraction in smooth muscle cells. The endoplasmic reticulum (ER) (sarcoplasmic reticulum (SR) in muscle cells) is an important Ca^{+2} storage organelle. SR provides 10,000 times more Ca^{+2} concentration within itself

than cell cytoplasm (Berridge, 2002).

Ca^{+2} is released from SR in response to the G protein-coupled receptors (GPCR) that stimulate the production of Phospholipase (PLC) and inositoltriphosphate (IP3) in the activated plasma membrane (PM). Calcium flow from SR to cytosol occurs spontaneously after IP3 binds to receptors in SR and this flow enables Ca^{+2} to rise rapidly in the cytoplasm (Berridge, 2002).

There are studies reporting that increased endothelin-1 (ET-1) or decreased nitric oxide (NO) levels are among the causes of direct contraction of vascular smooth muscle and affect Ca^{+2} regulation (Jeanmart et al., 2002; Zoja et al.,1986; Ramzy et al.,2005). However, in recent years, in the contraction of vascular smooth muscle cells, it is thought to be activated by ET – 1 and NO-related signal pathways or can be effective only with SOCE is emphasized (Kawanabe et al., 2002; Teubl et al., 1999; Erac et al., 2009).

SOCE

Putney demonstrated two-component GRPC agonists for the first time in 1986 that found in PM due to the reduction of Ca^{+2} in the endoplasmic reticulum. GRPC agonists are store operated Ca^{+2} entry that start with the release of Ca^{+2} from ER to cytosol and provide continuous Ca^{+2} entry from PM to cytosol (Kawanabe et al., 2002). Ca^{+2} entry from PM into the cell is determined according to the Ca^{+2} occupancy of the ER. With the emptying of Ca^{+2} reservoirs from ER, Ca^{+2} flows from two places from the cell PM towards the cytosol through SOCEs. The functions of SOCEs; are the discharge of Ca^{+2} from ER, and the activation of store-operated Ca^{+2} channels (SOCC) through receptors in PM after the discharge of ER, filling ER with Ca^{+2}, and the inactivation of SOCCs via receptors after the ER is filled with Ca^{+2} (Putney, 2007; Putney, 2009), respectively.

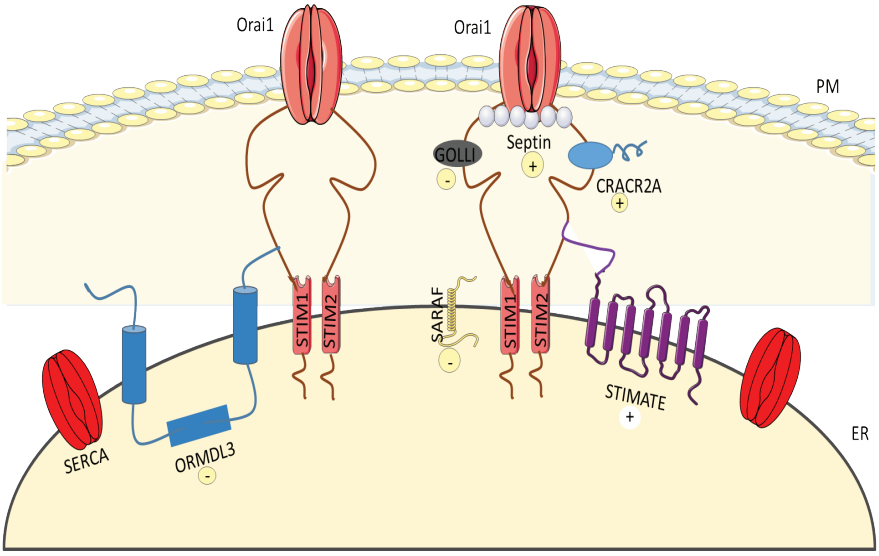
Two store operated channels in PM that regulate Ca^{+2} passage from the extracellular matrix to cytosol via PM have been defined. These;

1) The subunits of the Icrac family, which are called Ca^{+2} release-activated Ca^{+2} channels (CRAC) with high selective Ca^{+2} channels, are ORAI 1, ORAI 2 and ORAI 3,

2) The subunits of the TRPC family which are called transient receptor potential canonical channels (TRPC) are non-selective calcium channels, TRPC 1, 2, 3, 4, 5, 6, and 7 (Eraç et al., 2009).

Stromal interaction molecule (STIM), which is thought to be effective in opening these two channels and is found in the ER, is a multifunctional protein. It has two types, Stromal-interacting molecule 1, (STIM-1) and Stromal-interacting molecule 2, (STIM-2). In addition, STIM molecules

that enable communication with SOCE proteins have a Ca^{+2} binding domain in the lumen part of the ER, which enables the detection of Ca^{+2} changes in the ER. When the Ca^{+2} level decreases in ER, it oligomerizes and changes place by entering into a structural change and enables the subunits of Icrac family and TRPC family to open by creating an ER-PM link (Cahalan, 2009). Thus, Ca^{+2} flows from the extracellular matrix to the cytosol (Figür 2).



Figür 2. Overview of the SOCE modulators. Upon Ca2+ store depletion, STIM1 conformational change is stabilized by the ER-resident protein STIMATE, and the STIM1–Orai1 interaction at ER–PM junctions is supported by the proteins CRACR2A and septins. (Quoted from Albarran et al., 2016).

THE ROLE OF SOCES IN VASCULAR RESISTANCE

It has been shown in many publications that the increase in Ca^{+2} level in vascular smooth muscle cells, which is the reason for vascular contraction, may be due to deficiencies that may develop in the functions of SOCEs (Lampre, 1999; Niki et al., 1996; Dietrich et al., 2007; Giachini et al., 2007). Recent studies show that TRPC1 and ORAI 1 in smooth muscle cells can be SOCE proteins that can play a role in vascular resistance. These studies showed that SR-PM connections formed by STIM 1 were dislodged when SR was filled with Ca^{+2}, TRPC 1 and ORAI 1 channels were closed and the flow of Ca^{+2} from PM to cytosol is stopped (Liao et al., 2008; Kim et al., 2009).

There are also studies stating that blocking of ORAI 1 reduces the activity of SOCE, its modification may cause changes in SOCE components

(Liao et al., 2007) and also that ORAI 1 can control the opening of SOCC on one hand and provide inactivate of other channels on the other hand (Liao et al., 2009). In some studies, it has been reported that blocking the expression of ORAI 1 prevents Ca^{+2} transmission over TRPC 1 channels, and ORAI 1 may be a regulatory sub-unit for TRPC 1 (Liao et al., 2008; Kim et al., 2009; Liao et al., 2007; Liao et al., 2009). In addition to these, in a study, it was reported that Ca^{+2} input from ORAI 1, which starts with the emptying of tanks from ER, provides non-selective cationic current activation carried by TRCP 1 (Bergdahl et al., 2005). Giachini et al. (2011), stated that STIM1 / Orai1 expression is important for vascular dysfunction associated with arterial hypertension (Giachini et al., 2011). They also reported in another study that the aorta of hypertensive rats had increased intracellular Ca^{+2} influx and responsive vascular contraction compared to normotensive ones. STIM1 and Orai1 expression was increased in aorta of hypertensive rats, and that SR storage emptying could induce greater SOCE activation in these arteries (Giachini et al., 2009). Unlike these studies, (Gwozdz et al., 2008) a study reported that there may not be a direct relationship between STIM1 and Orai1 proteins that provides calcium flux and another sub-unit of the CRAC family could play a role. In this study, Ca^{+2} was discharged from SR in human embryonic kidney 293 (HEK293) cells treated with Thapsigargin (TG), and the expressions of STIM1 and Orai1 proteins were investigated by immunofluorescence staining method. Study findings showed that the correlation of STIM1 and Orai1 proteins was low. In addition, Hulot et al. (2011), stated that Ca^{+2} does not only provide the excitation-contraction pair in cardiomyocytes, but is also a signal molecule that causes cardiac hypertrophy. They reported that SOCE may be a factor in the formation of cardiac hypertrophy with STIM 1 dependent Ca^{+2} input (Figür 3).

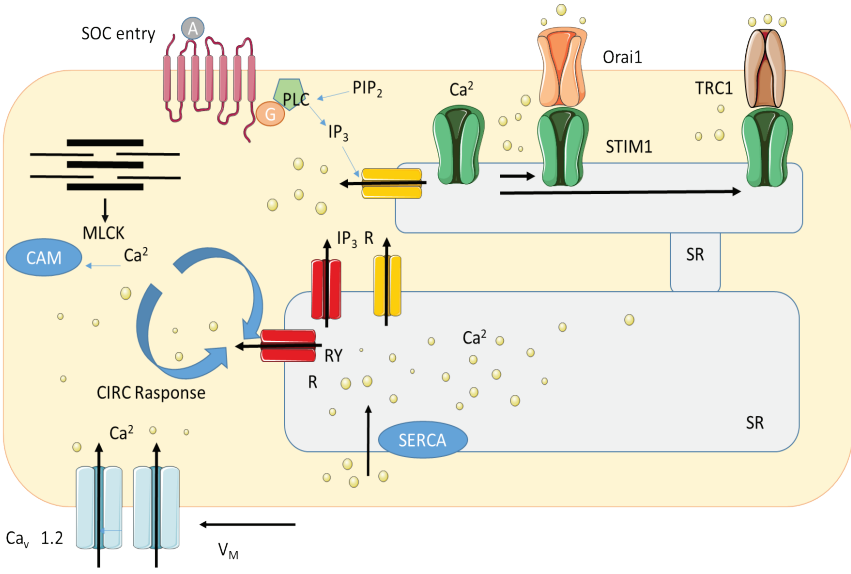


Fig. 3. Proposed mechanisms for metabolic syndrome-induced dysfunctional Ca2+ handling in coronary VSM. (Quoted from Plomaritas et al., 2016).

CONCLUSION

In the light of all this information, it is important to examine the pathways that enable and prevent the activation of the ORAI 1 and TRCP 1 and STIM 1 molecule, including the other molecules such as ET-1 and NO that may affect them, and to survey the relationships between these pathways. More detailed and clinically relevant histological, ultrastructural and molecular studies examining this relationship can be conducted. In this way, complications such as hypertension, decrease in kidney glomerular filtrate rate and renal dysfunction due to vascular resistance can be prevented, and as a result, the quality of life of patients can be increased and the cost of healthcare costs can be reduced.

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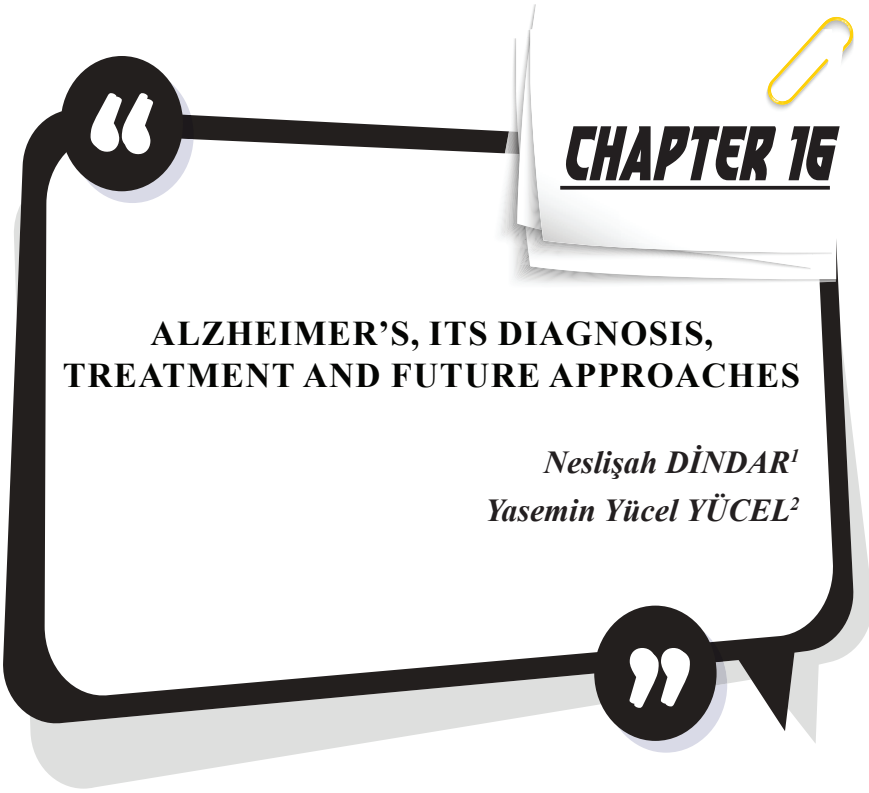

CHAPTER 16

ALZHEIMER'S, ITS DIAGNOSIS, TREATMENT AND FUTURE APPROACHES

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1. INTRODUCTION

Dementia is mainly caused by Alzheimer's disease (AD). AD is a gradual, irreversible, and progressive neurodegenerative disorder that leads to impairment of cognitive functions and eventually causes death. The first AD case was described in 1907 by Alois Alzheimer from a patient called Auguste D. when she was sent to the hospital with the symptoms of deteriorated mental dysfunction and behavioral changes, up to her death, she has monitored for 5 years her brain used for an autopsy. In the clinical study of her brain, aggregation of amyloid plaques and neurofibrillary tangles were concluded as the hallmarks of lesions in AD. And at present, in the USA, AD is the sixth major reason of death and the fifth leading reason of death in Americans who are over 65 years old (Thies & Bleiler, 2012). There is a dramatic increase in Alzheimer's disease in elderly patients worldwide, and it becomes a major public health problem. According to Cummings & Cole (2002), the number of new Alzheimer's cases per hour is estimated at 40 equating to 360000 new cases per year. At present, 55 million people are estimated to have Alzheimer's.

In this article, the history of AD, clinical presentation, diagnosis, epidemiology, hypotheses about pathophysiology, etiology, prevention, and treatment process will be explained and the latest information on the prospect of AD will be discussed. The reason why I choose this topic is I found it very interesting to lose a memory with your loved ones.

2. GENERAL INFORMATION

2.1. THE HISTORY OF ALZHEIMER'S DISEASE

Dementia is a scientific term that comes from the Latin word demens (meaning out of mind). Since ancient Greeks, Dementia is well known that an age-related mental disorder (Cipriani, C. et al., 2010). The collapse of the Greek-Roma Empire and increased young age deaths caused the loss of interest in dementia. In the eighteenth century, the term was used for young people who had serious head injuries and was later associated with stupidity. In the 1800s, senile dementia becomes separated from the other types of dementia with the help of physicians Pinel and Esquirol. They separated the term dementia and amentia (idiocy) and they described dementia as the loss of mental faculties resulting in disease (Cipriani, C. et al., 2010). Up to the nineteenth century, dementia becomes an inevitable part of the aging process. The first Alzheimer's disease case was defined by Alois Alzheimer who was born in 1864 in Germany. He studied medicine and worked as an assistant doctor at the Municipal Asylum for the Mentally ill and Epileptics, where the director was Emil Sioli. He worked for that clinic for 14 years. During those times, he became close friends with Franz Nissl, who developed fixation and staining methods for the microscopic

examinations of the nervous system (Cipriani, C. et al., 2010). Both made important searches of the pathology of the nervous system.

Alois Alzheimer's first patient was Auguste Deter who was born in 1850. Alzheimer first met with his patient in 1901. Before her acceptance to the mental asylum in Frankfurt, she was sitting with a blank expression. Her husband noticed progressive declines in her mental status. Her symptoms first showed when she was 51. Since then, her mental condition worsened, such as jealousy towards her husband, worsening mental disorientation, memory loss, and psychological impairments like someone wants to kill her. Her speech became unintelligible and for the last few months of her death, she slept most of the time. She died in 1906 because of septicemia. He moved to another clinic in Munich at that time, but he never forgot his first patient. So, he wanted her clinical reports and used her brain for the autopsy to examine her brain.

In clinical studies of her brain tissues, he examined a serious amount of neuronal loss besides, the aggregation of amyloid plaques and neurofibrillary tangles (NFT) in the cerebral cortex of the brain. He defined "miliary bodies" (Aβ plaques) and "dense bundles of fibrils" (NFT) as we recognize neuropathological hallmarks of AD (Blennow, K. et al., 2010). He published this clinical and pathological report under the title of ''A characteristic serious disease of the cerebral cortex''. Emil Kraepelin who is the director of the clinic in Munich introduced Alzheimer's disease as a complicated form of senile dementia that sometimes begins in the late 40s. He made a major distinguish the characteristic difference between senile dementia and AD according to young age, level of speech disturbances, and focal signs. After more than 50 years in 1906, Alzheimer considered extremely rare for young people whereas AD was considered abnormal aging because of the atherosclerotic changes in brain cells.

At present, the number of new Alzheimer's cases per hour is estimated at 40 equating to 360000 new cases per year. There are more than 50 million people living with Alzheimer's disease worldwide. And it is the sixth reason for death in patients who are over 65 years old.

2.2. Clinical Presentation

Alzheimer's disease symptoms are divided into 2 categories; Cognitive symptoms which needed brain-based skills such as perception, memory, and attention, and non-cognitive (behavioral) symptoms which are based on feelings, thoughts, attitudes, and motivations. Among them, loss of memory or in other words patient "forgetfulness" is the first symptom reported by Alzheimer's patients. Even before the clinical diagnosis of AD patients may complain about acquiring new information, and loss

of making plans (Förstl, H. & Kurz, A., 1999). In earlier stages, short-term memory loss from the patient's earlier years may be seen. Working memory and indicative memory decline gradually throughout the illness. Other reported cognitive symptoms are aphasia (loss of ability to make a speech and talk in mother tongue), apraxia (difficulty to initiate a speech) agnosia (loss of ability to recognize objects, faces, places, and voices), and impairment in the perception of time. Cognitive deficits develop in the mild stage of Alzheimer's. Noncognitive (behavioral) symptoms such as depression, hallucinations, getting upset or angry easily, impulsive behavior, physical and verbal aggression, and wandering could be seen in the earlier stage of illness and could be worsened during the moderate stage that causes the breakdown of family support. At the moderate stages of AD, even though the patient may be able to live independently they still need a variety of organizational matters to continue living because of the cognitive difficulties. In severe stages, loss of daily function (even in eating) and physical and verbal aggressions such as someone wanting to kill someone, jealousy, and suspicion of everything are seen because the patient mostly spends their time in bed. Pneumonia, myocardial infarction, and septicemia are the most widespread cause of death in Alzheimer's patients (Förstl, H. & Kurz, A., 1999).

2.3. Diagnosis

The first diagnosis starts with a family member who often brings memory problems of the patient to the clinicians because Alzheimer's patients highly deny memory problems and their disease. Elderly patients presenting gradual decline in memory and other impairment in their cognitive functions such as aphasia, apraxia, and agnosia are doubted of AD. In these patients, the Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA), widely used 30-point assessment tools for AD, and neuropsychological testing should be performed. Clinicians specifically neurological doctors do direct examinations of brain tissues at autopsy or biopsy. The diagnostic criteria of AD have been done by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) and Statistical Manual of Mental Disorders, fifth edition (DSM-V) (Dubois et al., 2007).

Since 1984, NINCDS–ADRDA provides criteria for "possible", "probable" and "definite" AD. The definite AD diagnosis based on NINCDS-ADRDA is made when there are neuropathological confirmations/ biomarkers such as the presence of amyloid plaques and tau proteins even without symptoms via autopsy pr biopsy. Probable AD includes a decline from usual daily activities, social interferences, and memory disorder with at least one or more impairments in other cognitive disorders such as

aphasia, agnosia, apraxia, and insidious onset with the gradual decline of symptoms. MMSE and neurophysiological testing should be performed. Possible AD is differentiated from probable AD in terms of onset, and underlying disorder. A possible AD includes atypical, sudden onset with documentation of progressive decline in cognitive abilities, with no co-morbid diseases to produce dementia. NINCDS– ADRDA indicates three stages of AD:

Stage 1 is the preclinical stage which is asymptomatic but neuropathological evidence such as accumulation of amyloid plaques and tau proteins are seen. Stage 2 is mild cognitive impairment (MCI) which is mild memory loss but there is not enough impairment in daily activities. Stage 3 is the dementia stage with gradual loss of functional abilities.

According to The Alzheimer's Association, 10 key signs of Alzheimer's are memory impairment, difficulty performing familiar tasks, language problems, time and place disorientation, poor judgment, problems with abstract thoughts, misplacing things, mood swings, changes in behavior, loss of initiative (Salloway & Correia, 2009). The difference between normal aging and Alzheimer's disease is normal people may forget some parts of the events or past but can recall the missing details whereas, people with AD likely forget all the experiences and cannot remember them later (Salloway & Correia, 2009). So, for differentiating the disease, clinicians use tests called MMSE or MoCA. MMSE test is used for cognitive function among elderly people to test their six domains of cognition such as orientation, learning/memory, social cognition, complex attention, sensory-motor function, language, and executive functions. Each of these six domains is divided into sub-domains so we get a 30-point assessment tool used to indicate which stage AD patients belong to. For orientation, clinicians ask about year, season, month, date, time, country, town, and hospital. For attention/calculation, subtract 5 from 50 or spell "pencil" backward: licnep. For learning, the examiner names three objects (orange, chair, penny) and asks the patient to repeat them. For memory, asks for the three objects learned earlier. For language, the examiner asks patients to read and obey a written command on the paper and then wants to write a sensible sentence. For intelligence and judgment, the examiner asks the patient to copy a pair of intersecting objects like pentagons. If the score is between 30 and 24 means patients have no cognitive impairment, a 23-18 score means mild cognitive impairment (MCI) patient has difficulty remembering recent events. They may get lost while driving. They begin to pull off from hobbies and hard tasks. They may reject memory issues. 17-10 means moderate AD which the patient needs assistance in daily living. Often disorientated to time (date, year, season). The memory of the latest events is strongly damaged. May become suspicious, jealous, or tearful. May forget some details of their past life. 9-0 score means

severe AD patient completely loss of daily function. May spend most of the time in bed. Aggression towards everything may be seen. There is also a cognitive assessment test released in 1966 called Montreal Cognitive Assessment (MoCA) which is very similar to MMSE, even scorings are the same, but MMSE takes 6-8 minutes while MoCA takes 10-12 minutes. Neither test is very detailed, they are only used for the initial process. It consists of five-word recall, clock-drawing, and executive and visuospatial items making it a more effective diagnosis for mild AD (Salloway & Correia, 2009). Alzheimer's is the biggest reason for dementia. American Psychiatric Association (APA) launched a glossary for mental diseases called DSM-I criteria in 1952. DSM-1 talked about "reactions". The mental disease shows the reactions of the personality to psychological and social factors. Then, this manual has undergone five revisions. DSM-II was published in 1967 and is very similar to DSM only eliminating the "reaction" term. In 1979, DSM-III was launched and provided explicit criteria for all the mental disorders listed in the manual. In 1999, DSM-IV was launched which provided a classification of neurocognitive disorders such as delirium, major neurocognitive disorders, and mild neurocognitive disorders and, released a chapter "Delirium, Dementia, and Amnestic and Other Cognitive Disorders". Dementia is described as the development of many cognitive impairments (including memory) caused by multiple etiology due to direct psychological effects. Today we use DSM-V criteria, published in 2013, to provide examples of symptoms and observations for each of the six cognitive domains.

The diagnosis of neurocognitive disorders due to AD based on the DSM-V approach is divided into two categories: Minor neurocognitive and major neurocognitive disorders of dementia due to AD.

The major neurocognitive disorder requires at least two or more declines in cognitive domains. One must be learning, and memory and other cognitive domains impairment cause the symptoms of agnosia, aphasia, and apraxia required for the diagnosis of major neurocognitive disorder. While for mild neurocognitive disorder learning and memory impairment are enough for the diagnosis.

Both neurocognitive disorders are characterized by the insidious onset with a gradual decline in at least one of the cognitive abilities. These criteria are made for possible and probable AD. For probable AD, only the evidence of genetic/causative mutation from family history and gradual decline in memory and learning abilities with one of the domain cognitive functions are present. For the diagnosis of possible AD, typical features are enough.

The most accruable diagnosis of AD is after death by autopsy, but it only confirms the disease. Brain biopsy has been confirmed to be accurate

in showing the pathological changes of AD (Khachaturian, 1985). So, biomarkers are used for determining the disease, but they are not valuable when establishing a diagnosis. They only increased the specificity of diagnosis. For Alzheimer's disease, the evaluation of biomarkers is complicated because of the variability in clinical features and progression, multiple molecular etiology, and long asymptomatic prodromal stages (Irizarry, n.d.). Cerebrospinal fluid (CSF), plasma, and imaging biomarkers are used (Reitz & Mayeux, 2014) for detecting the AD pathophysiology in biological fluids (Irizarry, n.d.). Other used markers can be urine, saliva, or fibroblast cell structures (Khachaturian, 1985). Among them, cerebrospinal fluid (CSF) provides the highest yield for the detection of AD which shows the composition of the brain's extracellular space (Irizarry, n.d.). Patients will undergo lumbar puncture. CSF's main biomarkers are phosphorylated tau (p-tau), β-amyloid1–42, and neurotransmitter loss. Neurotransmitter losses especially the loss of cholinergic neurons resulting in the degradation of the choline acetyltransferase (ChAT) (Khachaturian, 1985) detected in CSF is a useful marker because acetylcholine plays important role in memory and learning functions. The neuropathological causes of AD are the aggregation of amyloid plaques or in other words senile plaques (Aβ), Amyloid Precursor Protein (APP), and the microtubule-associated protein called Tau phosphorylation which causes neurofibrillary tangles (NFT). Aβ occurs in two prominent forms, containing 40 (Aβ40) or 42 (Aβ42) amino acids (Irizarry, n.d.)**.** Aβ42 deposited in the brain and more toxic in vitro studies (Irizarry, n.d.). In AD CSF or mild cognitive impairment (MCI) level, Aβ40 and Aβ42 are decreased while tau protein levels are increased compared to cognitively healthy people. CSF biomarkers are divided into two categories; basic and core biomarkers (Blennow et al., 2010). Primary biomarkers are useful when there is a concomitant cerebrovascular disease but not useful in pure AD. Primary biomarkers contain assays for Blood-Brain Barrier (BBB) (Blennow et al., 2010). Only small and lipophilic proteins can be able to cross BBB. Standard BBB function consists of the CSF: serum albumin ratio (Reitz, 2014). There is a steady-state level of the production and deposition of cerebral amyloid plaques in the lobes of the brain (Blennow et al., 2010). An increase will cause several types of vascular dementia (Blennow et al., 2010). On the other hand, core biomarkers have been developed to specify the main neuropathogenic processes (amyloid plaques and tau proteins levels) in AD. A decrease in CSF Aβ1–42 level is the aggregation of amyloids into plaques resulting in decreased availability of senile plaques to diffuse into CSF (Blennow et al., 2010). CSF total tau (t-tau) level reflects the neuronal damage in the brain. Patients who experienced acute disorders like stroke were reported to have an increase in CSF t-tau level. Plus, a high t-tau level has been associated with the high transition from MCI to severe AD and according to post-mortem NFT load, proposing that NFT might contribute to the high CSF t-tau level according

to post-mortem. Phosphorylated tau (p-tau) reflects the production of neurofibrillary tangles in the brain. So, AD patients have a marked increase in p-tau and t-tau levels and a significant reduction in Aβ1- 42 levels. Additional important CSF biomarkers are Visinin-like Protein 1 (VLP-1) and Apolipoprotein E (APOE) ε4 allele (a genetic risk factor for AD) (APOE4). VLP-1 is a calcium sensor protein and the gene assay analysis found out AD patients have a high amount of CSF VLP-1 level and APOE4 was also high. These genotypes highly correlate with late-onset AD. CSF biomarkers with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) increase the accuracy of the diagnosis of AD (Blennow et al., 2010).

Despite everything, CSF cannot routinely collect in the assessment of AD because its invasive and expensive. The most applicable are plasma biomarkers because they are not invasive and expensive (Irizarry, n.d.). Amyloid plaques can also be detected in the blood. Since BBB is limited only to small and lipophilic molecules, brain proteins are passed into the plasma. A cross-sectional study that investigated the plasma Aβ in AD patients found that Aβ40 was slightly increased in a study of 78 AD and 61 control cases and Aβ40 and Aβ 42 correlate with aging (Irizarry, n.d.). High plasma Aβ42 level may be a risk factor for the development of AD. But they are not sensitive or specific diagnostic criteria of AD because they correlate poorly with the severity of AD. But there is a steady-state level of the production and deposition of amyloid plaques in the brain. In other words, cognitively healthy individuals reflect the Aβ level in the brain to the Aβ in plasma (Reitz, 2014). Imaging biomarkers such as hippocampal atrophy on structural and functional MRI, Positron emission tomography (PET), and single-photon emission computed tomography (SPECT) may help the diagnosis of AD, but they are not sensitive and specific for the diagnosis of AD. PET using fluorodeoxyglucose shows the sample of brain metabolism and AD patients typically show hypometabolism in the temporal and parietal cortices (Salloway & Correia, 2009). Other markers are blood cells (erythrocytes) which may be a source of choline and cholinesterase levels. Both have been reported to be increased in red blood cells. Cell dyes such as Congo red, a safe stain, which can cross the BBB might show abnormal proteins to be detected by MRI (Khachaturian, 1985). The diseases may be the source of markers such as a cardiovascular disease like hypertension, diabetes may increase the deposition of Aβ, which can lead to the impairment of cognitive functions. High cholesterol level has been reported to increase the incidence of AD cases (Irizarry, n.d.). Cholesterol level is affected by APOE genotype, age, sex, and severity of AD. Impaired insulin signaling in the brain, oxidative stress, vascular disease, mitochondrial dysfunction, and excitotoxicity may the reason for AD. Laboratory tests may be helpful for the diagnosis.

Genetic factors also play important role in the development of early-

onset of AD (EOAD) and late-onset of AD (LOAD). Mutations of a gene located on chromosome 14, which produces a protein called presenilin 1, and mutations of a gene on chromosome 1, which produces presenilin 2 may be useful in the diagnosis. Because they encode for membrane proteins that may be involved in amyloid precursor protein (APP) processing. Mutations result in decreased activity of γ-secretase, an enzyme significant in β-amyloid peptide (Aβ) formation. Mutations in APP processing have been found to cause rare familial AD (FAD) (age at onset < 65 years). APOE4 is the most powerful risk factor gene for Alzheimer's disease. Even one APOE4 gene increases the risk of developing AD two- to threefold. 40-65% of Alzheimer's patients have the APOE4 gene. Late-onset of AD is primarily linked to the APOE4 genotype.

2.4. Epidemiology

Currently, in the USA, AD is the sixth major reason of death and the fifth leading reason of death in Americans who are over 65 years old (Thies & Bleiler, 2012). There is a dramatic increase in Alzheimer's disease in elderly patients worldwide, and it becomes a major public health problem. According to Cummings & Cole (n.d.), the number of new Alzheimer's cases per hour is estimated at 40 equating to 360000 new cases per year. And At present, more than 52 million people are estimated to have Alzheimer's. AD is associated with an approximately $172 billion healthcare cost per year in the USA (Reitz et al., 2011) with $20.5 billion associated with long-term care services (Castellani et al., 2010). In the next 50 years, the population of AD patients will nearly quadruple (Cummings & Cole, n.d.). The prevalence of AD duplicates every five years after the age of 60 rising from a prevalence of %1 among those 60- to 64- years old to %40 of those aged 85 years and older(Cummings & Cole, n.d.). The disease is more common in women compared to men by a ratio of 1.2 to 1.5. In Turkey, estimated Alzheimer's patients are more than 300.000 people. It would not be an exaggeration to say that this disease will be one of the most important health problems in the coming years like 30-40 years, since the young population is high. Based on its age of onset, AD is divided into two categories: EOAD (onset <65 years) accounting for %1-5 of all cases, and LOAD (onset >65 years) accounting for %95 of all cases (Reitz et al., 2011). Other than age, other risk factors are family history of dementia, head trauma, genetic factors (APOE4), being female, low education level, vascular disease, and environmental factors (Castellani et al., 2010).

TABLE 2.4.1 / *Prevalence and incidence of dementia in developed and developing regions*

Region	Consensus dementia prevalence at age ≥60 years (%)	Estimated annual incidence of dementia (per 1,000 individuals)	People with dementia aged ≥60 years in 2001 (millions)	Estimated increase proportion of peopl with dementia from 2001 to 2040 (%)
Western Europe	5.4	8.8	4.9	102
Eastern Europe (regions with low adult mortality)	3.8	7.7	1.0	169
Eastern Europe (regions with high adult mortality)	3.9	8.1	1.8	84
North America	6.4	10.5	3.4	172
Latin America	4.6	9.2	1.8	393
North Africa and Middle Eastern Crescent	3.6	7.6	1.0	385
Developed western Pacific	4.3	7.0	1.5	189
China and developing western Pacific	4.0	8.0	6.0	336
Indonesia, Thailand and Sri Lanka	2.7	5.9	0.6	325
India and south Asia	1.9	4.3	1.8	314
Africa	1.6	3.5	0.5	235
Combined values	3.9	7.5	24.3	234

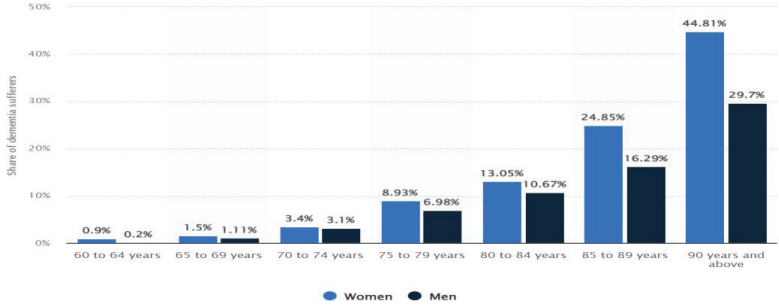
Data taken from Ferri *et al.* (2005).[3]

(Reitz et al., 2011)

In 2005, 24.2 million people had dementia and among %70 of them were attributed to AD. Individuals who are over 60 years old from North America and Western Europe displayed the highest prevalence of dementia. Meanwhile, annual regional dementia incidence rates are highest in North America followed by Western Europe (Reitz et al., 2011). The most notable rise of dementia was through the seventh or eighth decades of life. AD's prevalence and incidence rate also increase with age. The highest estimated increase of dementia in 2040 is from Latin America followed by North Africa /Middle Eastern Crescent and China and other developing western Pacifics (Reitz et al., 2011). As you can understand from the table, Alzheimer's disease is more common in developed countries due to the reduced contact with a diverse range of microorganisms which may cause the poor development of the immune system.

The other reason is diet. Asian people tend to eat rice more while European and American people tend to eat meat that has more fat content. For example, in Japan, with the increase in meat consumption while the decrease in rice consumption disappears the incidence of disease (Schwarcz, 2017).

TABLE 2.4.2 */ Share of population living with dementia in Europe in 2019, by gender and age*



(Estimating the Prevalence of Dementia in Europe, n.d.)

In 2019, almost 45 percent of women and 30 percent of men aged 90 years and above in Europe were living with dementia. Generally, the share of women living with dementia in Europe was higher in comparison to men. worldwide, women with dementia outnumber men with dementia 2 to 1(Mielke, n.d.). Because of this, there are so many theories of why women with dementia more than men with dementia are. The biggest theory is since females tend to live longer than men and for Alzheimer's disease, the greatest risk factor is age, the older you are, the more possible you are to develop AD. One study in Sweden followed 16,925 people and found that females were more likely to be diagnosed with AD than males of similar age. And another study in Taiwan shows that developing AD over six years was much more in women compared to men. So, women living longer than men is not a whole answer as to why women are more likely to diagnose with AD compared to men. Besides, one study in Sweden founds out the incidence of dementia without AD is not greater in women. So, this suggests that there must be a specific relationship between AD and gender. Many scientists believe the female hormone called estrogen which affects brain functions, mental health, cardiovascular system, and more is one of the reasons. They suggest that a woman with more estrogen throughout her life will be less likely to have dementia. For instance, if a woman starts her period at a younger age and goes through menopause later (Mielke, n.d.). Another reason is the deposition of amyloid plaques in AD may be the part of the brain's immune system for fighting infections. That's why they generally have more strong immune system than men and they end up having more amyloid plaques than men.

TABLE 2.4.3 */ Death Rate per 100,000*

Rank	Country	Rate
1	TURKEY	57.64
2	LEBANON	56.14
3	LIBYA	53.21
4	FINLAND	50.84
5	EQU. GUINEA	50.09
6	TUNISIA	49.00
7	YEMEN	46.37
8	JORDAN	46.33
9	SAUDI ARABIA	45.17
10	MOROCCO	44.13
11	NIGERIA	43.10
12	QATAR	42.74
13	IRAN	41.62
14	INDONESIA	41.55
15	SYRIA	41.30
16	CAMBODIA	40.49

Death rates per 100,000 people with standardized age show that poor countries and developing countries have higher mortality and morbidity rates of AD. It is mainly related to their socioeconomic levels such as industrialization and education. Second, public health measures to access professional caregivers, cost, adequate nutrition, and physicians.

Unfortunately, Turkey has the highest amount of death caused by AD. To decrease the amount of death, we need need to be more careful about the people around us and increase our education level (World Health Organization, 2018). According to health data, in Turkey, Alzheimer's becomes the fifth reason for death. It increases by %35.6 from 2009 to 2019 for all ages combined. The total prevalence of dementia in Turkey is 528,547. Men consist of 184,407 and women consist of 344,140. %0.65 of the total population have dementia in Turkey in 2018 **(**Estimating the Prevalence of Dementia in Europe, n.d.).

TABLE 2.4.4 */ Estimated prevalence of dementia in Turkey in 2050*

Turkey 2050						
Age ranges	Total population	Men	Men with dementia	Women	Women with dementia	Total number of people with dementia
30–59	37 102 965	18 705 537	29 929	18 397 428	16 558	46 487
60–64	5 795 866	2 850 984	5 702	2 944 882	26 504	32 206
65–69	5 616 766	2 718 864	30 089	2 897 902	43 480	73 569
70–74	5 003 743	2 364 379	73 240	2 639 364	89 810	163 050
75–79	4 071 312	1 847 855	128 926	2 223 457	198 484	327 410
80–84	2 897 316	1 246 819	133 003	1 650 497	215 429	348 433
85–89	1 722 927	669 529	109 085	1 053 398	261 776	370 861
90+	995 862	295 425	87 727	660 437	295 937	383 665
Population 30–90+	63 166 757	30 699 392	597 701	32 467 365	1 147 978	1 745 679
Total population	97 139 565	% of total population 1.80				

(Estimating the Prevalence of Dementia in Europe, n.d.)

The total prevalence of dementia in Turkey is estimated to be 1,745,679. Men with dementia will consist of 597,701 and women with dementia will consist of 1,147,798 people. %0.76 of the population have AD.

TABLE 2.4.5 / *Estimated Total Cost for the Treatment of Alzheimer's Disease in 2020 (Wong, 2020)*

TABLE. Estimated Total Cost of Care for the Treatment of Alzheimer Disease in 2020

Payment Source	Cost in Billions (B)
Medicare	$155 B
Medicaid	$51 B
Out of pocket	$66 B
Other	$33 B
Total cost	$305 B

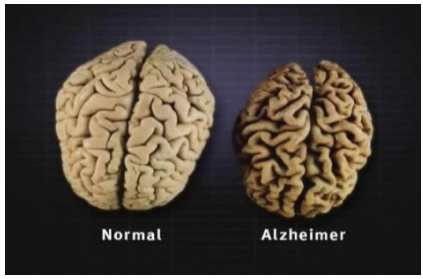
The total cost of healthcare for the treatment of AD is 305B dollars. These include doctor visits, emergency department, and hospital admissions, long-term care or skilled nursing facility care, and medicines. Total costs are predicted to increase to more than $1 trillion by 2050 (Wong, 2020).

2.5. PATHOPHYSIOLOGY

The main hallmark lesion in AD is the deposition of the protein beta-amyloid (Aβ) plaques outside neurons and twisted strands of the microtubule-associated protein tau inside neurons become neurofibrillary tangles (NFTs) in the cortical and hippocampus parts of the brain (More Than Normal Aging: Understanding Mild Cognitive Impairment, n.d.) and lipid-carrier protein apolipoprotein E, apoE4 (Roberson & Mucke, 2006) which can activate a neuroinflammatory cascade in the microglial cells (resident immune cells in CNS). However, continuous activation of microglial cells is toxic to neurons and glia. So, these changes lead to the degeneration of neurons and synapses and, cortical atrophy (brain size getting smaller). Aβ plaques and tau proteins are normal cellular components but in AD, they are abnormal fibrillary structures with poor solubility in water (Duyckaerts et al., 2009) formed pathogenic oligomers (Roberson & Mucke, 2006). The development of beta-amyloid plaques occurs earlier in the frontal cortex and medial temporal lobe of the brain (Jagust, 2018). Neurofibrillary tangles composed of the microtubule-

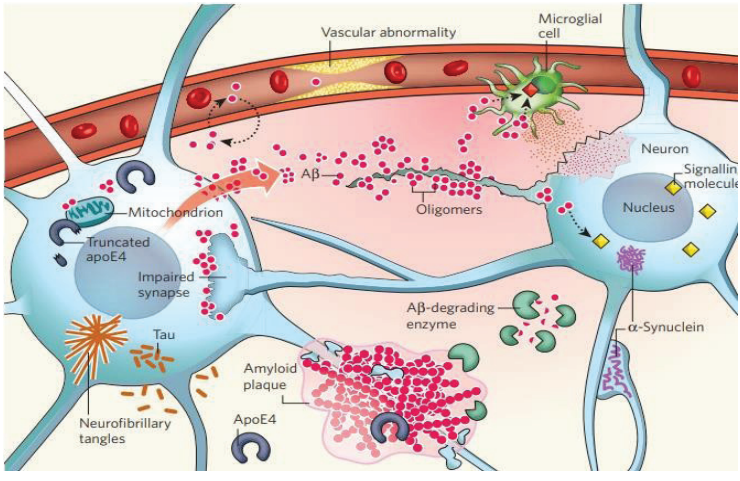
associated protein tau inside neurons correlate highly with the development of cognitive problems and are found in about %80 of individuals who are over 70 years old (Jagust, 2018). They generate Aβ peptides, especially in the brain and other organs, released from the APP after splitting by β-secretase and γ-secretase enzymes. Aβ self-aggregates into oligomers to plaques over the years and tau proteins self-aggregates into oligomers to tangles. So, this means all Alzheimer's patients have plaques and tangles in their brains. The most important neurochemical disturbance in AD is a deficiency of a specific neurotransmitter system called acetylcholine (ACh). The anatomical basis of the cholinergic deficit is atrophy and degeneration of subcortical cholinergic neurons.

FIGURE 2.5.1 / The brain atrophy of two people who lived to be the same age, same-sex, and the same size (Dementia Care Central, n.d)



Alzheimer's patients have considerably smaller brain sizes, the folds between AD patients are narrow and compact, and the gaps between the folds are enormously wider. This is called cortical atrophy.

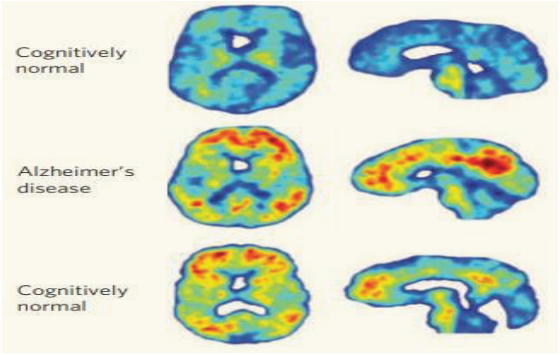
FIGURE 2.5.2 / Some key players in the pathogenesis of AD (Lots of People Are Forgetful. Are There Any Particular Warning Signs of Alzheimer's Disease?, 2009)



Aggregation of amyloid plaques in the brain may result from raised production of the protein beta-amyloid, and reduced activity of beta-amyloid degrading enzymes. Deposition of Aβ impairs the synapses between neurons and distorts neuronal functions in assisting

memory and cognitive functions (Roberson & Mucke, 2006). Because they interact with the cell-surface membranes and receptors by triggering the release of neurotoxic mediators by microglia plus inflammation, reduction of energy, and the collapse of calcium homeostasis (Roberson & Mucke, 2006) are the causes of the impairment in cognitive functions and neuronal losses. APOE4 elevates abnormal Aβ formation and reduces the Aβ clearance and causes the impairment of mitochondrial functions within neurons. Tau proteins form pathogenic oligomers by aggregating intra-neuronal and displacing vital organelles in the cell such as mitochondria.

FIGURE 2.5.3 / *The challenge of finding AD biomarkers*



(Lots of People Are Forgetful. Are There Any Particular Warning Signs of Alzheimer's Disease?, 2009). A study made by Gil Rabinovici at California University used two normal controls (top and bottom) and same-aged AD patients (middle row) who were given an IV injection of the radioligand PIB, which binds to fibrillar Aβ deposits (*Lots of People Are Forgetful. Are There Any Particular Warning Signs of Alzheimer's Disease?*, 2009) detected by PET. The cooler colors show low levels of PIB binding and most cognitively normal people showed cooler colors. AD patients show warm colors which means high levels of PIB binding. But some cognitively normal people showed an elevated level of PIB binding (bottom). So, this means amyloid plaques are not enough to impair cognitive functions. And cognitively normal people with a high level of PIB binding will develop AD in the future is unknown.

2.5.1. Amyloid Cascade Hypothesis

In the early 90s, the amyloid hypothesis was suggested to better understand the main hallmark of AD pathogenesis. This hypothesis proposed that Aβ accumulation is not the effect of AD, it is the cause of AD (Finder, 2010). Their accumulation in the brain forms soluble oligomers and amyloid plaques and causes neurotoxicity in the brain. Major evidence of this hypothesis comes from the analysis of Down syndrome patients. In 1984, Aβ was identified as the main reason of senile plaques in AD and Down syndrome patients develop senile plaques usually at the age of 15 or 16 in their life. In the early 90s, analysis of Down syndrome patient's meningeal blood vessels who have an additional AβPP allele and develop early-onset familial AD (FAD) presumably because of a dose-dependent effect (Finder, 2010) recognized as the main component of senile plaques of AD patient brain tissue (Hardy & Selkoe, n.d.). This makes it helpful to identify and compare the chromosomal abnormalities of patients with Down syndrome who develop AD with patients who don't develop AD. The susceptible gene of AD is chromosome 21 (Khachaturian, 1985) so, the studies showed that the cloning of the gene encoding the Aβ precursor protein (APP) in the chromosome 21, coupled with the earlier identification of trisomy 21 (Down syndrome) (Hardy & Selkoe, n.d.). The pathological and chemical changes seen in the AD and Down syndrome patients are virtually like each other (Khachaturian, 1985). This sets the proposal that Aβ aggregation is the main hallmark of AD pathogenesis. In addition, the mutation in APP could cause Aβ deposition outside of the parenchyma in the brain. Mutations in APP are cleaved by proteases called β-, γ- secretases to form Aβ, an aggregation that accumulates in the brain in AD (Hardy & Selkoe, n.d.). And the cloning of presenilin proteins (PS) and AD-causing mutations in PS-1 and PS-2 increase the processing of APP to form amyloid. Cloning of PS1 and PS2 and the evidence that they change the APP metabolism through a direct effect by proteases called γ- secretases. There have been made four important observations that support this hypothesis. First, the gene encoding tau protein undergoes mutations that cause frontotemporal dementia with parkinsonism. This neurodegenerative disorder includes the severe aggregation of tau proteins in NFTs in the brain, but no aggregation of amyloids. This finding suggests that even severe effects of tau alteration are not enough to induce Aβ plaques of AD. Thus, NFTs in the brain are presumed to have been deposited after changes in initial Aβ plaque formation, rather than before (Hardy & Selkoe, n.d.). Second, a study about transgenic mice overexpressing mutant human APP and mutant human tau protein showed the increased formation of tangles made by tau while the number of their Aβ plaques is unchanged. This means that APP processing occurs earlier before changes in tau. Aβ toxicity depends on tau proteins. Third, APP transgenic mice crossing with

apoE–deficient mice showed a markedly decreased amount of amyloid deposition in the offspring in the brain. This provides strong evidence of human apoE is involved in amyloid metabolism. Fourth, genetic variability in Aβ catabolism and clearance likely contribute to the risk of LOAD. In conclusion, Aβ accumulation in the brain is the main action in AD, and the rest of the actions resulting from the unbalance between Aβ formation, deposition, and clearance (Hardy & Selkoe, n.d.).

The concern about this hypothesis is the lack of details. For example, as it was shown in figure 2, the number of cerebral amyloid deposition does not correlate well with the cognitive impairment and cognitively normal people may have cerebral amyloids. Only the degree of dementia in AD correlates well with cerebral amyloids. Another concern is that AD-causing mutations in APP, PS1, PS2 increase amyloid depositions but the degree of the mutations affecting amyloid productions show no correlation with aging. Since there are no in vivo studies, it remains a controversial hypothesis. However, cerebral Aβ accumulation in the form of soluble oligomers (not fibrils or monomers) showed neurotoxicity and these soluble oligomers injected into living rats with human Aβ revealed oligomers inhibit long-term potentiation in the hippocampus, which is required for the memory and learning functions. Another concern is that transgenic mice don't show clear neuronal loss (Hardy & Selkoe, n.d.).

To summarize this hypothesis, missense mutations in APP, PS1, and PS2 genes on chromosome 21 increased amyloid production and accumulation. Amyloid oligomerization and deposition form plaques. Amyloid oligomers on synapses cause progressive and irreversible synaptic and neurotic injury while they also persistently activate microglial cells and cause neuroinflammation and neuronal loss. Changes in neuronal ionic homeostasis caused by oxidative stress change kinase/phosphatase activity and this change is the cause of tangles then the widespread neuronal dysfunction and cell death with neurotransmitter deficits such as ACh cause dementia (Hardy & Selkoe, n.d.).

2.5.2. Tau Hypothesis

Tau is a protein that maintains the structure and functions of the neurons. They are highly abundant in the axons of neurons. The tau gene is localized in chromosome 17 in humans (Kametani & Hasegawa, 2018). There are six isoforms of tau expressed in the human brain as the conclusion of mRNA splicing with or without exons 2, 3, and 10 (Kametani & Hasegawa, 2018). Their primary function is to stabilize the microtubules (MT) and is therefore called a microtubule-associated protein (MAP). Microtubules consist of highly conserved tubulin motifs which contain the carboxy-terminal (C-terminal) half of the protein, followed by a basic proline-rich region and an acidic amino-terminal (N-terminal)

region (Ballatore et al., 2007). The longest isoform in the brain has four repeats (R1, R2, R3, R4) but 3R and 4R are the core of protein tau fibrils. Exon 10 has a microtubule-binding region. The addition of exon 10 affords 4-repeat (4R) tau isoforms whereas, 3-repeat (3R) tau isoforms are made without exon 10 (Kametani & Hasegawa, 2018). This hypothesis suggests the main causative factor of AD is tau protein. Hyperphosphorylation of 3R and 4R tau causes an abnormal elevation in the levels of the unbound tau fraction (Ballatore et al., 2007). In the hyperphosphorylation state, twisted strands of tau are observed, and these pathological inclusions are called neurofibrillary tangles (NFT) formed in dendrites and axons. In 1991, tau pathology was staged by Braak and Braak. They show up in the transentorhinal region (stages I and II), then extend to the limbic region (stages III and IV) and neocortical areas (stages V and IV) (Kametani & Hasegawa, 2018). An increase in stage number directly correlates with the impairment in cognitive progress. It has been indicated that subjective cognitive decline is correlated with early pathogenesis of tau in the medial temporal lobe, especially in the entorhinal cortex. In addition to this, tauopathy occurs earlier than amyloid accumulation. That's why this hypothesis supports AD is mainly associated with tau pathology. They can be seen in other neurodegenerative dementing disorders such as frontotemporal dementia and parkinsonism. This evidence suggests that tau abnormalities cause tau accumulations, although the initial trigger is still unclear, a huge amount of tau is accumulated. Abnormal interaction of stabilized tau with filamentous actin causes synaptic impairment and defects in mitochondrial integrity.

Therefore, when they undergo abnormal phosphorylation, they can't bind to microtubules efficiently and this causes the collapse of microtubules. Microtubules can't support transportation and skeletal support system in the cells. A study with cultured mice showed abnormal tau conversion into normal tau in an abnormal way. So, this suggests tau aggregation starts in a small number of brain cells and then they spread to the other regions (Kametani & Hasegawa, 2018). But the transmission mechanism remains unclear. Recently found out that APP mutations accelerate tau accumulation and propagation rather than amyloids (Kametani & Hasegawa, 2018). A few years ago, tau and amyloid levels in an Alzheimer's patient could only be measured after the patient had died. But now living patients' tau and amyloid levels can be measured by analyzing samples of CSF and PET scans may show the possible interaction between tau and amyloid.

2.5.3 The Choline Hypothesis

Alzheimer's Disease's main pathologies are the accumulation of senile plaques, neurofibrillary tangles, and loss of neurons. It is accompanied by a loss of cholinergic activity and acetylcholine (Ach) level in the brain,

which are responsible for the cognitive functions in the brain (Lombardo & Maskos, 2015). Different studies showed that basal forebrain and rostral forebrain cholinergic pathways to the thalamus serve important functional roles such as memory, attention, and conscious awareness (Francis et al., 1999). The studies in AD patients showed an abnormality in these pathways and the level of cognitive decline directly correlates with these abnormalities. Since choline is needed to produce acetylcholine (ACh), a neurotransmitter that binds to two receptors, nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs) and regulates the cognitive process in the brain (Lombardo & Maskos, 2015). The studies used [3H]-nicotine and [3H]-Ach showed a reduction in nicotine and ACh binding sites in the cerebral cortex of the AD patients. In addition to this, an enzyme called choline acetyltransferase (ChAT), which plays important role in the production of ACh, decreased in AD patients (Lombardo & Maskos, 2015). So, it is possible for them to cause toxic effects. As a result, the cholinergic hypothesis was made. It states that loss of cholinergic activity is associated with the degree of AD (Cummings & Back, 1998). the number of acetylcholine is decreased, and there is a loss of nicotinic receptors in the medial temporal lobe of the brain. Presynaptic nicotinic receptors control the release of ACh, as well as other neurotransmitters important for memory such as glutamate. Besides, some studies support the existence of the direct effect of amyloid plaques acting as agonists or antagonists on nAChRs. So, it is possible for them to cause toxic effects. But this proposal needs to be detailed.

2.5.4 The Inflammatory Mediators

The cerebral amyloid accumulation is often correlated with local inflammation and immunologic changes. It is believed that Aβ may have direct neurotoxicity and indirect result of an Aβ protofibril-induced microglia (immune of the brain) activation and astrocyte recruitment. This hypothesis focused on the major role of inflammatory mediators in the immunopathogenesis of AD. Adaptive immune system cells can pass through the BBB, which is structurally different in AD patients compared to healthy individuals. In electron-microscope observations of AD patients showed that Aβ plaques are correlated with activated microglial cells. Their permanent activation is responsible for the ongoing neuroinflammatory process in the brain through the release of cytokines, chemokines, and neurotoxins (Azizi et al., 2015). Furthermore, they produce anti-inflammatory cytokines via direct interaction with T-lymphocytes. Inflammation starts when the innate immune system detects damaged tissue on the surface of cells.

Inflammatory mediators especially chemokines are responsible for the recruitment of immune cells to the damaged area. Degenerated cells

and accumulation of abnormal insoluble materials such as Aβ peptide 23 deposits and NFTs are the responsible for most common stimuli for the inflammation (Azizi et al., 2015). Inflammation of AD happens in two pathological pathways. First, early immune response to the changes in the brain of AD patient; inflammations occur when recruitment of the immune cells to the damaged cells or tissues because of the activation of microglial cells and the release of cytokines, chemokines, and other signaling inflammatory mediators. Second, a minor amount of permanent inflammation in the brain. This inflammation is considered the impaired adaptive immune response and leads to chronic inflammation.

2.6. Treatment of Alzheimer's Disease

There are two treatment strategies for AD. First, symptomatically treatment of cognitive difficulties and protect the patient's function as long as possible. Second, managing psychiatric and behavioral sequelae.

2.6.1. Non-pharmacological Treatment

A wide series of non-pharmacological interventions are made for the treatment of the symptoms of Alzheimer's disease. These are mostly for behavioral and psychiatric symptoms like sleep disturbance, urınary incontinence, provocation, wandering, and offensiveness. The key factor of non-pharmacological treatment is creating a warm and calm environment while removing stressors and triggers (*Overshott2004*, n.d.). Physical exercise, aromatherapy, phytotherapy, music therapy, massage, relaxation techniques, memory training, mental and social stimulation are examples of non-pharmacological treatment. In addition, personal discomfort may cause behavioral changes, so it is necessary to monitor for pain, constipation, skin irritation, fears, hunger, and frustrations. Education of the patient and caregiver is an important part of non-pharmacological interventions (Overshott2004, n.d.). The fundamental principles of care for AD are considering vision, hearing, or other sensory problems, being supportive and calm, decreasing choices, keeping requests, and demands of the simple things from the patient, and avoiding hard tasks that lead to disappointment. The responsibilities of caregivers are assisting patients with daily activities including dressing, and toileting. Serves meals and medications. Encourage patients for proper eating. Remove stress-triggering situations/ places. Improve patient's life quality.

2.6.2 Pharmacological Treatment

AD treatments have not been shown to cure AD or reverse the pathological process. So, pharmacological treatment is aimed at relieving symptoms. Increasement in the cholinergic transmission in CNS is currently the mainstay of AD treatment. The other pharmacological

treatment is preventing excitotoxicity produced by the overstimulation of N-methyl-D- aspartic acid (NMDA)-glutamate receptors in selected brain areas.

2.6.2.1 Cholinesterase Inhibitors

Cholinesterase inhibitors are the first-line therapy for the symptomatic treatment of the impairments of the cognitive functions in mild or moderate AD. There are three cholinesterase inhibitors used in AD treatment. These are donepezil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl). Use of them based on people with AD who have ACh production deficit leading to cortical cholinergic dysfunction (Massoud et al., 2011). Cholinergic neurotransmission happens when ACh is released from presynaptic neurons to bind nicotinic or muscarinic postsynaptic ACh receptors (Grossberg, 2003).

Cholinesterase occurs in two forms butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE). BuChE and AChE degrade ACh in the synaptic cleft. Cholinesterase inhibitors inhibit this action and maximize the levels of ACh available for postsynaptic stimulation (Massoud et al., 2011).

They improve the cognitive function of people with AD. Choice of cholinesterase inhibitors is according to the ease of the patient's choice, expense, and safety problems such as possible drug interactions with other drugs, and foods. They have similar adverse effects, and they are usually well-tolerated. The most common side effects are GI distress such as nausea. Other side effects are muscle cramping, fatigue, anorexia, insomnia, and abnormal dreams. They are contraindicated for people with bradycardia and incomplete heart block because they enhance vagal tone. Besides, women who are pregnant should not use this drug.

Donepezil (Aricept) specifically and reversibly inhibits AChE. It doesn't have any effect on BuChE. AChE catalyzes the hydrolysis of ACh to produce choline and acetate ions (Sharma, 2019) The concentration of ACh rises in brain synapses. It comes as a tablet or an orally disintegrating tablet. There are few studies about the pharmacokinetics of donepezil. It reached peak plasma concentration in 3-5 hours. Donepezil is well absorbed and donepezil has %100 bioavailability. This means it is not affected by the presence of food or administration time Since its elimination half-life is 70h, once daily is enough. Plus, no need for a dose-modification with donepezil in elderly patients or in the case of renal and hepatic impairments (Grutzendler & Morris, n.d.). It is a high protein-bound (%96). It's metabolized by the hepatic isoenzymes CYP2D6 and CYP3A4. But the outcomes related to the use of donepezil metabolization with these hepatic isoenzyme deficiencies are unknown. Starting dose should be the lowest

dose (5mg). And the maintenance dose, after 4-6 weeks, should be 10mg daily (Hafez, 2021). Dose-dependent side effects occur more with the use of donepezil. The most common are GI complications. That gradual dose titration over several weeks can increase tolerability.

Rivastigmine (Exelon) is a pseudo-irreversible inhibitor of AChE and BuChE (Js & Evans, 2015). BuChE only represents the %10 of AChE activity in the human brain but some studies showed this enzyme is capable of the hydrolysis of ACh and plays important role in cholinergic transmission than previously thought (Grossberg, 2003). They are cleaved by the enzyme and come in a covalent modification of the enzyme. It comes as a capsule or a transdermal patch. The transdermal patch form allows the titration to the highest and the most effective doses of the medication while minimizing the possible cholinergic side effects (Massoud et al., 2011). A Cochrane review showed a positive effect on cognitive functions. But it does not have any effect on behavioral functions (Birks, 2006). The outcomes with rivastigmine showed that improvement in cognitive function and is independent of the inhibition of AChE and BuChE (Grossberg, 2003). The positive effects were observed mostly in high doses (6 to 12mg daily). The starting dose for the oral dosage form is 1.5 mg twice a day and the maintenance dose is 6 mg twice a day (increased in 2- to 4- week intervals) and should be taken with food to prevent GI complications. The starting dose for transdermal patch form is 4.6 mg daily and the maintenance dose is 9.5 to 13.3 mg a day (increased in monthly intervals). Transdermal patch form can cause a rash. To prevent this rotating site of the patch is necessary. The elimination half-life is 1.5h and they are metabolized by their target enzymes with minimal effect of hepatic CYP450 enzyme. So, they are likely used with the drugs metabolized by CYP450 enzymes (Grossberg, 2003). The protein bounding is %40. If the treatment is interrupted for several days, the patient should be restarted at the lowest dose and titrated to the current dose. There is no proof of a difference between donepezil and rivastigmine on cognitive functions. In a Cochrane review fewer patients suffer adverse effects on donepezil than on rivastigmine (Birks, 2006). Compared to donepezil it has less tolerability and causes muscle cramps or weakness. But rivastigmine shows a particularly favorable side during the treatment of AD.

Transdermal patch application sites are the back, abdomen, upper arm, and legs. Rotation is important to avoid side effects.

Galantamine is a selective, reversible, and only competitive inhibitor of AChE. Also allosterically binds nicotinic ACh receptors (nAChR) and increases the action of acetylcholine on nicotinic receptors. It has two formulations; an immediate-release tablet or solution and an extended-release capsule. The starting dose for tablet form is 4 mg twice daily and the maintenance dose is 12 mg (increased in monthly intervals). The

starting dose for capsule form is 8 mg once daily and the maintenance dose is 24 mg once daily (increased in monthly intervals). The cognitive benefit of galantamine is above 16 mg daily (Joe & Ringman, 2019). The protein binding is low (%18) and metabolized via hepatic CYP2D6 and CYP450 enzymes so caution is necessary with the concomitant use of other drugs metabolized by CYP450. The elimination half-life is 7h and it has a low protein binding drug (%18). Their long-term administration rises AChE activity, which may exacerbate AD progression (Grossberg, 2003). If treatment with galantamine is interrupted for several days, the patient should be restarted at the lowest dose and titrated to the current dose.

These 3 cholinesterase inhibitors are approved by FDA to use in the treatment of AD. But there are other cholinesterase inhibitors used but removed in the 2000s because of the serious side effects they caused. Tacrine is one of them, it caused hepatotoxicity. Metrifonate is another example, it caused behavioral changes such as agitation and hallucinations.

2.6.2.2. Memantine

Memantine (Ebixa) is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA)- type glutamate receptor. Glutamate is the key excitatory neurotransmitter in the brain. Glutamate and its receptor are important in the learning and memory process, but overstimulation of glutamate may result in neuronal injury and causes excitotoxicity. Excitotoxicity leads to neuronal calcium overload and results in neurodegeneration (Reisberg et al., 2003). Memantine protects neurons from neurodegeneration and acts as a neuroprotective from glutamate-induced excitotoxicity. It's used as an alternative to cholinesterase inhibitors in AD or as an adjunct. They are used in the treatment of moderate to severe AD. It has a modest benefit on cognitive functions for early initiation (Joe & Ringman, 2019). It occurs in two forms immediate- and extended-release formulations. It is usually well-tolerated. But the dose-limiting side effects may be mild headache, dizziness, or confusion (Massoud et al., 2011). The starting dose for the tablet or oral solution form is 5 mg once a day and the maintenance dose is 10 mg (increased in weekly intervals). The starting dose for the extended-release capsule form is 7 mg daily and the maintenance dose is up to 28 mg daily (increased in at least one-week intervals). Dosing of 5 mg twice a day for tablet or oral solution form and 14 mg in a day for the extended-release capsule is recommended in patients with intense impairment of kidney/renal (creatinine clearance of 5-29 mL/min) (Hafez, 2021). The amyloid hypothesis is the most accepted hypothesis of the cognitive impairment in AD. And there are several potential roles for the NMDA receptor in Aβ-related mechanisms such as NMDA receptor may be the receptor for Aβ or indirectly interacts with the molecules that bind Aβ, or NMDA receptors may be necessary for the actions of Aβ on synaptic transmission

and plasticity (Malinow, 2012).

Studies found out that Aβ-induced hyperactivation was associated positively with neuronal activity and Two-photon glutamate imaging showed soluble dimers of Aβ could inhibit the re-uptake of glutamate at the synapses and potentiate glutamatergic transmission and mimicked the effects of Aβ on neuronal activity (Wood, 2019). This provides evidence that the effects of Aβ were being mediated by glutamatergic synaptic excitation (Wood, 2019).

Memantine may be used in combination with a cholinesterase inhibitor in patients with advanced disease. Usually combined with donepezil (Namzaric). A Cochrane review showed that their combination showed a benefit on an MMSE score, more effective than cholinesterase therapy alone, and well-tolerated (Standridge, 2004). Dosage reduction is only necessary in the case of severe renal impairment.

2.6.2.3. Antipsychotic medications

In Alzheimer patient's (non-cognitive) behavioral and psychiatric (BPSD) changes occur such as depression, hallucination, physical and verbal aggression, agitation, and wandering. The first-line management is non-pharmacological such as education of caregivers, improving environmental qualities, being supportive, and exercise. Second-line management in the case of BPSD is psychotic medications. Selective serotonin reuptake inhibitors (SSRI) such as citalopram are used and compared to tricyclic antidepressants (TCAs) they are more preferred because of the fewer side effects (Fillit et al., 2006). And in case of psychosis and agitation in AD, atypical antipsychotics such as risperidone are used but monitoring is very important (Sepehry et al., 2012). Anticonvulsants such as phenobarbital are used in the treatment of dementia with agitation. But it is important to take psychiatric medications without anticholinergic effects because they may worsen the cognitive function and interfere with cholinesterase inhibitor treatment. Starting from the possible lowest dose is necessary and slowly titrate the dose with monitoring while minimizing the duration of treatment.

2.6.2.4. Aducanumab

In 2021, a human monoclonal antibody that selectively binds Aβ fibrils or soluble oligomers called aducanumab is approved by FDA for treating AD (Schneider, 2020). It's the first drug for the underlying pathogenesis of AD rather than the symptoms (*More Than Normal Aging: Understanding Mild Cognitive Impairment*, n.d.-b) The mechanism of action is clearing amyloid plaques. A study with 165 patients with prodromal or MCI due to AD received a monthly dose of IV 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg of aducanumab and showed a reduction in amyloid plaque numbers in

a dose-dependent and time-dependent manner. After one year, half of the patients who received 10 mg/kg of aducanumab had no positive cerebral amyloid PET scans. Cognitive results on MMSE provide a decrease in cerebral amyloid confers a clinical benefit (Sevigny et al., 2016). This is not a cure and cannot use in every Alzheimer's patient, only can use in early AD for now. Half of these patients discontinue the treatment due to serious side effects at higher doses was occurred and it's called amyloid-related imaging abnormalities (ARIA), which can be an indicator of swelling of the brain (Schneider, 2020). So, close monitoring to prevent ARIA is highly necessary. But still, there is no data about safety and effectiveness. Since aducanumab is delivered by intravenous infusion, infusion nurses are the key factor in the dementia workforce (More Than Normal Aging: Understanding Mild Cognitive Impairment, n.d.-b).

2.7 Future Approaches

Since the identification of Alzheimer's disease, the focus was amyloid plaques but currently, scientists are looking for new treatments beyond amyloid plaques. Many scientists don't believe amyloid plaque clearance is enough for the treatment of AD, especially after the controversial approval of Aduhelm (aducanumab) for the treatment of AD by the FDA in 2021. In the future, the focus won't be on amyloid plaques. Currently and in the future, scientists will explore more into immune cells of the brain (glial cells), brain waves, and toxic tau proteins tangles.

Tau protein accumulation occurs inside neurons and causes microtubule dysfunction. Tau protein accumulation is directly associated with cognitive complaints. Besides, tau tangles show up in the entorhinal cortex of the brain which is the part of the brain that involves memory function and then systematically moves to other parts of the brain. Scientists aim is to produce medications that remove tau tangles. Until now, the only problem is making these medications cross BBB. So, scientists at Denali makes a study that successfully works in a model system using living human brain. They used iron to cross BBB via transferrin protein and reached transferrin receptors to let iron to reach brain tissue. Then, they designed a transport vehicle to carry different medications across BBB by interacting with transferrin receptors. So, with that system, they want to deliver a human monoclonal antibody to remove tau tangles.

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Scientists at MIT have been studying gamma waves which are electrical pulses in the brain important for learning and memory. They found out that people with AD have weaker gamma waves compared to normal same-age and same-sex people. Then they studied mice. They exposed mice to sounds and lights that causes to increase in the power of gamma waves. The result showed benefits. And after treatment, mice's brains were removed from amyloid and tau proteins, and the function of the immune cells of the brain improve. After the successful treatment in mice, they studied this on 15 people with mild Alzheimer's disease. They used portable devices that produce lights and sounds at a frequency of 40 Hz. AD patients used this device for one hour a day every day for three months. The results showed none of the patients had cortical atrophy. So, in the future, scientists hope to do with a large population of AD patients.

Glial cells are the first defense mechanism against toxic substances including tau and amyloid accumulation in the brain cells. They are the immune system of the brain. So, according to this, AD patients have weak immune cells in the brain and the improvement of immune cells will prevent AD. Scientists have been working on ways to immune boost.

3. CONCLUSION

Alzheimer's Disease is a neurodegenerative, progressive, irreversible disease seen in elderly people, where the acetylcholine injury in the hippocampus and entorhinal cortex of the brain causes memory and cognitive problems. It's characterized by the accumulation of proteins such as amyloid (Aβ) and MAP-tau in CSF. Cholinesterase inhibitors are used to increase acetylcholine in the brain to improve cognitive function. NMDAR antagonist protects neurons from the excitotoxicity of glutamate. Aducanumab clears amyloid plaques. These drugs don't reverse Alzheimer's. These drugs help to relieve the symptoms and help to improve the patient's life quality. Aging is the main risk factor for AD, but AD is not linked with normal aging. So, in the future, AD will become a more serious issue. The number of deaths caused by AD will increase. Age will continue to be the greatest risk factor. But with the development of new treatment strategies, I believe the quality of life of an AD patient will improve more in the future.

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CHAPTER 17

SUCCESSFUL AGING AND HAPPINESS

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The Turkish Language Association (2022) defines 'age' as the indicator of time starting from the emergence of an event or phenomenon and its measurement in years.

Aging continues from the intrauterine period until death, is not a static process, and varies from individual to individual. The aging process is dependent on several factors. *Chronological* aging measures a person's calendar age (Kılavuz, Savaş, and Akçiçek, 2019). *Biological* aging covers all anatomical and physiological developments, starting with the formation of a zygote. *Pathological* aging is associated with diseases. *Psychological* aging emphasizes the changes in one's cognitive abilities in older ages. *Social* aging is related to a person's changing roles in society. Finally, *economic* aging describes the changes in one's economic life due to changes in income level (Aslan and Hocaoğlu, 2017).

It is incorrect to evaluate aging only by the appearance of the symptoms of aging, but it is necessary to evaluate aging as a holistic process since it covers all periods throughout one's life.

Successful aging is related to avoiding diseases, high physical and mental functionality, having an active role in life, absence of diseases, psychological well-being, life satisfaction, financial security, and having a positive view of life (Hazer and Özsungur, 2017).

Successful aging refers to the rejection of the onset of advanced age, replacing the lost relationships and roles in middle-age with a new standard of living that can provide life satisfaction and well-being. (Tufan and Durak, 2017).

The Scope and Significance of Successful Aging

In Turkey, health problems of the elderly cover several issues, including inability to be an active, participatory, and productive, along with chronic diseases which accompany old age and a lack of care providers (Akkuş, 2019). Social and governmental support is important to cope with such problems, whereby competent people play a significant role in informing and guiding the elderly about successful aging.

Considering the issue from the perspective of health status, there is a limited number of studies conducted in Turkey on successful aging. There are only a few studies that evaluate people's perception of successful aging. Therefore, current studies on this issue are indicative (Vural, Özen, and Yazıcı, 2018).

One study examined how the elderly with chronic diseases evaluate their free time and found that they tended to act passively due to their illness. The study concluded that the elderly with chronic diseases did not

know how to spend their free time (Ilyas and Lapa, 2022).

In the transition to advanced adulthood, there is a significant gender-related difference in personal characteristics regarding the successful aging levels of individuals — females have higher successful aging scores than males (Kars, Fertelli and Deliktaş, 2019).

Kars, Fertelli and Dilektaş (2019) have reported that not only females, but also practicing Muslims have high rates of successful aging as they are patient in times of distress and have a positive approach to diseases as they believe the disease and difficult times are given by God. They have concluded that religious beliefs are an effective way of coping with difficulties.

In sum, perceived successful aging varies due to several factors such as culture, age, gender, education, income, occupation, and health status.

Factors Affected by Successful Aging

Successful aging affects social, mental, and physiological factors, health, and physical activity. Therefore, determining the effects of these factors on aging has led to the emergence of differing views on successful aging. Successful aging is associated with one's ability to reach their goals and objectives, be socially and physically active, and have an idea about their life.

Successful aging is also related to several factors throughout the more advanced aging process, such as coping with financial problems, having social opportunities, the ability to overcome health problems, making efforts to take necessary precautions for possible health problems, and creativity. Successful aging is interconnected to one's access to informational resources, ability to read and understand information, and their capacities to protect and improve health (Hazer and Ateşoğlu, 2019).

Bosnes et al. examined the lifestyle determinants of successful aging, which are considered the middle-age determinants such as smoking, obesity, physical activity, alcohol use, and social support, and found that non-smoking and being physically active were associated with successful aging but there was no relationship between successful aging and alcohol use. The authors concluded that having more than one positive lifestyle determinant positively affected successful aging (Bosnes et al., 2019).

In their study on healthy aging, Atallah et al. have emphasized that individuals should acquire healthy lifestyle habits during middle-age for healthy aging by demonstrating the individual contribution of each healthy lifestyle factor to healthy aging. However, further interventional studies are needed to confirm these results (Atallah et al., 2018).

Prevention of substance use is an issue that needs attention in all age groups for a successful aging process. To ensure active and successful aging, trainings on non-smoking can be expanded in childhood and adolescence. In older age groups, prevention or quitting of substance addiction is important. Social spaces can be created for all age groups where they can gain skills to prevent substance use or can be directed to sports and art activities.

Smoking is considered a risk factor in healthy aging as studies have shown that smoking accelerates aging and affects several body systems, including cardiovascular and respiratory systems. Smoking poses a risk to successful aging by jeopardizing not only life expectancy but quality of life. There is not a significant difference between smoking and being exposed to smoke, so the first way to avoid this habit is to keep away from smokey environments. Today, several methods have been developed to quit smoking, including pharmacological or non-pharmacological interventions. Therefore, it is possible to increase the rate of successful aging by choosing one of these methods appropriate for each smoker (Mauro, Malta, Lasco and Basil, 2010).

Regardless of one's age, physical activity is necessary to protect their health. Physical activity is a cost-effective method that can be used by any age group with ailments for relaxation purposes (Sarman, 2018). Successful aging is an active process; therefore, being physically active is as important as not using harmful substances.

The variety of exercises suitable for different ages and health conditions depends on the social environment together with a balanced diet. While exercising, it should be considered that if the individual has not frequently exercised before, they should start with light exercise movements and gradually increase, continuing without tiring the body. Walking is the most suitable exercise, but it is important to walk on tracks and soft soil surfaces. Type and frequency of exercises should be taught by physiotherapists or sport specialists. It is recommended to exercise in a clean, calm, open, and green space, which helps both body coordination and psychological relaxation (Akın, 2017).

To maintain muscle mass and strength, it is necessary to have adequate intake of protein and energy. Studies show that elevated levels of dietary protein are required to prevent age-related changes in protein metabolism. For elderly individuals, it is recommended to get a daily protein intake of at least 1.0-1.2 g/kg/day. Higher protein intake is associated with an increase in muscle strength and mass. The recommended intake of protein can be increased especially for those with acute or chronic diseases. An equal distribution of protein at breakfast, lunch, and dinner is beneficial

for protein synthesis.

Successful aging is associated with adequate and balanced nutrition. A study conducted by Yazıcı to determine the views of different age groups on successful aging reported that 91.4% of the participants completely agreed with the item of "a healthy and balanced diet is necessary for successful aging" (Yazıcı, 2018).

Like, adequate and balanced nutrition, fluid intake is necessary for successful aging. Adequate fluid intake is associated with cellular activities, prevention of infections, and the removal of waste from the body. A sufficient amount of fluid should be consumed by calculating the amount of liquid consumption according to individual body needs.

In their study on the consumption of antioxidant-rich foods and healthy aging, Assmann et al. suggest that antioxidant-rich food sources, which should be included in the daily diet in sufficient quantities, may have a beneficial role in healthy aging (Assmann et al., 2015).

Social, physical, and mental activities, along with regular contact with social circles, continuity of daily life, and having a hobby, are associated with successful aging for all age groups (Tereci et al., 2016).

To maintain healthy and successful aging, all age groups should have equal opportunities for health and care services. In their study on successful aging, Yazıcı found that 93.4% of the participants completely agreed on the importance of accessibility to health and care services (Yazıcı, 2018).

An ability to manage stress factors is recommended for a cognitively healthy life. If stress is mild and controlled, it appears to have a positive effect on learning and adapting. Finding a way to manage stress is crucial for reducing stress levels and improving sleep quality and mood. Meditation and mindfulness practices reduce the release of cortisol at a high rate and increase the cerebral blood flow to the frontal lobe, which is particularly important for cognitive functions. In addition, yoga improves stress management, daily focus, and memory management. Adequate sleep for lifelong brain health is considered to be 7-9 hours for adults under the age of 65 years and 7-8 hours for those above the age of 65 years. In sum, adequate sleep is considered essential for healthy cognitive aging (Krivanek, Gale, McFeeley, Nicastri and Daffner, 2021).

Successful aging is a continuous process that is facilitated by physical activity, healthy and balanced nutrition, adequate fluid intake, participation in daily activities, participation in artistic and cultural activities, and hobby acquisition at all life stages.

What is Happiness?

Bentham defines happiness as the combination of joy, pleasure, and pain one gets from life (Veenhoven and Dumludag, 2015). Socrates argues that people can reach happiness by living virtuously throughout their lives (Keser, 2018).

The concept of happiness has been essential throughout history. In modern times, happiness is an important research topic throughout various disciplines such as philosophy, psychology, economics, and sociology. The link between happiness and health allows one to have energy, be healthy, and have the opportunity for a satisfying life — being healthy positively affects well-being (Ibrahim and Ekşi, 2018).

Happiness is a key component of having a healthy life. One study on the factors related to happiness was conducted in Malaysia and included a total of 1,204 participants 60 years of age. In the study, 79.2% of the participants considered themselves happy. The authors argued that sociodemographic characteristics such as being male aged between 60-74 years and living in urban areas were significantly associated with happiness and that emotional support and active social interaction had a positive effect on the levels of happiness in the elderly (Shah et al., 2021).

The Scope and Significance of Happiness

Efforts to define and give a meaning to happiness date back to ancient times. According to Aristotle, happiness is the feeling one gets when they are motivated or able to meet their needs in necessary matters. The Turkish Language Association defines happiness as "the state of being proud of reaching all aspirations completely and continuously" (Çelebi, 2020).

Studies on happiness have revealed that individuals are affected by their personal issues, and that environmental factors are determinant in happiness (Çelebi, 2020).

In their study on the relationship between loneliness, perceived social support, and happiness in the elderly living in nursing homes vs. their private homes, Akyıl et al. have revealed that the place of residence does not affect the level of loneliness in the elderly, but those with good physical and psychological feelings are subjectively happier. Likewise, social support positively affects the level of happiness in the elderly (Akyıl et al., 2018).

Technology provides convenience in daily life, and its use is constantly becoming more widespread. One study examined the level of happiness in middle-aged and elderly individuals regarding their opinions on the internet and social media and found that the elderly used the internet

and social media platforms for several activities such as entertainment, obtaining information, recreation, socialization, and communication with their loved ones, which they considered a source of happiness. Therefore, technology and social platforms have contributed positively to the happiness of the elderly, allowing them to have a happy life with their loved ones (Tekkedere and Arpacı, 2016).

Considering the relationship between television and elderly individuals, their duration of watching television is quite high compared to other age groups. One study revealed that increased life satisfaction increases happiness, which is compatible with the literature. The study suggested that making relevant programs would positively affect the level of happiness in the elderly (Tiryaki, 2019).

Successful Aging and Happiness in the Elderly

Successful aging refers to positive physical and mental competencies (Vural, Özen and Yazıcı, 2018). On the other hand, the level of happiness is affected by several factors depending on the level of life satisfaction. In this context, successful aging, in other words, knowing the components of successful aging and having a healthy lifestyle, will affect the state of happiness by increasing life satisfaction.

A study on the successful aging of elderly black African immigrant women in Montreal has found that although older immigrant women face many challenges due to their age, ethnicity, gender, and identity, the majority view aging as the normal process of life and as a privilege. By defining themselves as resourceful, hardworking, determined, and combative, they emphasize essential elements for successful aging. In addition, the elderly black African women have reported that change and innovation are always important in their development, whereby they work hard to adapt to these new situations (Noubicer & Charpentier, 2013).

One study of elderly Koreans has determined that participation in leisure activities supports life satisfaction and successful aging. The study used three main themes, including experiencing psychological benefits, creating social support, and improving physical health. The study concluded that a significant participation in physical activities would result in successful aging and various health benefits (Kim, Yamada, Heo and Hani, 2014).

Another study on the sedentary life and happiness among adults and older adults living in a rural area of Japan found that spending more time watching television was associated with a lower happiness score, while those who spent time doing different leisure activities had higher happiness scores. The study suggests that less television viewing, talking

with others, and engaging in hobbies are associated with greater happiness (Yasunaga et al., 2021)

Many studies suggest that there is a positive relationship between successful aging and levels of happiness. In this context, establishing successful aging standards and encouraging the elderly to continue their lives in accordance with these standards are important for their life satisfaction and happiness level.

The Role of Nurses in Boosting Successful Aging and Happiness

In addition to protecting their physical and mental health, it is necessary to support elderly individuals in preventing diseases, ensuring compliance with diseases and treatment, creating a healthy and safe environment, taking safety measures to prevent accidents, and providing hygiene requirements (Kalyoncu and Kartın, 2021).

Nurses not only deal with all health-related matters but also play significant roles in protecting the health of society. By moving away from disease-centered care, nursing care and services are involved in every step to protect and improve the health of the individual and the public.

Considering the positive physiological, sociological, and psychological effects of successful aging, a multidimensional aging model is important for nurses to administer relevant intervention by choosing the appropriate nursing diagnosis in line with the data they obtain after evaluating the patient physiologically, sociologically, and psychologically. In sum, nurses have important roles in maximizing successful aging (Lucena et al., 2020).

Today, harmful habits such as an unhealthy diet, a sedentary life, and smoking have a negative effect on successful aging by reducing the quality of life. At this point, nurses have a significant role in health education and regular health checks.

All of these harmful factors can be corrected by lifestyle changes. Achieving behavioral change can be achieved by providing individuals with adequate training and raising awareness of these issues. Nurses have an educational role in protecting public health and helping people learn healthy behaviors due to today's changing living conditions. Health education allows individuals to take responsibility for their own health, creating healthy societies (Özpulat, 2010).

Nurses should provide training in accordance with whatever physical activity is appropriate for all age groups. Due to the advance of age and the accompanying health problems, it is important to choose appropriate activities and present them to individuals by providing them with relevant training instead of just compelling activities.

By making use of developing technology, a study of a telehealth physical activity program in South Carolina in the United States has produced successful results in increasing mobility in older adults and emphasized both mental and physical health to promote successful aging due to the prevalence of social isolation and depression (Vanravenstein and Davis, 2018).

Another study has reported that owning a dog increases walking activity. The study has also revealed that individuals who walk with their dogs are more physically active, which is one of the essential elements of successful aging. In the study, those who had dogs walked an average of 22 minutes more per day than those who did not. The authors argued that regular contact with pets had a positive impact on depression, anxiety, and loneliness (Friedmann et al., 2020).

Studies have revealed the importance of lifestyle habits and social supports in successful aging. From this point of view, it is necessary for individuals to gain healthy lifestyle behaviors and strengthen social relations for successful aging (Yılmaz, 2020).

Nursing is a multifaceted profession. The nursing profession cannot be restricted to only treating diseases. Nurses are needed to provide the necessary training and arrangements not only for individual health but also for the health of society. As role models, nurses' professional responsibilities, educational roles, and lifestyles affect society. Therefore, nurses have a great responsibility in the promotion of health activities as there is a relationship between the progress in the promotion of health activities, successful aging, and increased happiness. As successful aging concerns both individuals and society, nurses have the responsibility for understanding successful aging and raising awareness of individuals.

Further studies should be conducted to create a society consisting of individuals who live in harmony with successful aging.

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CHAPTER 18

EPIDERMOLYSIS BULLOSA: CLINICAL DENTAL SYMPTOMS AND DENTAL PROCEDURES

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Introduction

Epidermolysis bullosa (EB) is a group of several genetic disorders which are genetically transmitted autosomal dominant or recessive diseases, characterised by the formation of bullae as a result of repeated mechanical and chemical trauma causing an increase in the fragility of the skin and mucosa (Karaduman, 2011; Fine, 2010; Wright et al., 1994). Bullae are fluid-filled swellings that form in the skin and mucosa. The diameter of these blisters is >0.5cm and smaller formations are known as vesicles.

Bullae occur because of mutations in the genes encoding protein structures in the basal membrane structure (dermoepidermal junction) or epidermis tissue (Karaduman, 2011; Kulalı et al., 2019). Approximately 20 genes have been identified associated with these mutations and more than a thousand mutations have been determined (Laimer et al., 2015; Sánchez-Jimeno et al., 2018)

EB is very heterogenous both clinically and genetically, and the bullae which form are classified in four main forms according to the skin layer: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (KS) (Mariath et al., 2020). Each main EB type has different subtypes which can show differences in respect of genetic, biological, or clinical characteristics (Bruckner-Tuderman, 2019). The primary diagnostic methods are light microscopy, transmission electron microscopy (TEM), immunofluorescent antigenic mapping (IAM), and mutation analysis (Karaduman, 2011). These methods are also used for prenatal diagnostic purposes. The presence or absence of basal membrane proteins examined in the amniotic fluid is important for diagnosis (Karaduman, 2011; Fine, 2010).

EBS is the most commonly seen type of EB disease (Mariath et al., 2020), constituting approximately 75-85% of EB cases (Laimer et al., 2015; Laimer et al., 2019). Keratoderma, alopecia, nail dystrophy, and mucosal involvement are very rare. Healing through scar formation, milia (superficial keratin cysts) and nail dystrophy are seen less in EBS than in JEB and DEB (Karaduman, 2011). EBS is separated into subtypes. In the classic subtype of EBS, there is separation in the epithelium along the basal layer. In more uncommon forms of EBS the separation of tissues is suprabasal (Karaduman, 2011).

JEB is characterised by separation of the lamina lucida in the dermo-epidermal junction (Karaduman, 2011). In most cases, the inherited transmission form is autosomal recessive (Laimer et al., 2015). There is a spectrum ranging from life-threatening severe forms to relatively more mild forms (Karaduman, 2011).

DEB has two forms of inherited transmission as autosomal dominant and autosomal recessive (Laimer et al., 2015). The findings of the autosomal dominant form are clinically milder than those of the recessive form (Karaduman, 2011).

Kindler syndrome is inherited as autosomal recessive, and among the skin findings are the formation of acral bullae, webbed fingers and toes, skin fragility, and at advanced stages, poikilodermic pigmentation and photosensitivity that can develop after exposure to even very mild sunlight (Karaduman, 2011; El Hachem et al., 2015). Gingival inflammation is common, and in addition to oesophageal and urogenital stenoses, gastrointestinal symptoms are possible. Moreover, there is a greater risk of the development of skin tumours (Laimer et al.,2015; Has et al., 2011).

EB patients are generally referred to as "butterfly children"as the skin is as fragile as butterfly wings. This inherited disease is not limited to the formation of bullae on the skin but they may also develop in mucosal membranes such as in the oral cavity, and in the respiratory, gastrointestinal and genitourinary pathways (Laimer et al.,2015; Has et al., 2011).

Extracutaneous symptoms and complications in other epithelialised organs cause EB to be a multi-system disease associated with significant morbidity and mortality (Laimer et al.,2015).

The basic clinical symptom is moderate or severe epithelial fragility. This results in bullae formation and secondary lesions such as erosions, ulcers, scars, and generally atrophic scars (Karaduman, 2011). Other symptoms, especially in dermolytic subtypes of EB, lead to complications such as strictures, stenoses, and pseudosyndactyly (Figures 1a, 1b). Milia, pigment disorders, microbial superinfection, nail dystrophy, and alopecia are other characteristics of various EB types (Karaduman, 2011). In severe EB forms, systemic symptoms may be seen as malnutrition and as a metabolic condition in the form of growth retardation. In addition, systemic symptoms in severe EB forms may include chronic inflammation, infections/sepsis, anemia and other symptoms corresponding to specific organ involvement (Laimer et al., 2015).

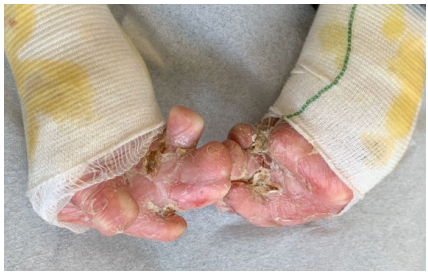

Figure 1a:

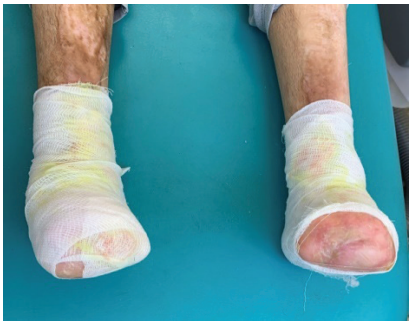

Figure 1b:

Figure 1: In dermolytic subtypes of EB, strictures, stenoses, and pseudodactyly (1a: fingers, 1b: toes).

The main risk factor for this disease is a family history of EB. When prevalence is examined, it can be found in every race and ethnic group, and affects both sexes equally (Limmer et al.,2019; Mello et al., 2016; Rao et al., 2012). Epidemiological data related to the global incidence and spread of EB are extremely variable in different studies (Mariath et al., 2020). The rate of EB has been reported to vary between 8.2 – 19.6 per 1 million live births, and thus EB is defined as a rarely seen disease (Fine, 2010; Laimer et al.,2015; Mariath et al., 2020).

EB is a complex disease group affecting people worldwide for which there is no effective treatment (Prevalence, 2016; Prodinger et al., 2019).

Among the keystones of the healthcare service structure, which varies dynamically, are definitive and early diagnosis, requiring a multidisciplinary approach and co-ordinated specialised centres. Specific personalised patient care, optimised symptomatic treatments and the development of healing therapeutic treatment strategies are of the greatest importance (Laimer et al.,2015).

As there is no great awareness on the subject of this genetic disorder, EB patients and their families experience psychosocial problems in society. While still in childhood, these individuals are physically and psychologically isolated from social life because of skin fragility and the complications which develop. It is important that societal awareness is increased on this subject to increase comfortable living and for these individuals to be integrated into society and to live comfortably within their community. The families of EB patients should be referred to physicians and institutions who can provide genetic counselling about the inherited transmission of this genetic disorder.

These lesions are generally seen on the skin but oral mucosal lesions are also common (Figure 2). An accurate diagnosis is necessary before

planning any form of treatment and in providing this diagnosis, oral symptoms are invaluable (Kudva and Jain, 2016).

Oral findings of EB:

- Perioral-intraoral bullae and lesions
- Ankyloglossia (tongue-tie)
- Microstomia
- Vestibular sulcus obliteration (shallow vestibular sulcus)
- Enamel hypoplasia
- Widespread decay
- Maxillary atrophy
- Predisposition to candida infections
- Squamous cell carcinoma (Kudva and Jain, 2016; Chrcanovic and Gomez, 2019; Feijoo et al., 2011).

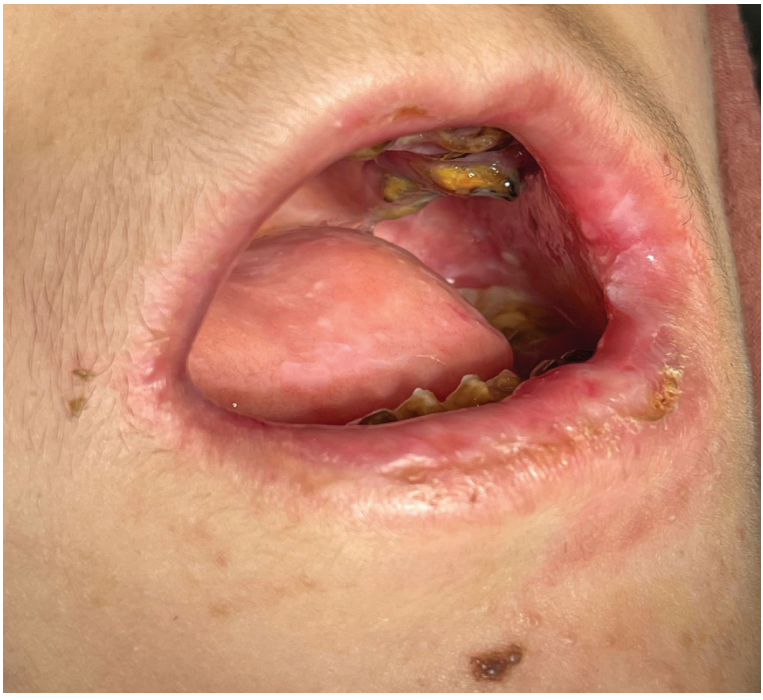

Figure 2: Perioral-intraoral bullae and lesions.

Dental Treatment Procedures for Clinical Symptoms of Epidermolysis Bullosa

Even the most simple dental procedures such as oral and dental examinations may cause the development of bullae in the oral mucosa and tissue. Therefore, dental practitioners should allocate longer periods of time for dental appointments for these patients to be able to treat them more comprehensively and carefully. Sessions may also last longer than normal because of discomfort or dental fear.

A preventative dental treatment approah is accepted as the current dental treatment approach for EB patients (Krämer et al., 2012). By eliminating as far as possible the pain and infection associated with possible dental caries, preventive dental treatment is also important in preventing possible difficulty in food intake and malnutrition, which is one of the most common complications in EB patients (Finke et al.,1996; Wright et al., 1993).

It is important that infants diagnosed with EB are referred to the dentist for the first consultation at the age of 3-6 months. Several published case reports have stated that these patients unfortunately only go to the dentist when they have many caries or dental pain (Krämer et al., 2012; Azrak et al., 2006).

If oral hygiene management is examined, it has been reported that most EB patients, their parents, and dentists have general concerns about the use of toothbrushes and that there could be potential damage in the oral mucosa as a result (Krämer et al., 2012). Although tooth brushing is possible in all EB patients, it is more appropriate to prefer tooth brushes with the following characteristics: the toothbrush should have a small head and soft bristles (Wright et al., 1993; Azrak et al., 2006). In addition, toothbrush bristles can be further softened by soaking in warm or hot water (Krämer et al., 2012).

The 0.12% form of chlorhexidine is widely recommended for the prevention of oral diseases in EB patients (Laimer et al.,2015; Limmer et al., 2019; Mello et al., 2016). As a preventative and protective treatment protocol, it is seen to be appropriate to use this twice a day for two weeks once every 3 months (Kudva and Jain, 2016). In patients with oral mucosal lesions, alcohol-free formulation forms in particular are recommended (Krämer et al., 2012; Olsen and Bourke, 1997).

In EB patients at high risk of caries, the topical application of high-dose fluoride varnish is recommended at every dental examination or once every 3 months (Krämer et al., 2012). Fissure sealants are recommended for preventive and therapeutic purposes, in order not to encounter an advanced

caries condition (Nowak, 1988), although some clinical specialists have concerns about this treatment option as the application of fissure sealant requires great technical sensitivity and full co-operation of the patient and may therefore not be an appropriate option for some EB patients because of limited collaboration (Krämer et al., 2012). For the non-invasive treatment of early decay lesions in EB patients, other remineralisation agents may be used, such as Recaldent (CPP-ACP) Tooth Mousse (Krämer et al., 2012).

It should be aimed to prevent decay by starting a non-cariogenic diet program at as early an age as possible (Silva et al., 2004).

Dental treatment of EB patients is challenging because of the difficulty of surgical techniques and the risk of causing blisters in the oral mucosa, and because of microstomia due to sciatrisation of injuries in the oral epithelium (Nava et al., 2014). In cases of severe microstomia, to develop and maintain good mouth opening, daily mouth opening exercises should be performed and the improvement in mouth opening will also facilitate phonation and swallowing (Krämer et al., 2012). Improving mouth opening with exercises performed half an hour before dental treatment will make it easier to reach the oral region (Krämer et al., 2012).

While drugs are prescribed in tablet form to patients with severe EB, it is important to consider that swallowing tablets may be difficult because of oesophageal stenosis and could cause oesophageal trauma. Therefore, it is important that drugs prescribed are in soluble suspension or liquid form (Krämer et al., 2012).

Of the complications encountered in EB, as the predisposition to the development of intraoral squamous cell carcinoma (OSSC) increases with age, this is a condition to which attention must be paid in the examinations of patients with severe EB in particular from the second decade onwards, and biopsy should be performed of any unusual ulcers or red or white marks to ensure that they do not represent precancer or cancer in the mouth (Krämer et al., 2012).

Before any dental procedure, the lips should be lubricated with vaseline or other suitable oils to prevent forces separating tissues and forming bullae. It is also recommended that in the operating theatre, a non-flammable, water-soluble lubricant is used instead of vaseline. When using cotton wool rolls, the rolls should be lubricated with vaseline or water-soluble lubricants to increase the oral mucosa lubrication before placement in the mouth, and after the procedure, the cotton wool rolls should be removed by first wetting with water to prevent them sticking to the mucosa when being removed from the mouth (Krämer et al., 2012). It is recommended that the saliva aspirator is bent over hard tissue, in other words the occlusal surfaces of the teeth or over a wet cotton wool roll, and

an air spray can be used but with great care (Krämer et al., 2012).

Application of the periapical technique in EB patients has been shown to be extremely difficult especially because of microstomia in the posterior region, ankyloglossia and scarring of the sublingual area (Krämer, 2010). The use of digital panoramic radiographs has been observed to be easier than the periapical radiographic technique in EB patients.

Local anaesthetic fluid should be injected deep into the mucosal tissues at an extremely slow rate to avoid mechanical separation of the tissue and the subsequent formation of bullae (Krämer et al., 2012). When dental treatments under general anaesthesia are planned, the medical doctor attending the patient must be consulted. The availability of an anaesthetic team with EB experience is of great importance.

To minimise the risk of ulcers in the mucosa and to avoid the formation of iatrogenic oral mucosa bullae and blisters, restorations and prostheses must be applied with care and must be polished extremely well (Siqueira et al., 2008). Endodontic root canal treatment can be applied to all patients that have no problems of access within the mouth for reasons such as limited mouth opening (Krämer, 2010). In addition, it may be necessary to change the entry path to the pulp chamber in patients with severe microstomia. For example, the entry cavity of anterior region teeth may require opening from the vestibular surface of the tooth (Krämer et al., 2012).

Tolerance to tissue-supported mobile prostheses depends on the EB subtype and degree of oral mucosal fragility of the patient (Krämer et al., 2012). Fixed prosthetic restorations have been reported to be a successful treatment option which improve oral function and increase patient confidence and aesthetics in severe EB types (Siqueira et al., 2008). In cases with generalised enamel hypoplasia, full crowns of all the teeth may be necessary, and computerised, digital intraoral scanning can be a non-invasive measurement method for taking measurements without harm for implant planning and placement, and for surgery and prostheses (Krämer et al., 2012). The use of stainless steel crowns has been reported to be a successful treatment method (Krämer et al., 2012; Lindemeyer et al., 2009).

There is no problem in applying periodontal treatment to all patients with EB, but as there may be a great amount of bleeding during the procedure, it should be performed with as atraumatic an approach as possible. Sutures can be used in all EB patients but must be placed with care (Krämer et al., 2012).

Current oral and dental health concepts aim to first provide preventative and protective dental treatments. However, extraction may be necessary in some cases for reasons such as severe decay or severe closure

problems which orthodontically impair occlusion (Krämer et al., 2012). When planning tooth extraction, especially if it is necessary to extract more than one tooth, attention must be paid to the the possibility of deep anaemia, which is a complication seen in these patients. As this condition can make the tooth extraction more difficult, consultation with the doctor attending the patient is recommended (Krämer et al., 2012). For multiple tooth extractions, it is recommended that anterior region teeth are extracted first then posterior region molars to be able to provide optimal access to the posterior region. Light pressure on the area with gauze is required to halt any bleeding, and the gauze should be wet to prevent tissue adhesion (Krämer et al., 2012). When planning this type of rehabilitation, approval must be obtained from the medical doctors as there could be comorbid pathologies such as severe anemia, a poor prognosis, or SCC (Krämer et al., 2012).

Conclusıon

EB is a rarely seen genetic disorder with many oral findings, of which dentists should have knowledge, and which require careful and precise approaches in dental treatments.

It must not be forgotten that the clinically observed findings of EB patients cause significant health and psychosocial problems.

It must be known that EB cases require a treatment approach with a multidisciplinary understanding including genetic, psychiatry, dermatology, cardiology, hematology, and nutrition specialists, and dentists.

The main aim in EB cases is to improve the quality of life for patients by reducing the effects of the clinical symptoms of this genetic irregularity.

The aim of dental treatments is to prevent the development of malnutrition associated with potential feeding problems by eliminating pain and infection associated with dental problems and regaining function, phonation, and aesthetics.

The aim of this review was to inform dental practitioners about the clinical symptoms of epidermolysis bullosa and to create an awareness about the dental treatment procedures for patients with epidermolysis bullosa.

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CHAPTER 19

VITAL PULP AMPUTATION AND TECHNIQUES

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The pulp is a structure found in the crown and root of the tooth and has shaped, nutritive, sensory, and protective properties. Pulp tissue is a vascularized connective tissue of ectomesenchymal origin and surrounded by a nerve network. The coronal pulp is surrounded by enamel and dentin, and the root is surrounded by dentin and cementum. These hard tissues surrounding the pulp provide mechanical support and protect the pulp against microbial rich in the oral environment. pulp tissue; It is a vascularized connective tissue of ectomesenchymal origin and surrounded by a nerve network, which has shaping, nourishing sensory and protective functions. (1)

Vasodilation and increased vessel permeability caused by inflammation in the pulp tissue cause an increase in pressure in the pulp tissue. This pressure increase activates the local feedback mechanism. According to this mechanism, increased interstitial pressure in the inflamed pulp reduces the transcapillary hydrostatic pressure difference and prevents filtration.

In addition, increased interstitial pressure helps capillaries in adjacent tissue where inflammation is not present to absorb interstitial fluid and increases lymph flow. With these two mechanisms, the fluid that causes the increase in tissue pressure is removed from the affected area, thereby reducing the tissue pressure. The fact that the pulp can protect itself for a long time after inflammation and that the inflammation regresses and pulpal health can be regained when suitable conditions are provided can be explained by these mechanisms. For the pulp tissue to be repaired, it is necessary to maintain the vitality of the pulp. For this reason, the importance of vital pulp treatments is increasing. (2-4)

Although a tooth can remain functional without being vital, it should be aimed to preserve the vitality of the pulp (5) In recent years, clinicians and scientists; emphasized the importance of maintaining the vitality of the pulp while cleaning deep caries. (6)

Our priority in pulp treatment; is to maintain the vitality of the pulp affected by caries, trauma, or other reasons. (5)

The indications, goals, and type of pulp therapy depend on whether the pulp is vital or not. A healthy pulp should be clinically asymptomatic and respond to vitality tests. (7)

amputation treatment; It is the covering of the infected or affected coronal pulp tissue with a coating agent that will help maintain the vitality of the radicular pulp, which is left behind after surgical removal and that will help maintain the vitality of the radicular pulp, which is predicted to be alive. It is one of the most applied endodontic treatments. (8-10)

The features that an ideal pulp coating agent should have can be listed as follows; (11,12)

- Should be bactericidal
- It should help the radicular pulp to heal.
- It should be biocompatible for pulp and surrounding tissues.
- It should not interfere with the physiological root resorption process.

Various materials have been tried to find an ideal capping agent in amputation treatments from past to present, unfortunately, an ideal amputation agent has not been defined among these available materials yet. (13,12)

Indications for Vital Pulp Amputation (5, 13)

- teeth where the pulp is perforated by caries or mechanical injury.
- There should be no spontaneous complaints of pain.
- There may be pain related to meals, especially sugary foods.
- Soft tissue findings (swelling, abscess, fistula) should be normal,
- There should be no radiological signs of infection.
- When bleeding is controlled with cotton pellets after a few minutes, there should be no purulent, necrosis, or excessive bleeding.

Vital Pulp amputation contraindications; (14)

- The patient has any systemic disease that will prevent treatment,
- The tooth is in a condition that cannot be restored,
- Spontaneous and ongoing pain complaints,
- Sensitivity to percussion or palpation,
- Pathological mobility,
- Presence of swelling, abscess or fistula due to infection in the pulp,
- Presence of serous or purulent drainage in the pulp,
- Pathological radiological findings:
- ✓ Pulp calcifications,
- ✓ Internal and external resorption,
- ✓ Radiolucency in furcation or periapical,

- ✓ Physiological root resorption reaches 2/3 of the root.

While performing amputation treatment, the color and duration of the bleeding in the pulp and the vitality of the pulp are important issues to be considered. Bleeding should stop within 5 minutes after the coronal pulp is removed. This indicates that the remaining root pulp is healthy. (11.14)

Targets in amputation treatment; (5)

Radicular pulp is asymptomatic, there should be no pain, fistula, and swelling in the tooth,

- ➢ Postoperatively, external resorption should not be observed radiographically.
- ➢ If there is internal resorption, it should be monitored if it is self-limiting and stable, if perforation occurs and causes bone resorption or whether there is clinical infection.

Amputation treatments are classified according to their goals (15):

Devitalizing therapy (mummification, cauterization) (formocresol, electrosurgery, laser)

- ✓ Preventive therapy (minimal devitalization, non-inducer) (glutaraldehyde, ferric sulfate, zinc oxide eugenol (ZnO))
- ✓ Regenerative therapy (inducer, reparative) (calcium hydroxide, bone morphogenic protein (BMP), mineral trioxide aggregate (MTA).

Devitalizing treatment: Devitalization was the first aim of amputation treatments for primary teeth. In the multi-session formocresol technique applied for the first time by Sweet (15), the aim is to completely embalm the tissue. They suggested that when the tissue is completely fixed, the root pulp is theoretically lifeless and sterile, so there will be no infection and internal resorption problem (15). In the following years, it was understood that the number of sessions did not affect the success of the treatment (17). (15, 17)

Preventive treatment: In this treatment, it is aimed to keep the radicular pulp completely alive, although a regeneration process has not started (Ranly 1994). Zinc oxide eugenol (ZnOE), which has been widely used since the first periods of dentistry, has also been used in amputation treatments (15) and has become the first material used in preventive treatment applications. Since ZnOE, which has a therapeutic effect when placed on healthy dentin in indirect pulp coatings, has a cytotoxic effect when placed directly on the pulp, its use as an amputation material has been abandoned (18). He suggested that glutaraldehyde and ferric sulfate materials, which are different, should also be evaluated within this group.

Because glutaraldehyde is difficult to prepare and store, glutaraldehyde applications have not become widespread compared to formocresol. (15)

Regenerative treatment: In ideal amputation treatments, radicular pulp; should be vital, healthy, and completely covered by the dentin barrier formed by odontoblasts. In this case, the pulp tissue will be isolated from the damage of the restorative materials under the formed dentin barrier, thus reducing the risk of internal resorption. In addition, odontoclasts in the healthy pulp participate in the exfoliation process on time, and this process continues within physiological limits. For these to occur, repair dentin must be created by the amputation agent. (15). Over time, various pharmacotherapeutic agents and techniques have been proposed to provide treatments that meet the criteria for success. Some of these are formocresol, calcium hydroxide, ferric sulfate, glutaraldehyde, electrosurgery, laser irradiation, and growth factors. Formocresol is the most popular of these methods due to its ease of use and high clinical success (18, 19).

Partial Pulpotomy (Cvek Amputation)

It is a treatment that aims to maintain the health of the remaining coronal and radicular pulp by removing a small part of the crown pulp. (8)

When deciding on the pulpotomy treatment, the type of tooth (permanent or primary tooth), the etiology of pulp perforation (trauma or caries), root development (open or closed apex), the type of fracture (simple or complicated crown fracture), trauma-related bone and gingival injury. decide whether it is not. In primary teeth, when the pulp is perforated with caries, a total pulpotomy is preferred. On the other hand, if there is pulp exposure in permanent teeth due to trauma or caries and pulpal bleeding can be controlled within a few minutes, cvek amputation is preferred. (20-22)

After local anesthesia is applied to the tooth, it is isolated. If there is an exposure due to caries, all carious tissue in the cavity is cleaned from the periphery towards the center. Using a sterile diamond bur, the pulp tissue under the pulp exposure area is lifted by 1-3 mm to reach the healthy pulp underneath. If there is a pulpal opening due to trauma, it is removed to reach the healthy pulp of 1-3 mm in the same way. After the pulpal bleeding is controlled, the exposed pulp surface is covered with a pulp coating agent and the tooth is restored. (23)

After the Cvek amputation treatment applied by Fuks AB et al. on 63 permanent incisors whose pulp was exposed due to complicated crown fracture; It was reported that no clinical and radiological pathology findings were observed in 59 teeth, a positive response was obtained in electrical pulp tests, and dentin bridge was formed radiographically, while necrosis findings were observed in 4 teeth. With this study, it was

concluded that partial pulpotomy treatment showed good clinical and radiological success. (24)

Materials and Methods Used in Vital Pulpa Amputation Treatment

Over time, various pharmacotherapeutic agents and techniques have been proposed to perform amputation treatments that meet success criteria. (18)

Formocresol Amputation

Formocresol, first introduced by Buckley in 1904 and applied by Sweet in 1930, is a strong germicide drug that provides fixation of living tissues. (18) Formaldehyde penetrates organic structures and dentin canals, fixes the bacteria present in this region, and is thought to prevent tissue destruction by binding to these proteins. (25)

After clinical studies by Buckley, a treatment protocol was developed by modifying the technique described by Sweet (19), shortening the application time of formocresol and applying it for 3-5 minutes. They reported that formocresol suppressed metabolism by playing a cytotoxic role in fixation, and following formocresol amputation in the histological sections taken, homogeneous eosinophilic tissue in the coronal 1/3 of the radicular pulp, coagulation necrosis in the middle 1/3 and vital tissue in the apical 1/3 were found. (18, 19), however, Beaver et al. stated that there are no histologically separated regions in the root pulp, and concluded that there may be different tissue responses such as fixation, internal resorption and necrosis. (18)

As a result of many studies on the toxicity and systemic spread of formocresol used in amputation treatment, this material has been accepted by many researchers as having immunogenic, mutagenic and carcinogenic effects. (11,26) For this reason, it was thought that formocresol should be diluted. It was stated that the use of 1/5 concentrations did not affect the clinical success of this material. (19.27)

Thus, despite the devastating effect of diluted cresol on vital tissues, it has been argued by researchers that the systemic spread of this material is insignificant after amputation treatment (28).

Failures of formocresol amputations are mostly detected radiologically. The first sign of unsuccessful treatment is internal resorption, usually localized at the root, close to the area where formocresol was applied. Especially in the later periods, external resorption may accompany it. With the increase in resorption, excessive mobility is observed in the tooth and a fistula usually occurs. It is rare to see pain symptoms in the failure of formocresol amputation (19).

Due to its high clinical success rate, formocresol amputation is an

amputation technique that is frequently preferred in clinical practice today.

Increasing concerns about the toxicity and potential carcinogenicity of formocresol have led researchers to alternative methods to find an ideal pulp coating agent. (29)

Clinical Application

The tooth is isolated using local anesthesia. The access cavity is opened by following the endodontic rules. The coronal pulp chamber is removed with a sharp and sterile instrument. Bleeding is controlled with cotton pellets for 3-4 minutes. If bleeding continues, root canal treatment is started. Cotton pellets impregnated with formocresol are kept in the canal mouths for 3 minutes. After the drug is removed, the pulp should be non-bleeding and have a brown appearance. Following these procedures, zinc oxide eugenol cement is placed in the pulp chamber and restored with PÇK (stainless steel crown) or other restoration materials. (18)

Calcium Hydroxide Amputation

In an ideal amputation treatment, it is expected that the remaining pulp is healthy and vital, and it is covered with an odontoblast-limited dentin layer. In this case, it becomes important to use an amputation agent that will stimulate the formation of reparative dentin. (18). Calcium hydroxide was the first material used in the treatment of amputation, which was shown to have the capacity to induce dentin regeneration (15).

Today, Ca(OH)2 is widely used as a capping agent because it is the most classical agent that can stimulate dentin regeneration and keep the root pulp alive. Ca(OH)2 has a high pH value and has a bactericidal effect. It can neutralize bacterial acids and lipopolysaccharides in dentin. Thus, it causes the release of growth factors attached to dentin. (30)

Due to the high pH of the hydroxyl ions in the calcium hydroxide content, it causes a chemical injury to the pulp. Following this injury, superficial coagulation necrosis and moderate inflammation occur. This is the superficial, germ-free, slow death of pulp tissue. The proteolytic ferments in the necrosis layer are eliminated and there is also a coagulation in the pulp. This necrosis layer formed; induces differentiation of adjacent healthy pulp tissue, fibroblasts, or undifferentiated mesenchymal cells into odontoblasts. This event is especially seen during the healing events observed in vital pulp treatments such as vital amputation and direct capping. (31.32)

In the repair process, after the inflammatory response, the area of necrosis is filled with dystrophic calcifications and the repair dentin, namely the dentin bridge, is formed (Carrotte 2005, Briso et al 2006, de Souza Costa et al 2008). The resulting calcified tissue is called osteopontin.

The alkaline pH of calcium hydroxide not only neutralizes the lactic acid released from osteoclasts but also prevents the dissolution of the mineral components in dentin. At the same time, it plays an important role in the formation of hard tissue by activating alkaline phosphatase (32-34). The stimulation created by calcium hydroxide is in a delicate balance between repair and resorption (15). However, when the balance is disrupted destructively, the process fails the treatment. (18)

Particular attention should be paid to pulpal bleeding after pulp amputation, especially in calcium hydroxide amputations (35). In the clinical and radiological evaluations of calcium hydroxide amputations, a success rate of 57-80% has been reported (36,37). In clinical studies comparing calcium hydroxide and formocresol, it has been reported that calcium hydroxide is not as successful as formocresol (35). Internal resorption is the main cause of failure of calcium hydroxide amputations. Therefore, it has been reported that calcium hydroxide is not a strong alternative to formocresol as a material that is not preferred much with low clinical and radiological success in primary tooth amputations. (18,36) There is no strong consensus regarding the use of this material due to failures due to failure to prevent internal resorption. (15)

Ferric Sulfate Amputation

20% ferric sulfate (Fe2(SO4)3) (Monsel solution), a strong hemostatic and non-aldehyde group compound was first used in France in 1857. In dentistry, it is generally used to stop bleeding in surgical operations and for gingival retraction. Although it is not fixative, it has only bacteriostatic properties. To control bleeding, gentle application of a 15.5% solution of ferric sulfate is recommended, intermittently for about 15 seconds. The short application time provides clinicians with great convenience when treating children. (38) Due to the low pH of the solution due to its direct application to the pulp, iron and sulfate ions appear. Although it has been reported that these ions cause the precipitation of blood proteins as a result of the interaction of iron and sulfate ions with the blood, causing mechanical blocking of the cut vessels, the actual mechanism of action is still debated.

The metal-protein complex formed in the pulp tissue acts as a non-toxic barrier against irritants. (39) Since ferric sulfate provides hemostasis by precipitating blood proteins without forming blood clots, it is thought that failures such as chronic inflammation and internal resorption can be prevented by eliminating failures that may occur due to extravascular clot formation. (40) However, internal resorption and radiolucency areas in furcation were also observed in ferric sulfate amputation. (39)

In previous studies, the presence of a clot formed between the pulp

tissue and calcium hydroxide was mentioned as the cause of calcium hydroxide failure. For this reason, the necessity of improving bleeding control was considered and the use of ferric sulfate together with calcium hydroxide was investigated. In later periods, ferric sulfate began to be used alone in the treatment. In clinical studies, success rates similar to formocresol were seen for ferric sulfate in amputation treatments in primary teeth. (39,41).

Clinical Procedure

The tooth is isolated by performing local anesthesia. The pulp chamber is opened by adhering to traditional endodontic rules. The coronal pulp is removed with a sharp and sterile instrument. Bleeding is controlled within 3-4 minutes with cotton pellets. If the bleeding cannot be controlled, the next treatment step, root canal treatment, is planned. In the canal mouths, 15.5% ferric sulfate is contacted with the pulp tissue for 10-15 seconds. If bleeding control is not achieved in the first application, it can be applied a second time. After this stage, it is thoroughly washed with physiological saline. The cavity is thoroughly dried with cotton pellets and the tooth is restored by placing zinc oxide eugenol. (18)

Glutaraldehyde Amputation

Glutaraldehyde, due to its strong antimicrobial and low toxicity properties, is used in deciduous tooth pulpotomy treatments and establishes tight bonds with amino acids of proteins, and provides a more stable and irreversible fixation than formocresol.

Since it has a larger molecular size than formocresol, it diffuses more slowly into tissues. In addition, it can stabilize proteins faster, irreversibly, and completely. In addition, since its spread is limited to hard tissues, it does not cause periodontal irritation. Although it is less toxic and a better fixative, this material is not widely used because it is not as clinically successful as formocresol. Due to the instability of glutaraldehyde in its pure form, the preparation of buffer solutions is considered among its disadvantages. (18,38,42)

Clinical Procedure

The tooth is isolated by performing local anesthesia. The pulp chamber is opened by adhering to traditional endodontic rules. The coronal pulp is removed with a sharp and sterile instrument. Bleeding is controlled within 3-4 minutes with cotton pellets. If the bleeding cannot be controlled, the next treatment step, root canal treatment, is planned. Cotton impregnated with 2% glutaraldehyde is kept in the duct mouths for 5 minutes. After the drug is removed, the pulp should be non-bleeding and have a brown appearance. Zinc oxide eugenol is placed and the tooth is restored. (18)

Mineral Trioxide Aggregate (MTA) Amputation

In recent years, the use of MTA as a preferred material for pulp treatments has come to the fore. It was described by Torabinejad et al. It was approved for use in humans by the FDA in 1998. Clinical studies have shown that MTA has similar or better success than formocresol and ferric sulfate. (13, 43-45)

Its chemical components are related to Portland cement, and it is formed by mixing dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetra calcium aluminum afferent, and gypsum, and bismuth oxide is added to increase its radiopacity. (38)

While it was originally developed as a retrograde filler in periapical surgical treatments, today it has many uses, including in the treatment of perforations, vital pulp treatments, and apexification. (46.47)

MTA applied to pulp tissue; It causes proliferation, migration, and differentiation of cells responsible for collagen matrix production to this region. With the mineralization of the formed matrix, first osteopontin and then tertiary dentin are formed. (48-50) Ainechchi et al. When they compared pulpotomy treatments made with calcium hydroxide and MTA, it was reported that although the hard tissue formed with calcium hydroxide contains deficiencies and tunnel defects such that bacterial leakage may occur, this situation was not observed in the dentin bridge formed with MTA. (51) Histologically, according to Ca(OH)2 of MTA; It was observed that a thicker dentin bridge formed, less pulpal inflammation, hyperemia, and pulpal necrosis. (52)

Difficult to manipulate the material, long curing time, causing discoloration on the crown, and high cost was seen as disadvantages, and researchers started to search for new materials. (53)

Clinical Procedure

The tooth is isolated by performing local anesthesia. The pulp chamber is opened by adhering to traditional endodontic rules. The coronal pulp is removed with a sharp and sterile instrument. Bleeding is controlled within 3-4 minutes with cotton pellets. If the bleeding cannot be controlled, the next treatment step, root canal treatment, is planned. Following the company's recommendations, powder and liquid are mixed and placed in the channel mouths in 3-4 mm thickness. It is condensed with the help of a damp cotton pellet. Zinc oxide eugenol is placed in the pulp chamber and the tooth is restored or preferably 1 day later. (18)

Electrosurgical Amputation

The systemic spread and toxic effects of some agents used in primary tooth pulpotomy suggested that bleeding control should be performed

with non-pharmacological approaches such as electrosurgery and laser during direct pulp treatments. (54) When the success of pulpotomy applied with formocresol and electrosurgery methods were compared; Some investigators reported that there was no difference between the clinical and radiographic success of formocresol and electrosurgical pulpotomy. (55.56) Öztaş et al. formocresol pulpotomy are histopathologically superior to the electrosurgery method (57); El Meligy et al. reported that less histopathological reaction occurred in pulpotomy treatment with electrosurgery. Although the study of this procedure is limited, encouraging results have been reported. (58)

Clinical Procedure

The tooth is isolated by performing local anesthesia. The pulp chamber is opened by adhering to traditional endodontic rules. The coronal pulp is removed with a sharp and sterile instrument. Bleeding is controlled within 3-4 minutes with cotton pellets. If the bleeding cannot be controlled, the next treatment step, root canal treatment, is planned. A U-shaped electrode to the pulp tissue at the canal orifices is coagulated with a high-frequency current of the pulp at the canal orifices with brush-like strokes. After the application, the pulp should be non-bleeding and have a brown appearance. Zinc oxide eugenol is placed and the tooth is restored. (18)

Laser Amputation

After long studies, FDA allowed the use of laser technology in 1997, cleaning and preparation of carious cavities, aesthetic dental treatments, periodontal surgery, periimplantitis treatment, treatment of aphthae and herpes, reduction of dentin hypersensitivity, evaluation of pulp vitality, direct pulp coating, pulpotomy, and root canal disinfection. It has many uses such as Laser technology has a wide variety of applications in pediatric dentistry. (18, 38, 59, 60)

Laser irradiation creates a limited area of coagulation in the pulp tissue it contacts. However, the underlying tissue is in good condition and is protected from the negative effects of the pulp base material. (18)

When the success of direct pulp coating with Ca(OH)2 after carbon dioxide laser application is compared with direct pulp coating performed with only Ca(OH)2, the clinical success in the laser applied group was 93% after 2 years of follow-up; In the control group, the success rate was 68%. (61)

Jayawardena et al. stated that coating the pulp with calcium hydroxide after the use of Er: YAG laser did not cause any pathological response and provided dentin bridge formation. (62)

However, in some studies, carbonization, necrosis, and inflammation

of the pulp were observed after pulpal amputations with laser, while the repair was minimal. (38) In a review, it was seen that there is weak evidence that laser amputation applications can improve the results of treatment. He concluded that further studies are needed. (63)

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