INTERNATIONAL THEORY, RESEARCH AND REVIEWS IN

HEALTH SCIENCES

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Chapter 1

ADIPOSE TISSUE IN HEALTH AND CARDIOMETABOLIC DISEASES

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INTRODUCTION

Obesity, a chronic disease that affects people's quality of life physiologically, psychologically and economically, is accepted as the primary health burden and global health problem of the 21st century(Cremonini et al. 2020; Manna and Jain 2015; Stolarczyk 2017). Obesity is associated with many diseases such as Dyslipidaemia, Cancer, Insulin Resistance, Hypertension, Non-alcoholic Fatty Liver Disease, Type 2 Diabetes Mellitus and Cardiovascular Diseases. Obesity-related risk factors are not only caused by excess body weight, but also the regional distribution of excess fat mass in the body is important (Manna and Jain 2015). Accordingly, it is known that abdominal fat is a risk factor for obesity-related diseases, and visceral fat accumulation stimulates pro-oxidant and pro-inflammatory states.

Since obesity is a disease characterized by an increase in the number and mass of adipocytes, the spread of the disease has increased the interest in adipose tissue. In obesity, change in the distribution of adipose tissue in the body and impairment of its function are associated with low-grade inflammation, and dysfunctional adipose tissue is the main link between obesity and cardiovascular diseases (Fuster et al. 2016; Lastra and Sowers 2013; Rana and Neeland 2022)

ADIPOSE TISSUE

Adipose tissue is a type of connective tissue consisting mainly of fat cells called 'adipocytes' and is an endocrine organ that can synthesize and secrete many substances that regulate energy balance and metabolic homeostasis. In addition to its role in energy storage and being an endocrine organ, adipose tissue is also recognized as an important immune organ (Stolarczyk 2017). Apart from adipocytes, adipose tissue includes preadipocytes, fibroblasts, macrophages, monocytes, innervation cells, and stroma vascular fraction (Raza et al. 2020).

Adipose Tissue Classification and Distribution

There are four types of adipose tissue that differ in function, coloration, vascularization and structure (Figure 1). These are White adipose tissue (WAT), Brown adipose tissue (BAT), Pink adipose tissue and Beige adipose tissue (Saxton et al. 2019).

White adipose tissue is a white-yellow tissue with less vascularization and innervation than brown adipose tissue. WAT cells are unilocular cells, varying in size between 20-200 μm, and containing a single, large lipid vacuole. WAT is the predominant adipose tissue in mammals (Declercq, Taylor, and Zahradka 2008). Many hormones, cytokines, growth factors and enzymes responsible for metabolism, energy expenditure, food intake, immunity and homeostasis are secreted from WAT (Pilkington, Paz, and Wankhade 2021).

Brown adipose tissue is more vascularized and contains many more mitochondria. BAT cells are multilocular cells containing many lipid vacuoles. These cells are polygonal in shape and their size is between 15-50 µm. BAT originate from muscle tissue precursor cells, not from white adipocytes. BAT has no energy storage function. BAT is responsible for the distribution of energy through thermogenesis. The amount of brown adipose tissue is especially high in new-borns (Frigolet and Gutiérrez-Aguilar 2020; Ying et al. 2023).

Adipose cells in beige adipose tissue have characteristics similar to brown adipocytes. Uncoupler protein (UCP-1) expression is also observed in beige adipose tissue with exercise, exposure to cold or some hormone stimulation (Frigolet and Gutiérrez-Aguilar 2020).

A fourth type of adipocyte, the pink adipocyte which are epithelial cells in the mammary glands seen throughout pregnancy and lactation and are responsible for milk production and secretion (Giordano et al. 2014; Valencak, Osterrieder, and Schulz 2017).

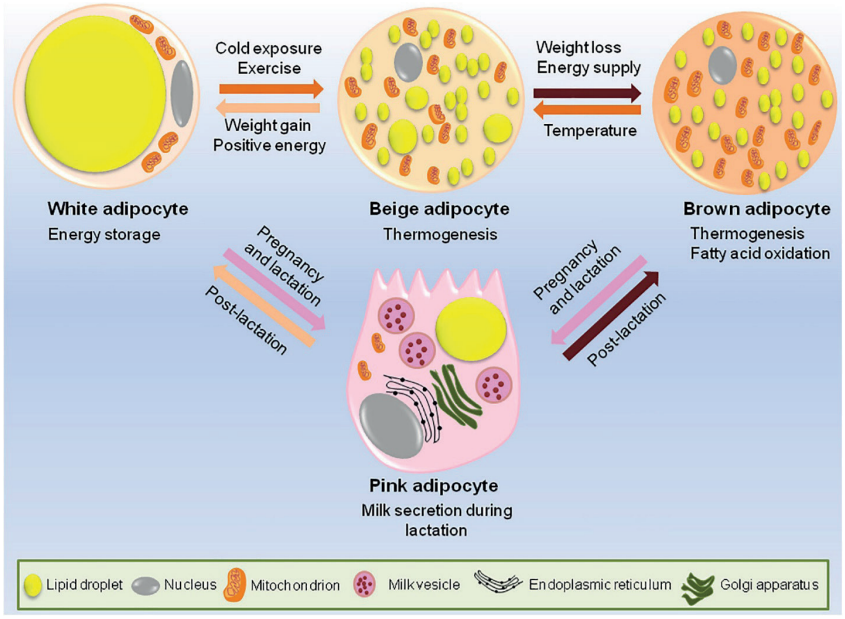

Figure 1: Four types and functions of adipose tissue *(Pant et al. 2021).*

Subcutaneous and Visceral Adipose Tissue

Anatomically, there are two main types of adipose tissue: visceral and subcutaneous adipose tissue (Figure 2). The adipose tissue lining around the internal organ is called visceral adipose tissue and that under the skin is called

subcutaneous adipose tissue (Ibrahim 2010; Oikonomou and Antoniades 2019). Visceral adipose tissue is grouped as intrathoracic and intraabdominal. Subcutaneous adipose tissue is classified as abdominal gluteal and femoral. In addition, they are grouped as superficial and deep (Ibrahim 2010; Oikonomou and Antoniades 2019). Visceral and subcutaneous adipose tissue differ in structure and function. The type of adipocyte and their endocrine functions are also different (Ibrahim 2010)

The placement and distribution of adipose tissue in the body are important in terms of cardiovascular risk. Epidemiological studies reveal a relationship between visceral adipose tissue expansion and cardiometabolic risk (Oikonomou and Antoniades 2019). In addition, subcutaneous adipose tissue is neutral in terms of cardiometabolic risk. It may even sometimes have cardioprotective properties (Oikonomou and Antoniades 2019).

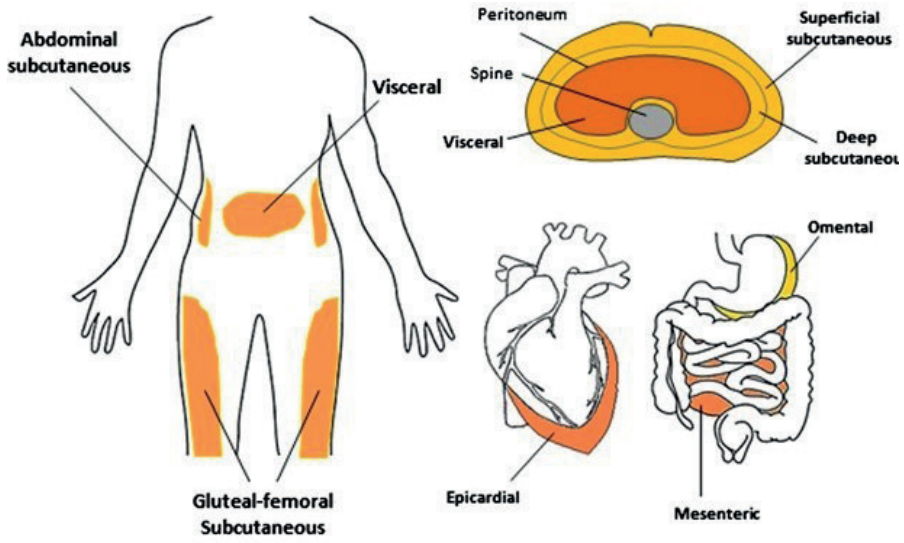

Figure 2: Two anatomic types of adipose tissue: Visceral and subcutaneous adipose tissue *(Kwok, Lam, and Xu 2016)*

Biochemistry of Adipose Tissue

Adipose tissue is a type of connective tissue consisting mainly of adipocytes and is an endocrine organ that can synthesize and secrete many substances that regulate energy balance and metabolic homeostasis (Figure 3). Adipose tissue has a major role in various physiological functions such as regulation of food intake and body weight, inflammation, coagulation, insulin sensitivity and vascular functions. Hormones secreted from adipose tissue, called adipokines, play an important role in regulating metabolism.

Apart from adipocytes, adipose tissue contains preadipocytes, fibroblasts, macrophages, monocytes, innervation cells, and stroma vascular fraction(Coelho, Oliveira, and Fernandes 2013).

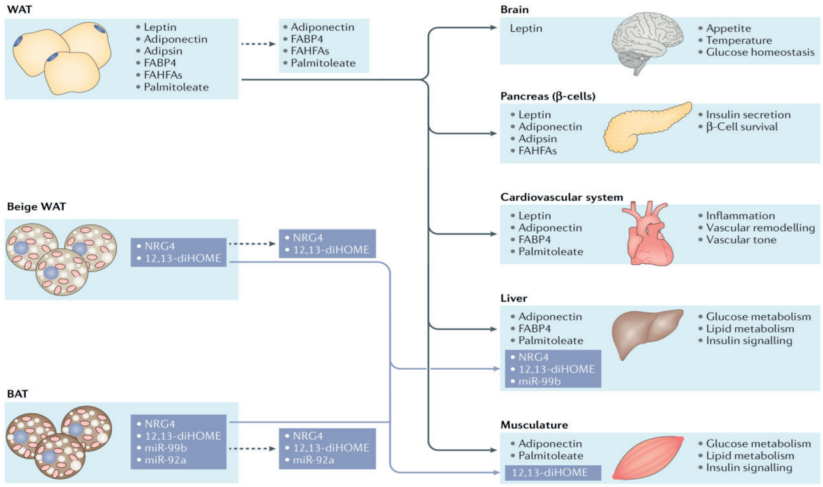

Figure 3. Substances secreted from adipose tissue and their systemic effects
(FABP4; Fatty acid binding protein 4, FAHFAs; Fatty acid esters of hydroxy fatty acids, NRG4; Neuregulin 4, 12,13 diHOME; 12,13-dihydroxy-(9Z)-octadecenoic acid, miR-99b; microsomal RNA- 99b, miR-92a; microsomal RNA 92a) (Scheja and Heeren 2019)

Cardiovascular Diseases

Cardiovascular diseases (CVD) are the leading cause of mortality and morbidity worldwide (Zhou et al. 2018). CVDs are a group of diseases characterized by cardiovascular disorders that affect the flow of blood to the heart, brain, and peripheral parts of the body. CVDs include coronary heart disease, cerebrovascular disease, rheumatic and congenital heart diseases (Hijová 2023).CVDs are the leading cause of premature death worldwide (Van Camp 2014). The prevalence of CVD increases with age, as well as modifiable factors such as tobacco use, alcohol consumption, obesity and physical inactivity (Lyngbakken et al. 2019).

CVD is a lifelong disease characterized by the development of subclinical atherosclerosis (Santhakumar, Battino, and Alvarez-Suarez 2018). Inflammation plays an extremely important role in the onset and development of atherosclerosis. Atherosclerotic cardiovascular diseases are among the chronic inflammatory diseases (Irwandi et al. 2022).

Factors that cause CVD are divided into two groups: preventable and non-preventable. Factors such as age, gender and genetic predisposition are not

preventable because they cannot be changed. In addition, lifestyle, nutritional habits, physical activity, blood glucose and lipid levels, which are changeable factors, are preventable factors (Hijová 2023). Studies have shown that a balanced diet plays an important role in preventing CVD risk (Anon 2013; Llorente-Cortés et al. 2010; De Lorgeril et al. 1999; Widmer et al. 2015).

Relationship Between Adipose Tissue and Cardiovascular System

Increased body mass index (BMI) is associated with the development of cardiovascular risk factors such as, hypertension, insulin resistance, dyslipidemia and Type 2 diabetes and the occurrence of cardiovascular diseases (Bastien et al. 2014). BMI and obesity are considered independent risk factors for cardiovascular diseases (Bastien et al. 2014; Svačina 2020).

Adipose tissue expansion and dysfunction lead to cardiovascular diseases through different mechanisms (Oikonomou and Antoniades 2019).

Obesity is a disease that supports the development of chronic inflammation and is characterized by an increase in the number and mass of adipocytes. Obesity, specifically abdominal obesity, is associated with an increased risk of cardiovascular disease (Declercq et al. 2008).The distribution of adipose tissue in obesity is extremely important. Visceral adipose tissue is associated with insulin resistance, dyslipidaemia, and hypertension, regardless of subcutaneous adipose tissue and BMI (Saxton et al. 2019).

According to studies increased adipose tissue mass contributes directly toward an increase in systemic inflammation. Also increased BMI correlates with an increase in inflammatory proteins in the systemic circulation. Systemic inflammation is also associated with increased cardiovascular risk (Berg and Scherer 2005).

Obesity induces hypertension due to both the renin angiotensin aldosterone system and the expansion of visceral adipose tissue, which mechanically puts pressure on the kidneys (Oikonomou and Antoniades 2019). Adipose tissue can also affect cardio metabolism with the adipocytokines it secretes. In dysfunctional adipose tissue, leptin secretion increases while adiponectin secretion decreases. Adiponectin has protective effects against cardiovascular disorder. When adiponectin levels decrease, insulin resistance increases and antioxidant and anti-inflammatory properties decrease (Zhao, Kusminski, and Scherer 2021). Leptin, which increases with obesity, affects the cardiovascular system by exhibiting pro-oxidant properties (Oikonomou and Antoniades 2019).

Adipose tissue can also affect the cardiovascular system through the vascular wall. Vascular smooth muscle cell migration, inflammation and endothelial function are regulated by substances secreted from adipose

tissue (Oikonomou and Antoniades 2019). Perivascular adipose tissue has an important role in vascular wall functions. In addition, epicardial adipose tissue affects the cardiovascular system through myocardium. Epicardial adipose tissue exhibits embryological and morphological similarities with visceral fat, located between myocardium and visceral pericardium (Ansaldo et al. 2019; Le Jemtel et al. 2019). In dysfunctional epicardial adipose tissue, the thickness and tissue volume increase and the levels of substances secreted from the adipose tissue changes (Le Jemtel et al. 2019). This causes an increase in inflammation.

As a result, adipose tissue is a link between obesity and cardiovascular diseases, and adipose tissue affects cardiovascular metabolism through endocrine, paracrine and vascular signalling mechanisms, as shown in the Figure 4.

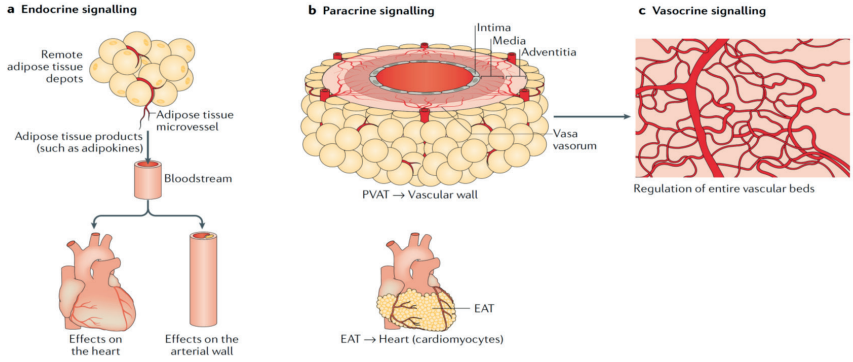

Figure 4. Effects of the adipose tissue in the cardiometabolic system *(Oikonomou and Antoniades 2019)*

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Chapter 2

E-SPORTS AND ADOLESCENTS: 21ST CENTURY YOUTH PHENOMENON

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Introduction

The last decade has seen remarkable growth in e-sports. E-sports is a global market valued at approximately $695 million in 2018, and this market is projected to exceed $5.40 billion by 2029 (Delello et al., 2021; Statista, 2023). However, this rapid growth brings with it some uncertainties. In the definition of e-sports, there are still grey areas due to the lack of physical activity, which is evident, and the debate continues on whether e-sports is a sport or not (Summerley, 2020). Although the concept of sports is often associated with physical activities, it is also argued that e-sports should be expanded and considered as sports given that e-sports have components of modern sports (Giakoni-Ramírez et al., 2021). While these discussions are going on, adolescents who actively play digital games, which have become a part of their daily lives (Blumberg and Fisch, 2013), have started to take their place in the e-sports ecosystem. With the growth and spread of e-sports, e-sports players, especially in adolescence, face some health problems. In the e-sports process, many physical and mental health problems affect the health of adolescents, especially obesity, due to the time spent sedentary in front of the computer for a long time (DiFrancisco-Donoghue et al., 2022; Mustafaoğlu and Yasacı, 2018; Trotter et al., 2020). During the average daily five-and-a-half hours of e-sports period of e-sports players, posture in the gaming chair, prolonged screen exposure, and hundreds of repetitive movements are among the factors that contribute to the occurrence of these problems (Giakoni-Ramírez et al., 2021; DiFrancisco-Donoghue et al., 2022). In their study, Maras et al. (2015) concluded that adolescents who spend sedentary and long periods in front of a screen have increased symptoms of depression and anxiety. In their study, Bayrakdar et al. (2020) examined the effect of e-sports on physical activity level and body composition and concluded that as the time spent in e-sports increased, body mass index increased and the number of physical activity steps decreased. Straker et al. (2007) concluded in their study that sedentary, prolonged screen time causes posture disorders in adolescents.

E-sports, which is growing rapidly on a global scale, has created a source of employment for e-sports players who develop and demonstrate their skills in digital games, with the recognition that it has sports qualities, and has offered career opportunities to professional players supported by broadcasting platforms and organizations (Giakoni-Ramírez et al., 2022). Career potential in e-sports is developing thanks to the participation of e-sports players in professional-level organisations and the possibility of broadcasting on online platforms. However, the career opportunity alone is not enough to explain why e-sports players are turning to this field and why their motivation to play digital games is increasing day by day. For these reasons, it is important to examine the issue of e-sports and adolescents in detail. From this point of view, it is aimed to address and examine the issue of e-sports and adolescents

and to offer suggestions on the subject. In line with this purpose, firstly, the question "Is e-sports a sport?" was examined in detail, and then e-sports and its types, e-sports and digital game psychology, and the effects of digital games on adolescents were examined.

Is e-sports a sport?

To understand e-sports as a new phenomenon, the discovery of the motivations of adolescents and young adults to play digital games is a priority. However, another important issue is whether e-sports is a sport or not. The literal meaning of sport is explained as "all movements performed individually or collectively intending to develop the body or mind, applied according to some rules" (TDK, 2023). Modern sport is addressed in its physical, organized, competitive, and institutionalized dimensions (Pizzo et al., 2018). In the broadest sense, it is possible to define sports as an individual and mass systematic movement activity that is carried out in a certain discipline, has a functional activity relationship with many institutions with its organizational structures, has regulations, and is subjected to a competitive evaluation (Kazaz, 2007; Tomecka, 2017).

When we look at the definition of e-sports, it is defined in different ways in the literature just like sports. Wagner (2006) defined e-sports as a sports activity in which individuals develop and train their mental or physical abilities using information and communication technologies. Argan et al. (2006) explain e-sports as a sport that requires both physical and mental effort, where players can come together by connecting to a common platform from different parts of the world via the internet, play mutual games or participate in large e-sports organizations and play games with the players in this organization.

It is still an ongoing debate whether e-sports meets these criteria and whether it is included in the concept of sport (Hallmann and Giel, 2018; Jenny et al., 2017; Mustafaoğlu, 2018; Witkowski, 2012). The issue of whether e-sports, which has gained wide international acceptance, especially in recent years, can be considered a sport, is still an area with resistance and conceptual complexity. In fact, this conceptual confusion is a situation that applies not only to the concept of e-sports but also to the concept of sports. The main reason for this situation is the lack of physical activity due to the nature of e-sports (Bányai et al., 2019; Hallmann and Giel, 2018; Hemphill, 2005). According to this view, for a game to be considered a sport, the game must require physical skills or abilities, and the successful application of these physical skills must have a direct impact on the successful completion of the task (Jenny et al., 2017; Parry, 2019).

There are also opinions in the literature that e-sports should be considered as a sport. This is mainly because e-sports has many of the same components as traditional sports, such as players, teams, managers, leagues, competitions,

major events, financial agreements, player transfer fees, university scholarships, and match-fixing, doping, and gender disputes (Jenny et al., 2017; Newzoo, 2018; Pizzo et al. 2018). In addition, in modern sports, there are tournaments and leagues in various categories such as football, basketball, and volleyball. In e-sports, there are also tournaments and leagues of digital games. The opinions that e-sports do not include physical activity are shown as examples of sports that include low physical activities such as billiards, darts and chess. Before the concept of e-sports emerged, many branches from horseback riding to skiing, which have a low level of physical activity, were accepted as sports (Taylor, 2012). Just as other modern sports branches have national and international federations, the e-sports branch also has national and international federations. The International E-sports Federation was founded in 2008 and since its establishment, it has undertaken the promotion, dissemination, support and acceptance of e-sports as a sport. More than 130 countries, including Türkiye, that accept e-sports as a sport are members of the International E-sports Federation (IESF, 2023). In line with all this information, although e-sports does not explicitly include the physical activity that is the main feature of many traditional sports, it complies with all the sports criteria set by international standards of all other modern sports. Just because the level of physical activity is low, there is still debate about whether e-sports is a sport or not.

E-sports and Its Types

With the development of technology, cost-effective and fast technological tools have become more accessible to everyone in society. This process has led to changes in the activities that adolescents do in their free time. Gaming with technological means is gaining more and more popularity among adolescents (Nippold et al., 2005). Gaming with technological means is defined by concepts such as computer games, video games, amusement machine games and electronic games (Karaman Yaşar, 2019).

Today, games played with technological means are called digital games by researchers (Bianchi-Berthouze et al., 2007; Ijsselsteijn et al., 2007; Kiili, 2005). However, not all digital games are considered e-sports. E-sports is the practice of competing to beat an opponent that requires personal interaction within certain game rules. E-sports is also defined as a form of sport in which the basic elements of sports are facilitated through electronic systems (Hamari and Sjöblom, 2017).

To better understand e-sports, it is necessary to first explain the types of digital games. Although digital game genres are basically divided into seven categories as action, adventure, role-playing, simulation, strategy, sports, and board games, their number is increasing with sub-categories (Demiral, 2018; Dündar, 2015). With the advancement of technology and the increase in platform diversity, differentiation and increase in sub-genres are also observed.

Action games are one of the oldest genres of digital games and are usually based on a script. Action games are a type of game that emphasizes physical challenges that require hand-eye coordination, timing, reaction speed and precision, with intense gameplay that includes movement and speed. The e-sports player is in a physical and mental struggle against one or more opponents with physical actions. The player needs to complete a level, collect various rewards, overcome obstacles and escape from enemy attacks. War and violence content is common in this type of game. Games such as Crysis, Counter Strike, Call of Duty, Grand Theft Auto (GTA), Red Dead Redemption 2 can be given as examples of action games.

Adventure games usually have a story, in which the player is treated as the protagonist of the story and the game proceeds within the framework of this story. The player is located in an unknown, often ancient or fantastical virtual world, trying to solve various problems by investigating, making discoveries and collecting objects. Such games are usually story-driven puzzle-solving games. Game graphics are very important in this game. Although the fight is in place, this game focuses on narrative, scenario and puzzle solving. Players have to interact with other characters from time to time while solving puzzles. Usually, the scenarios of such games are fixed and are designed to force the player to progress through this fixed story of the game. However, some adventure games may also include elements that give a certain autonomy and dynamism to the characters and plot. Games such as Lost of Us, The Longest Journey, Indiana Jones, Myst and Riven are examples of this genre.

Role-playing games stand out for their features such as the fact that the game characters constantly raise their level and gain experience. It is generally divided into two subcategories. The first subcategory is action role-playing games with a specific story and ending. Another subcategory is the massively multiplayer online role-playing game, or MMORPG, which is usually defined as games that have no end and where the characters in the game constantly gain level and experience points. Games such as Dungeons and Dragons, Diablo, World of Warcraft, and League of Legends are the most well-known examples of this genre.

Simulation games are games that are shaped according to the decisions of the player. For example, it gives the player the freedom to decide between eating and having fun. Games often distract individuals from stress and calm them down. These games are divided into subcategories as vehicle simulation, life simulation and management simulation. For example, in the management simulation genre, players try to perform certain tasks, such as constructing a building or managing a business with a limited budget. Its goal is not to fail financially when performing the tasks given in the game. This type includes situations where real-life situations are gamified and transferred to the computer environment. The player can build a city, fly an aeroplane, or

become the manager of a company. Players take part in these games with goals such as building a developed city on a vacant lot and even trading with neighbouring settlements. The most popular games of this genre include The Sims, Tom Clancy's HAWX, Trauma Center, and SimCity.

Strategy games are games in which the player manages a war or a business by making strategic decisions. The player participates in the game only as a manager and gives orders. Such games are divided into two categories, real-time and turn-based. Real Time Strategy (RTS) is a war game usually played between two sides and involves elements such as obtaining resources, establishing bases, and developing technology. Real-time strategy games are a real-time subgenre of tactical wargames that mimic military tactics and combat conditions. In real-time strategy games where resources are limited, players need to use these resources before their opponents and use them in a balanced way. Turn-based strategy games (TBS) are strategy games where players can make moves in a limited time. Each player, in turn, submits a report of the events of the previous round. The most well-known examples of such games include Chess, Dune 2, Tycoon Series, Warcraft, Starcraft, Age of Empires, and Civilization.

Sports Games are a type of game that offers the possibility to play popular sports such as football, basketball, and tennis, as well as various sports such as car racing in a digital environment. These games digitally offer physical sports. Players sometimes take the field as athletes and sometimes manage teams as coaches. Sports games can be found in the form of packages that include many sports such as Olympic sports, and winter sports, as well as games that include a single sport. Games such as football and basketball are of particular interest. Games such as FIFA, NBA, Skate, Tennis, Championship Manager, PES are the most well-known examples of this genre.

Board Games, on the other hand, are a type of game that individuals from all age groups can play, where negative effects (aggression, weapons, sexuality, etc.) are almost absent. Such games offer players the possibility to play against artificial intelligence or each other. Players often prefer such games to fill their limited free time. Games such as Candy Crush, Toy Blast, Angry Birds are well-known examples of this genre.

E-sports and Digital Game Psychology

Newzoo (2020)'s 2020 report states that e-sports, which has a revenue of about one billion dollars and more than 450 million viewers worldwide, is not a whim, but a technological and cultural phenomenon. Likewise, according to the OFCOM (2023) report, nine out of ten children between the ages of 3 and 17 have been found to have played digital games. Revealing young people's motivations to play digital games can help address the reasons why e-sports is growing rapidly and inclusively. In his study, Yee (2005) examined

the motivations of players who played multiplayer online role-playing games. According to the study, players explained that their motivation to play digital games was to communicate with other players and listen to other people's conversations in the game, to make progress in the game, to try to overcome situations that they could not cope with in the game before, to be someone else for a few hours and to reduce stress, to test their real-world social theories in the virtual world. Williams et al. (2011) also examined the prevalence, practices, and identity formation of players playing role-playing games in a virtual game world. In the study, it was concluded that players use such games for creative outlets and socialization.

One of the motivations of adolescents to play digital games is to provide equality to the players in the virtual world (Cairns et al., 2021). The concept of equality plays an important role, especially in online games. Online games are environments where players can communicate, interact and compete on a virtual platform. In these games, the influence of external factors such as social, economic or geographical factors is minimised and the main difference of the players is their ability to play. In their study, Jonasson and Thiborg (2010) aimed to relate the sporting qualities of e-sports to the definition of sports. In this study, the players competing in the virtual world compete under the same conditions regardless of gender, appearance, form and/or functionality. Social or economic inequalities that exist in the real world are disappearing thanks to online games. In the gaming environment, each player competes on equal terms with others in a fair competition environment, which encourages adolescents to develop their skills and provides an opportunity to compare themselves with other players. While digital games offer players a space of freedom, they also create an environment where everyone is equal in a democratic context. In the game, players cannot exercise their real-world economic power or status over each other, the rules of the game apply to everyone (Ulusal and Umunç, 2021). In conclusion, one reason for the interest in online games among adolescents may be that these games embrace the principle of equality and allow players to differentiate themselves only by their skills. This can allow adolescents to discover their talents, compete, and form social bonds.

In another study, adolescents' motivations to play digital games were explained by eight themes. According to them, games are evaluated in various important aspects such as bringing people together, making them relax, developing their skills, having artistic experiences, playing for entertainment purposes, becoming a lifestyle, being a universal activity and providing equality to all players. These themes are described below:

- **Connection:** Games have the potential to bring people together and build community. Games can be seen as a means of connecting friends and family.

- **Orientation:** Games can function as a kind of escape and direction by providing the opportunity to get away from daily stresses and engage the mind. Players can step into different worlds through games, allowing them to relax and reorient themselves.

- **Useful:** Gaming can offer players benefits such as developing skills or acquiring new knowledge that can benefit the outside world. Games can contribute to the self-development of players by providing skills such as problem-solving, strategy development, and collaboration, as well as exploration and learning experiences.

- **Art:** Games are recognized as a form of creative expression for both the developer and the players. In addition to expressing themselves in games, players can have valuable experiences in terms of art and storytelling.

- **Entertainment:** Games are considered an activity to be enjoyed. Players have the opportunity to relieve stress, engage mentally and have a good time by playing games for entertainment purposes.

- **A way of life:** For some players, games are becoming a way of life, not just a leisure activity. Gaming is part of their daily routine and an activity they are constantly interested in.

- **Universal:** Games are seen as a universal event that offers something for everyone. Regardless of language, culture or other differences, it can bring people together and bring them together on a common ground.

- **Enabling people with disabilities:** Games have the potential to provide equal opportunities to individuals with disabilities. Games that can overcome disability-related physical or mental barriers allow players with disabilities to compete or interact with other players on an equal level (Cairns et al., 2021).

As can be seen in the themes described above, games are recognised as elements that have the potential to offer experiences to people, not just games, by offering elements that people need, such as building communities, equality and interaction.

The Impact of Digital Games on Adolescents

Digital games are in our lives today as entertainment tools that appeal to a wide audience and their popularity is rapidly increasing. While digital games offer interactive experiences to players, they can also have some negative impacts. Digital games, which are widely played especially among children and young people, have the potential for both positive and negative effects ranging from physical health problems to psychosocial effects.

In society in general, it is commonplace for digital games to be seen as the antithesis of sports. In fact, the view that digital games cause digital game

addiction is widely accepted. The American Psychiatric Association (APA) (2023) made a statement by preparing a report on this situation and stated that there is an important distinction between players who play digital games passionately (enthusiastic and focused on the game) and people who are pathologically addicted to digital games, and they accepted e-sports players as individuals who show passionate gaming behaviour. Therefore, digital game addiction is not mentioned in this report.

Many studies in the literature have repeatedly shown that the time spent sedentary in front of the screen playing digital games triggers obesity. As a solution to this situation, it has been proposed to encourage players to engage in regular physical activity and limit digital play hours to maintain their ideal weight (Ballard et al., 2009; Gülü et al. 2023; Vandewater et al. 2004). In their study, Mustafaoğlu and Yasacı (2018) investigated the negative effects of digital gaming on the mental and physical health of children. As a result of the study, playing digital games in children can lead to mental problems such as anxiety, aggressive attitudes and depression, musculoskeletal problems, eye health problems such as dry eye, pain and redness, and physical health problems such as deterioration in sleep quality.

Another common view is the issue of digital games and violence. The view that violent games can increase aggression, especially in adolescent players, has been addressed in many studies. In their study, Adachi and Willoughby (2011) examined the effect of digital games, violence, and competitiveness on aggressive behaviour. In the study, they concluded that the competitiveness of the games affects aggressive behaviour rather than the violent elements in the context of digital games. Özmen and Aktaş (2019) investigated the effect of digital game playing on the level of aggression in their study and concluded that the aggression levels of children who play digital games are higher than those who do not play. It is also emphasized that the time children spend on digital games adversely affects social skills and group cohesion and causes physical fatigue (Toran et al., 2016). Horzum (2011) stated in his study that children who spend time on digital games for a long time cannot quit the game, cannot recognize the difference between games and real life, and do not want to take on the responsibilities they have to fulfil.

Along with these studies, some studies reveal the positive effects of digital games on young people. As a result of their study, Petri et al. (2018) stated that digital games contribute to the provision of pleasure, happiness and attention in the learning environment and encourage cooperation and sharing of ideas among students. Digital games incentivise players with motivational elements such as rewards, levelling up and leaderboards. This increases the motivation in the learning process and increases the interest. Çakır (2013) stated that digital games affect children's mental processes, and support stress coping skills and concentration. Yalçın Irmak and Erdoğan (2016) stated in their study

that educational and developer digital games make a positive contribution to child development if they are played within appropriate time limits and in a controlled manner.

As a result, in line with all these studies, digital games, which are the most basic component of e-sports, have both positive and negative effects on children and young people. In general, when the potential negative aspects of digital games are considered, long-term gaming can lead to physical health problems, reduce social interaction, and social isolation, and increase violence and aggressive behaviour. On the other hand, when the potential positive aspects of digital games are considered, they can contribute to the learning process, support cognitive processes, cooperation, communication and teamwork, and increase motivation and interest (Rasdi and Rusli, 2021).

A field that has gained popularity among the rapidly growing masses in recent years is also seen that e-sports is considered a career opportunity. With the rapid growth of e-sports, there is an increase in the number of e-sports players and it is becoming a career option in e-sports, especially among adolescents (Kocadağ, 2019). There is still not enough information in the literature about career development and advancement in e-sports. How players get involved in e-sports events, their journey to becoming a professional, and the challenges and opportunities they face in the process of career development are still not fully known (Meng-Lewis et al., 2022).

Although the career duration differs depending on the type of game in e-sports, the career duration of e-sports players, who can reach the professional level between the ages of 13-15, usually varies between 10 and 15 years. The fact that e-sports players who take part in tournaments organised around the world, especially with the acting and broadcasting revenues of e-sports, are between the ages of 16 and 28 can contribute to young people's career orientation in e-sports (Kocadağ, 2017). The career potential of e-sports has become a major source of attraction for young people. Some foundation universities now support talented players with e-sports scholarship programmes and encourage participation in competitive leagues at national and international levels. This situation enables young people to consider e-sports as a career and increases potential job opportunities in the future.

Broadcasting, which is a part of the e-sports industry, also constitutes a separate career field for e-sports players. On live streaming platforms such as YouTube and Twitch, players can share their games with viewers live, viewers can donate to players, subscribe to their broadcasts or interact with sponsored content. All of this brings revenue to e-sports players. Broadcasters can also collaborate with brands, even if they have very low viewership, to create advertising deals and commercial opportunities such as product placement.

Conclusion and Discussion

As a result, e-sports is accepted as a sport that has a federation, is supported and growing in many countries today. Although there is still debate around the world about whether e-sports is a sport or not, e-sports is growing its ecosystem day by day and increasing its popularity among adolescents.

When the negative aspects of e-sports are considered, many physical and mental health problems affecting the health of adolescents, especially obesity, are encountered due to the long periods of inactivity spent in front of the computer. E-sports components must take precautions in this regard because e-sports clubs, federations and sponsors need to support e-sports players to do sports healthily. Developing healthy lifestyle habits for e-sports players to prevent and manage health problems is not a matter to be left only to the initiative of e-sports players. E-sports players need to do physical activities such as exercising regularly, paying attention to sleep patterns, maintaining the correct posture and moving with frequent breaks, especially during the e-sports process. As a result, it is possible to minimise the negative impact of e-sports by taking precautions against potential physical and mental health problems and encouraging players to adopt healthy lifestyle habits.

When the positive aspects are considered, it is concluded that digital games contribute to the provision of pleasure, happiness and attention in the learning environment and encourage cooperation and idea-sharing among students. The e-sports process must be carried out healthily so that digital games are played in a balanced way and that young people develop healthy gaming habits.

One of the benefits of e-sports' growing ecosystem is its career potential. With the increase in the reputation of e-sports in the academic and sports world, career opportunities will also diversify. The e-sports ecosystem is growing day by day and constantly innovating and providing e-sports players with innovations and opportunities. That's why e-sports players need to improve themselves. It is also important to create games that will develop important skills such as discipline, strategy, teamwork and leadership.

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Chapter 3

BIOLOGICAL ACTIVITY OF COUMARIN COMPOUNDS

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In today's world, where technological developments continue unabated, although human beings are working to establish living spaces on new planets, there are still some areas where science, such as diseases, is insufficient. Even in this century, human beings are helpless in the face of death due to factors such as infectious diseases and cancer. Today, one of the most important public health problems in the medical sense is infectious diseases caused by microorganisms. Despite promising developments in the field of medicine, infectious diseases originating from microorganisms continue to be seen. As a result of cross-resistance caused by excessive use of antibiotics, bacteria become immune to existing antibiotics, the antibiotic types used in the clinic cannot be effective, the death rate from infectious diseases increases, and human beings succumb to this genetic compatibility of microorganisms. For this reason, antimicrobial agents that will be effective on microorganisms continue to be developed in order to fight infections [1]. Today, the medical world, faced with the loss of effectiveness of existing antibiotics as a result of antibiotic resistance, has accelerated the process of developing new antibiotic drugs. In this context, research has begun on many substances of natural and synthetic origin, which have the potential to create a source. It is thought that coumarin derivative compounds, which have multiple positive effects in studies, may be effective in this context and are worth studying.

Coumarins (2H-chromen-2-one, 2H-1-benzopyran-2-one) are odorless, colorless, crystalline heterocyclic organic compounds from the class of benzopyrans. They are formed as a result of condensation of the pyron ring with the benzene ring. Coumarin is an important structural component of various natural and synthetic products with important biological activities such as anticoagulant, antifungal, antibacterial, spasmolytic or stotoxic activity [2, 3], figure 1. Coumarin derivatives are widely found in nature. Coumarins are compounds of the isoflavonoid class and are found in citrus fruits, vegetables, many legumes and orchids. Coumarin and its derivatives are used in the preparation of food, perfume, pesticides and optical brighteners, fluorescence emission, laser dyes, optoelectronic materials etc. They use it in a very wide area. In addition, these compounds are natural products with pharmacological activity that can be used in the treatment of brucellosis, burns, rheumatic diseases and some types of cancer. Therefore, the synthesis of coumarin and its derivatives is of great interest [4, 5].

Coumarin, especially tonka bean (Dipteryx odorata), vanilla plant (Anthoxanthum odoratum), fragrant sticky grass (Galium odoratum), mullein (velvet plant) (Verbascum), sugar plant (Hierochloe odorata), cassia (Cinnamomum cassia), sweet clover (Melilotus ssp.) and deer tongue (Dichanthelium clandestinum) are found naturally in high concentrations in most plants.

It has a wide range of uses such as food additives, fragrances, pharmaceuticals and pesticides. It has a sweet scent, considered the scent of freshly mown

grass, and has been used in perfumes since 1882. Although generally banned as a fragrance and flavoring food additive due to concerns about its

hepatotoxicity in animal models, coumarin is used as a flavor enhancer in some alcoholic beverages [6].

Coumarin and its derivatives, one of the pharmacologically important skeletons; are compounds with remarkable biological activity against fungi, tumors, viruses, and particularly HIV protease. Although coumarin itself has non-anticoagulant properties, it is converted to the natural anticoagulant dicoumarol by some types of fungi. This occurs as a result of the production (in the presence of naturally occurring formaldehyde) of 4-hydroxycoumarin, a fermentation product and the mycotoxin anticoagulant dicumarol. This substance is the cause of the bleeding disease (historically known as the sweet clover disaster) in cattle that eat moldy sweet clover silage. One of the most important applications of coumarin derivatives is their use in anticoagulant drugs. They are also used as anti-inflammatory, analgesic, antioxidant. Anticoagulant drugs are drugs that prevent coagulation at various stages. Coumarins are used in the pharmaceutical field as a messenger molecule in the synthesis of anticoagulant drugs such as synthetic warfarin (brand name Coumadin). Because of these features, they have a wide range of use in vascular diseases [7, 8].

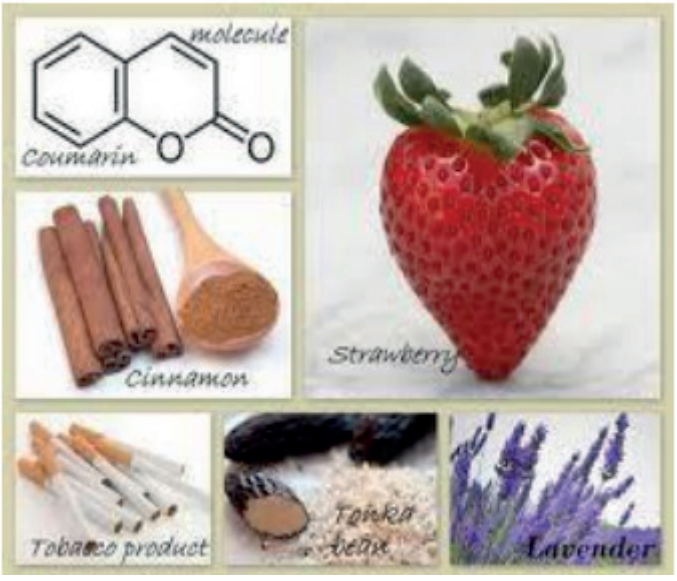

Figure 1. *Families of plants containing coumarin [3]*

Pharmaceutical coumarins were all discovered during studies of sweet clover disease. Coumarin has clinical medicinal value on its own as an edema reliever. Coumarin and 5, 6-benzopyrone, 1, 2-benzopyrone, diosmin etc. It is known that benzopyrones such as benzopyrones cause edematous fluids to dissolve faster and stimulate macrophages to decrease extracellular albumin [7, 8]. Coumarin is also used as a gain tool in dye lasers [9-11] and as a sensitizer in older photovoltaic technologies [12].

Absorption, Distribution and Metabolism of Coumarin Compounds in Humans

The absorption, distribution, metabolism and excretion of coumarin compounds in humans have been the subject of studies for many years. Toxicokinetic studies in humans have shown that coumarin is rapidly absorbed from the gastrointestinal tract after oral administration and is extensively metabolized by the liver in the first pass, with only 2-6% reaching the systemic circulation intact. Removal of coumarin compounds from the systemic circulation is rapid, with half-lives of 1.82, 1.46 and 1.49 seconds following intravenous doses of 0.125, 0.2 and 0.25 mg/kg bw. Coumarin compounds have also been found to be extensively absorbed after dermal application. In a study with human subjects, 60% of the 2.0 mg drug dose administered over 6 hours was observed to be absorbed [13].

Percutaneous absorption of coumarin has been shown to occur *in-vitro* in human skin. The presence of little or no coumarin metabolites in the urine and bile of human subjects given orally administered coumarin compounds suggests that coumarin compounds are rapidly excreted as 7-hydroxycoumarin conjugates [14]. There are marked species-specific differences in coumarin metabolism [15]. Metabolic pathways of coumarins in humans and animals are given in Figure 2 [13]. The main primary pathways of coumarin metabolism are 7-hydroxylation or ring opening of the lactone ring and carbon atom cleavage metabolism by donating carbon atoms.

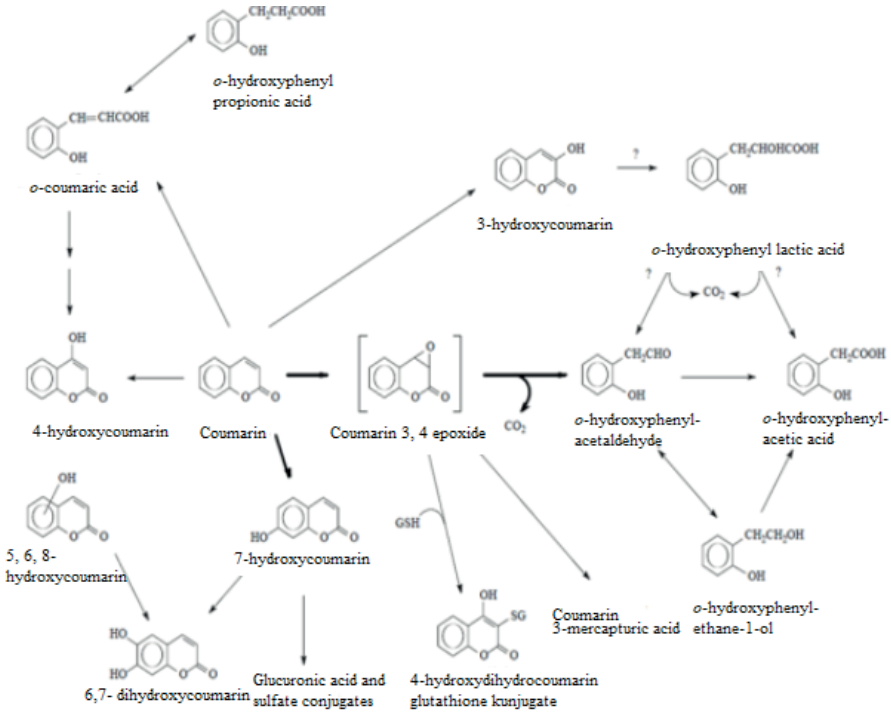

Figure 2. *Metabolic pathways of coumarin in humans and animals [13]*

Toxic Effects of Coumarin Compounds

Although various mild side effects have been reported following coumarin therapy, changes in liver function were noted in only a small proportion of patients. Toxicity reports are rare [16]. Trials with patients with cancer or chronic infection showed that only eight (0.37%) patients who received coumarin 100 mg daily followed by 50 mg daily for two years developed increased serum aminotransferase [17].

Reported an increase in serum alanine aminotransferase activity in two lymphedema patients given 90 mg of coumarin daily for five months. [18]. He reported a case of toxic hepatitis in a patient given daily coumarin for eight weeks. This condition is characterized by hepatomegaly and elevated serum enzyme levels. All signs of liver toxicity returned to normal upon discontinuation of therapy. Coumarin is non-toxic to *in-vitro* mononuclear human peripheral blood cells at concentrations up to 100 μg/mL. In another study, it inhibited cell proliferation in various human tumor cell lines after 48 hours of incubation [19].

Anti-inflammatory, Antibacterial and Anticancer Activities of Coumarin Compounds

Coumarin compounds exhibit anti-inflammatory properties and are used in the treatment of edema with this feature. This occurs by stimulating phagocytosis, enzyme production, and thus proteolysis, thereby clearing proteins and edema fluid from the injured tissue [20]. Esculetin was isolated from *Cichorium intybus* [21] and *Bougainvillea spectabilis* Wild *(Nyctaginaceae*). It exhibited anti-inflammatory activity in rat colitis induced by trinitrobenzenesulfonic acid [22, 23]. Coumarin itself has very low antibacterial activity, but against broad-spectrum Gram (+) bacteria such as *Staphylococcus aureus*, *Bacillus megaterium, Micrococcus luteus, Micrococcus lysodeicticus*, long-chain such as Ammoresinol, Andostruthin. Anthogenol.

Structurally similar to Novobiocin, Cojermycin is effective against *Escherichia coli* and *Staphylococcus aureus*, but about 50 times more potent. Coumermycin also inhibits DNA superhelix formation catalyzed by DNA gyrase in *Escherichia coli* [24]. Chartreusin has been isolated from *Streptomyces chartreusis* and has a rare nature. It is predominantly active against Gram (+) bacteria, but due to its toxicity, the compound has not been tested for therapeutic application [25].

Anticancer drugs have traditionally been targeted to damage cells that divide abnormally by interrupting the cell division process. Reagents used include DNA intercalating agents (eg Adriamycin), DNA crosslinking agents (eg Cisplatin), topoisomerase inhibitors (eg Campothecins), cytoskeletondisrupting agents (eg Vinblastine), and antimetabolites (eg mercaptopurine). These drugs are cytotoxic and therefore show serious side effects, especially in normally proliferating tissues such as the hematopoietic system. In most combination applications, several cytotoxic agents are combined in the treatment regimen and offer better results with fewer toxic side effects as they are carefully engineered to allow the recovery of non-malignant cells after drug exposure. Imperatorin, a coumarin compound, has exhibited anticancer effects.

Recent studies have investigated the efficacy of coumarin/Troxerutin combination therapy in protecting salivary glands and mucosa in patients undergoing head and neck radiotherapy. The results suggest that the combination of Coumarin / Troxerutin has a positive effect in the treatment of radiogenic sialadentis and mucositis. Interest in coumarin and 7-hydroxycoumarin as anti-cancer agents arose from reports of these agents having objective responses in some patients with advanced malignancies.

Antibiotics and Antibiotic Resistance Mechanisms

Substances that are formed by fungi and some microorganisms or that can be synthesized by chemical means, stop the development of other types

of microorganisms and kill those microorganisms at the same time are called antibiotics. Antibiotics are antimicrobial agents that are active against bacteria and are the most important type of antibacterial agent used to combat bacterial infections. Antibiotics can inhibit the growth of bacteria or kill the bacteria completely. A limited number of antibiotics also have antiprotozoal activity [26]. In conventional medical use, antibiotics can be produced naturally (to fight a microorganism such as penicillin), while non-antibiotic antibacterials (such as sulfonamides and antiseptics) are purely synthetic.

However, both classes have the same goal of preventing or killing the growth of microorganisms. Both varieties are involved in antimicrobial applications. There is evidence of antibiotic use since ancient times. Many ancient civilizations, such as the Egyptian, Chinese and Roman civilizations, used moldy bread to prevent and treat diseases, citing its positive effects. John Parkinson was the first to directly document the use of mold to treat infections. Alexander Fleming discovered modern penicillin in 1928 [27]. Antibiotics are generally classified according to their mechanism of action, chemical structure or spectrum of activity. Most target bacterial functions or growth processes [28].

Protein synthesis inhibitors (Macrolides, Lincosamides and Tetracyclines are generally bacteriostatic except for bactericidal aminoglycosides [29]. The mechanisms of action of antibiotics are given in Figure 3 [30]. With advances in medicinal chemistry, most modern antibacterials are semi-synthetic modifications of various natural compounds. These include beta-lactam antibiotics, which include penicillins, cephalosporins, and carbapenems. Compounds currently isolated from living organisms are aminoglycosides. Sulfonamides, quinolones and oxazolidinones are produced only by chemical synthesis [31].

The emergence of bacterial resistance to antibacterials is a common phenomenon. The emergence of resistance often reflects evolutionary processes that occur during antibiotic therapy. There are several molecular mechanisms of antibacterial resistance. Intrinsic antibacterial resistance may be part of the genetic makeup of bacterial strains [32]. For example, an antibiotic target may not be present in the bacterial genome. Acquired resistance is due to a mutation in the bacterial chromosome or the acquisition of extra chromosomal DNA. Bacteria producing antibacterial agent, resistance shown to be similar to and transferred to antibacterials resistant strains developed mechanisms [33].

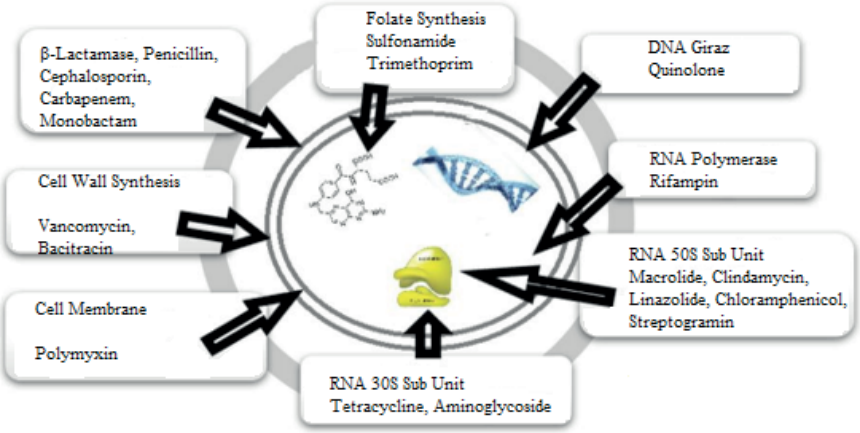

Figure 3. *Mechanisms of action of antibiotics [30]*

Antifungal Drugs and Antifungal Resistance

Antifungal therapy is the mainstay of treatment for acute and chronic mycoses. However, treatment options are limited due to the lack of antifungal drug classes against the variety of fungal species that cause infection. Fungal infections in general can occur, although less threatening superficial infections such as vaginal candidosis or oral candidiasis may occur. It also causes serious chronic diseases such as infections, cryptococcosis, aspergillosis, allergic bronchopulmonary aspergillosis or life-threatening acute diseases such as these.

Most invasive fungal infections occur as a result of immunosuppression. Recognizing the importance of fungal infections has increased the use of antifungal agents in the treatment and prevention of infection. Antifungal drug classes used in current antifungal therapies; pollens, azoles, allylamines, flucytosine and echinocandins. Azoles and allylamines inhibit ergosterol biosynthesis, while pollen binds to ergosterol in the plasma membrane. Flucytosine inhibits pyrimidine metabolism and DNA synthesis. Echinocandins are cell wall active agents that inhibit the biosynthesis of the fungal cell wall. According to their chemical structure (Table 1), according to their mechanism of action (Table 2), according to the patient group for which they are used (Table 3) [34].

Table 1. *Antifungal drugs according to chemical structures*

Polyenes	Amphotericin B, Nystatin, Endomycin, Griseofulvin
Azole derivatives (Imidazoles-Triazoles	Ketoconazole, Miconazole, Fluconazole, Itraconazole, Tioconazole, Bifonazole, Oxiconazole, Clotrimazole, Isoconazole, Econozole, Sulconazole, Buconazole, Variconazole, Fenticonazole
Allylamine	Terbinafine, Naftifine
Pyrimidine derivatives	5-Fluorocytosine
Echinocandins	Caspofungin, Micafungin

Table 2. *Antifungal drugs by Mechanism of Action*

Mechanism of Action	Medicines
Those that inhibit nucleic acid synthesis	Fluorocytosine
Influencing nuclear division. It acts through the inhibition of fungal mitosis.	Griseofulvin
They cause the death of fungal cells by binding to ergosterol, which is the main sterol of the fungal cell membrane.	Polyenes
They act by inhibiting ergosterol biosynthesis.	Allylamines
By inhibiting the synthesis of Ergosterol from lanosterol, they stop the reproduction of the fungal cell.	Imidazole and Triazoles

Table 3. *Antifungal drugs according to the patient group used*

Type of treatment	Medicines
In local treatment	Amphotericin B, Nystatin, Griseofulvin, Pimarisin, Bifonazole, Oxiconazole, Clotrimazole, Isoconazole, Econozole, Fenticonazole, Terbinafine, Naftifine, Griseofulvin
In systematic treatment	Ketoconazole, Fluconazole, Itraconazole, Variconazole, Flusizotin, Echinocandins, Miconazole

Fungi, like human cells, are eukaryotic. Therefore, it is difficult to find selective toxic agents for fungal cells. For this reason, fungal infections are more difficult to treat. Antifungal drugs have been developed that inhibit yeast cells without harming human cells. Antifungal drugs generally show their effects by inhibiting cell wall, ergosterol and nucleic acid synthesis and microtubule formation. Mechanisms of antifungal drugs Table 4 and it is given in Figure 4 [35].

Table 4. *Mechanisms of action of antifungal drugs*

Antifungal agents	Mechanism of action
Polyenes (Nystatin, Amphotericin B, AB Lipid complex (ABC), Liposomal AB	It increases cell wall permeability by binding to fungal cell wall ergosterol. In particular, the loss of intracellular K^+ causes the cell to lose its viability.
Azoles (Ketoconazole, Fluconazole, Itraconazole, Variconazole	Lanosterol 14 α demethylase inhibition, 24 methylene dihydro lanosterol demethylac inhibition
Allylamine and thiocarbamates (Terbinafine, Naftifine, tolnaftate) Morpholine, Amorolfine Pyrimidine (Flucisotin) Echinocandins Polyoxins (Polyoxin A, Polyoxin B)	Inhibition of oxidosqualene cyclase Sterol 14 reductase and 7-8 isomerase Inhibition It degrades the structure of RNA by turning into 5-fluorouracil, and DNA by turning into thymine. 1-3 beta D-glucan synthetase inhibitor Inhibitor of chitin synthesis in the cell wall

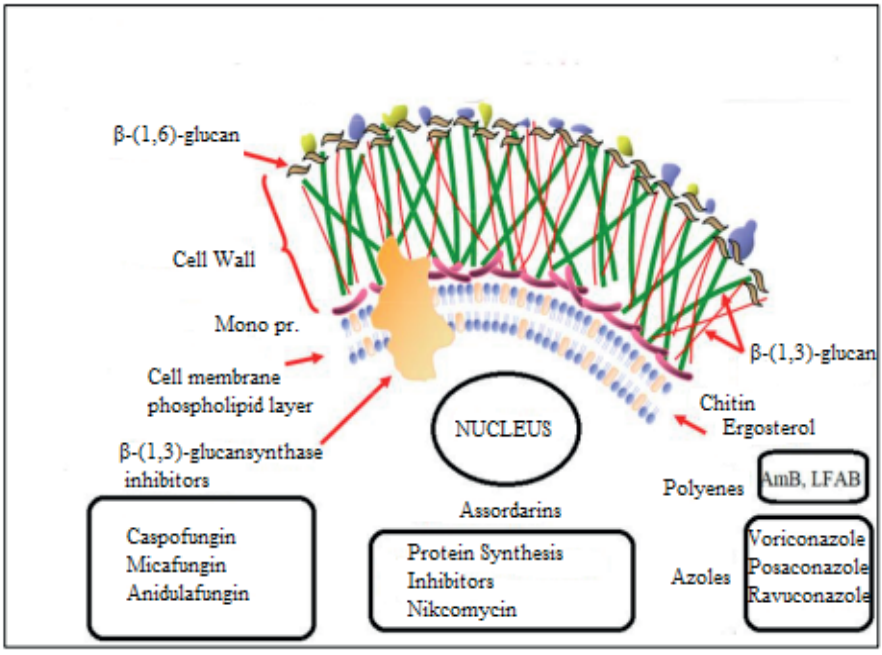

Figure 4. *Mechanisms of action of antifungal drugs*

Due to the increase in fungal infections, the widespread use of antifungal agents causes the emergence of resistant strains to these agents. The most frequently used antifungal drug classes today are polyenes, azoles, allylamines, 5-flucytosine, and echinocandins. Allylamines such as terbinafine target ergosterol biosynthesis. Polyenes such as amphotericin B bind to ergosterol in

the plasma membrane and form pores. Thus, they impair the membrane permeability. 5-Flucytosine inhibits pyrimidine metabolism and DNA synthesis. Echinocandins, on the other hand, inhibit β-1,3 glucan synthesis, thereby disrupting the cell wall structure [36]. The clinical treatment of fungal infectious diseases is hampered by the emergence of antifungal resistance, which counteracts the effects of existing drug classes. In this context, antifungal drug resistance can cause many risky situations.

The general molecular mechanisms of antifungal resistance are altered drug affinity and target abundance, reduced intracellular drug levels caused by efflux pumps, and biofilm formation. Most cases of antifungal resistance result from the evolution of resistant organisms that resist several different classes of antifungal agents, particularly among *Candida* species. *Candida* species develop resistance to antifungal drugs by preventing the accumulation of antifungal agents in the cell, creating changes in the structure and amount of proteins that are the binding target of antifungal agents, and conforming to sterol compounds [37]. In addition, when looking at antifungal resistance at the molecular level, the two most common mechanisms are increasing the expression of MDR and CDR genes, which are responsible for the formation of pumps responsible for excretion of substances from the cell, and point mutation in the gene encoding the ERG11 enzyme, which is the target compound of antifungal drugs [38].

Cancer

Cancer is another important public health problem that threatens human life today. Apart from chemotherapy, radiotherapy and surgical intervention, one of the methods that can be effective without reducing the quality of life of patients is the use of anticancer drugs. Compounds of various structures are constantly being investigated by researchers as anticancer agents. Most of the anticancer drugs have cytotoxic effects and prevent the development of cancer cells and cause their death. Effective treatment is possible by killing all cancerous cells in the body. However, such a treatment is unfortunately not available with drugs currently on the market [39]. Therefore, more research should be done to develop anticancer drugs that can be so effective. Coumarin compounds show promise in this field. Coumarins are a class of phenolic substances found in plants, which can be of natural or synthetic origin.

Studies have shown that coumarin compounds are effective in the medical field. Coumarin compounds are used in the treatment of edema because they exhibit anti-inflammatory properties. This occurs by stimulating phagocytosis, enzyme production and thus proteolysis, removing protein and edema fluid from the injured tissue. However, studies have shown that coumarin compounds have antibacterial, antifungal, antiviral, antiparasitic, anticancer, antigenotoxic, antioxidant, antihypertensive, anticoagulant, antihyperglyce-

mic, anticonvulsant, antitubercular and neuroprotective activities.

Cancer is used to describe forms of tissue proliferation characterized by uncontrolled growth and division of cells, leading to the formation of malignant tumors [40]. Cancer occurs as a result of failure in the regulation of essential tissue growth. To transform a normal cell into a cancer cell, genes that regulate cell growth and differentiation must be mutated, overexpressed or underexpressed [41]. Changes in more than one gene together or sequentially are sufficient for a normal cell to become cancerous [42]. Differentiation of the cell as a result of faulty DNA replication during cell division is also among the causes of cancer. In order for mutated cells to be cancerous, they must divide uncontrollably, grow, invade neighboring tissues, and have the feature of metastasis. Tumors that do not show the ability to metastasize are called benign tumors [43]. When cancer formation is examined at the molecular level, it has been observed that mutations that cause cancer occur as a result of mutations in some vital features of normal cells.

Cancer-causing mutagenic events occur as a result of failure in the regulation of basic tissue growth. To transform a normal cell into a cancer cell, genes that regulate cell growth and differentiation must be mutated. The underlying cause of malignancy is the over expression of oncogenes that provoke tumor formation or the deactivation of tumor suppressor genes. Errors in the regulation of the cell cycle also lead to the deterioration of the control of cell division and thus to the formation of cancer. The cell cycle is controlled by cyclin-dependent kinases (cdk, catalytic subunit) and cyclin (cyc, regulatory subunit). Cell cycle checkpoints are given in Figure 5 [44]. At cell cycle checkpoints, cells that are found to be damaged are usually transferred to the apaoptptic pathway without forming tumors and are eliminated in a programmed manner by the apaoptosis mechanism. In this context, the main tumor suppressor function in cancer development is the flawless execution of the critical pathways of DNA repair and apoptosis [45].

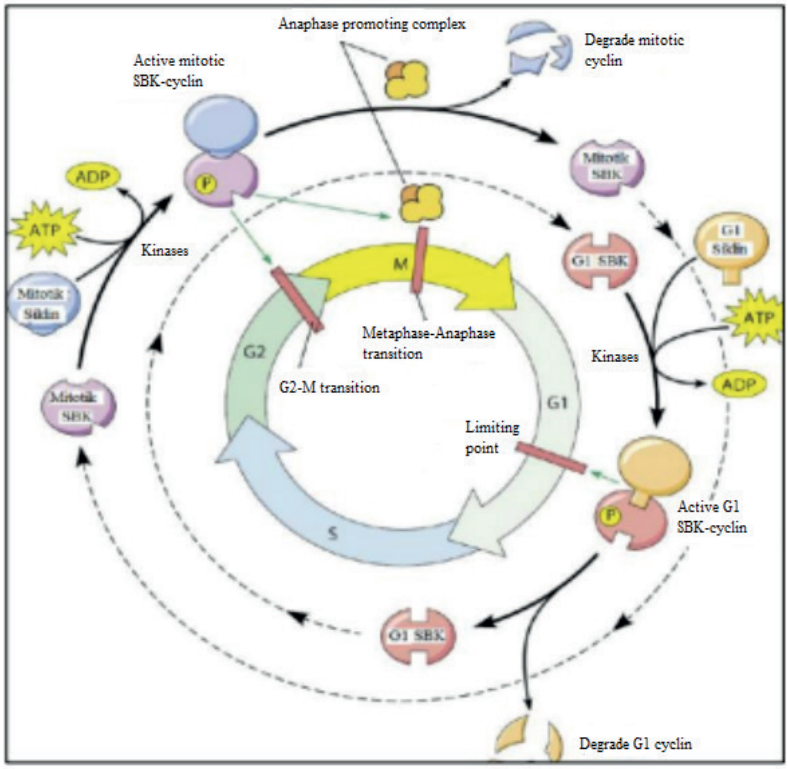

Figure 5. *Cell cycle checkpoints [44].*

A number of physiological and pathological stimuli can initiate apoptotic processes, including nutrient deficiency, activation of cell surface death receptors, chemicals, ionizing radiation, and direct physical injury. These stimuli initiate apoptosis by activating different pathways that lead to apoptosis. At the beginning of apoptosis, a family of proteins known as *Caspase* is first activated. These proteins are able to degrade important cellular components necessary for normal cellular function, including structural proteins within the cytoskeleton and nuclear proteins such as DNA repair enzymes. *Caspases* also have the ability to activate other enzymes such as DNases. Cells undergoing apoptosis show significant morphological differences in this process. First, the cell begins to contract following division of the laminin and actin filaments. Then the fragmentation of the chromatin in the nucleus begins to occur. The fragmentation of chromatin in the nucleus causes nuclear condensation and a horseshoe-like appearance of the apoptotic cell nucleus. In the final stage of apoptosis, the appearance of bubbles in the cell membrane and the formation of small vesicles called apoptotic bodies can be observed. The mechanism of apoptosis is as in Figure 6 [46].

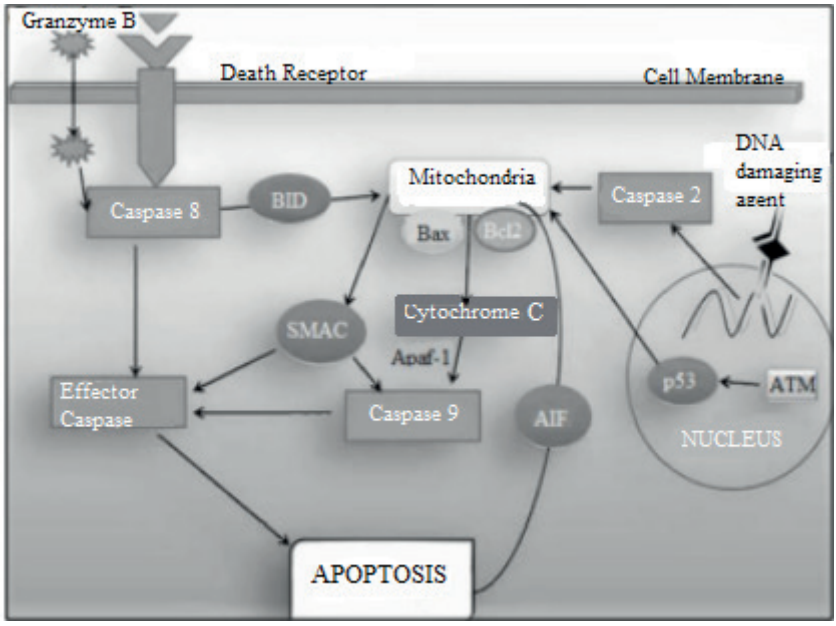

Figure 6. *Apoptosis mechanism [46].*

Diet, physical inactivity, and obesity are associated with appro ximately 30-35% of cancer deaths. It is thought that physical inactivity contributes to the formation of cancer not only with its effect on body weight, but also with its negative effects on the immune system and endocrine system [47]. It is a common feature of all cancers that mutations alter the expression of cancer-preventing tumor suppressor genes. In this context, cancer-related genomic changes range from rearrangements, duplications, and deletions of chromosomes, particularly the displacement of a single nucleotide [48]. Viral factors also have an effect on cancer formation. Liver and cervical cancers are among the leading human cancers caused by viruses. The most important viruses that cause cancer in humans; papilloma viruses (HPV16 and 18), human T cell leukemia virus (HTLV-1), *Hepatitis B* virus and *Epstein-Barr* virus.

Since many viruses use the host cell's necessary enzymes for DNA synthesis, they require cells in the active and growth phase. These viruses usually have genes that synthesize cell cycle-stimulating products. If the virus does not kill the cell, loss of control of the cell cycle occurs and tumor formation begins [49]. Bacterial and parasitic infections can also cause cancer. Bacterial infection caused by Helicobacter pylori may also increase the risk of gastric carcinoma [50] Parasitic infections closely related to cancer include Skistosoma haematobium, which is the cause of squamous cell carcinoma in the bladder, and *Opisthorchis viverrini* and *Clonorchis sinensis*, which is the cause of cholangiocarcinoma in the liver [51] Hormones, mainly estrogens. They are

important tumor stimulators in the development of human cancers. For example, estrogen stimulates the proliferation of endometrial cells in the uterus.

Excess estrogen increases the likelihood of developing endometrial cancer in women. As a result of many carcinogenic peripheral effects, mutations occur in DNA, DNA repair mechanisms, cell signal transmission, cell cycle regulation, apoptosis mechanism, proto-oncogene and tumor suppressor genes in normal cells, and as a result, cancerization is observed as a result of these effects. The abnormal proteins produced by the mutated cells in the body and the abnormal functioning of the receptors on the membrane of these cells are noticed by the immune system elements and these types of cells with mutant characteristics are eliminated. Therefore, very few of the mutant cells formed are likely to turn into cancer. If the immune system works properly and completely in a normal person, the probability of getting cancer decreases. On the contrary, the risk of cancer is considerably increased in people with diseases that cause immune system failure and in patients receiving immunosuppressive therapy [52].

CONCLUSION

The most important application and research area of coumarin derivatives is the study of their biological effects. Today, many natural and synthetic coumarin derivatives are being investigated with interest. Some of them are used clinically, and some are in the advanced testing stages. The best known biological effect of coumarin derivatives is that they act as anti-coagulants, and their anti-HIV, anti-microbial, enzyme inhibition and anti-cancer effects are also well known. In addition to these properties, it is known that coumarin derivatives have activities such as antidepressant, fat-reducing, anti-inflammatory, anti-oxidant, anticonvulsant, anti-virus, anti-Alzheimer's in various biological fields, and research continues.

Despite the advances in medicine in our age, diseases caused by microbial infections are quite high. For this reason, the synthesis of compounds that can be used as antibiotics, with very high anti-microbial properties and at the same time without side effects, becomes very important. The most researched biological properties of coumarin derivatives in recent years are their anti-microbial and anti-cancer properties.

Considering the results obtained in the studies conducted in the light of all these data, it is thought that the coumarin compounds to be synthesized can be a source in the production of new antimicrobial and antiviral drugs. It is thought that the data to be obtained as a result of this study will contribute to the design of antimicrobial and anticancer drugs that can be used in the treatment of various infectious diseases and cancer.

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Chapter 4

ROLE OF OVIDUCT ENVIRONMENT IN REPRODUCTION

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1. Introduction

In recent years, although great progress has been recorded in assisted reproductive technologies such as artificial insemination, in vitro fertilization and in vitro embryo production, the efficiency of these technologies is not sufficient compared to the success rates in in vivo conditions (1,2). To increase the effectiveness of assisted reproductive techniques, researchers aim to acquire new knowledge by examining the oviductal environment where the fertilization takes place and an early embryonic development is occurred and in which gametes are transported, stored alive and functional until the time of the fertilization (3). Now, we have more information about oviductal environment and our knowledge is increasing day by day with the help of the evolving possibilities and technologies. Recent studies have shown that oviductal environment has effects on gametes (4,5), fertilization (6,7) and early embryonic development (8,9). The point of present review is to share recent studies focused on the effect of oviductal environment on sperm, oocyte, fertilization and early embryonic development.

2. Oviduct and oviductal environment

Oviduct is an organ that is also known as fallopian tube. It is composed of uterotubal junction, ishtmus, ampulla and infundibulum respectively from uterus to the ovary (10). It was first described by Fallopius in 1561 (11). Different segments of the oviduct are specialized according to their function, for example, the fimbrias extending from the infundibulum leads the ovulated oocyte to be caught and directed into the oviduct (10). Oviductal environment is composed of oviductal fluid and the oviductal epithelium which is consist of cilia and secretory cells (10,12,13). While secretory cells contribute to the formation of the oviductal fluid, the cilia allows the oocytes to be transported in the oviductal fluid with the help of their hit and to form the sperm reserves in the isthmus region interacting with the spermatozoa (10,14,15,16). The content of the oviduct fluid is shaped by the secretions of oviduct secretory epithelial cells as well as blood plasma, small contributions from follicular and peritoneal fluid (10,16). Oviductal fluid consists of many different components, including different proteins, growth factors, hormones, enzymes, receptors, defense agents etc. (16). The protein and lipid concentrations (17,18), enzymatic activities (19,20), as well as gene expression of oviduct epithelial cells (21) change dynamically at different stages of estrus cycle. In addition, the content of oviductal fluid varies depending on the presence or absence of gametes (22,23). Furthermore, the oviductal fluid obtained from different regions of oviduct affects sperm functions differently (24,25). Thus, oviductal fluid dynamically changes during early reproductive events (such as gamete transport, fertilization, early embryonic development), and the idea is that it plays important roles in these events has recently gained importance. Gam-

etes reach from oviduct to the uterus as embryos that have undergone early embryonic development after the last maturation period of gametes and the fertilization process. The contribution of the oviductal environment to this journey starting in the form of sperm and oocyte and finishing in the form of embryo has been described under different headings in the following sections of the review.

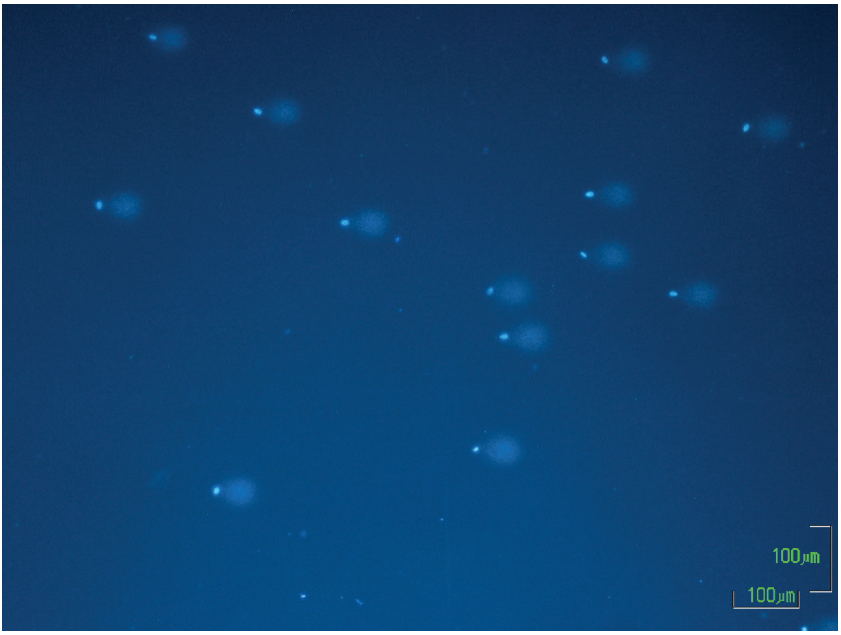
Picture 1. Dissected bovine oviduct.

2.1. Oviductal environment and sperm

In the natural reproduction process, most of the sperm are eliminated by the female genital tract before arrival of the oviduct (26). The spermatozoa passing through the utero-tubal junction and reaching the oviduct are stored in the isthmus region of the oviduct by binding to the cilia. This binding is mediated by carbohydrates surface of the oviduct epithelium and lectin-like proteins at the sperm head (27), and the molecules involved in this binding may differ between species (1). Sperm-oviduct binding not only provides the formation of sperm reserves and it also slowdowns the sperm movements (28), ensures maintaining the sperm survival for longer periods, and prevents sperm to undergo premature acrosome reaction before the fertilization (15). Although the mechanism that allows sperm to reach the fertilization zone by escaping from binding is not fully established, studies provide some information. It is believed that sperms are released from these reserves with the effect

of sending signals from the ovulated oocyte to oviduct epithelium (3), changes in the oviductal fluid content, progesterone and estrogen levels, and the capacitation and hyperactivation of sperm (29,30,31). And also, mechanisms that are involved in the detachment of bull and pig sperm from this reserves and capacitation of these sperm might be related to glycosidases activity in oviductal fluid (19,20). Additionally, Alterations in pH, calcium and bicarbonate levels of oviductal fluid before ovulation might affect sperm functions, stimulate leaving of sperm from oviduct epithelium, cause changes in sperm plasma membrane, and induce capacitation and hyperactivation (1,32,33,34).

When sperm are disposed of the female genital tract, they do not have the ability to fertilize. They undergo some biological changes known as hyper active motility, capacitation and acrosome reaction in the female genital tract (35,36,37). Certain proteins in oviductal fluid (HDL and osteopontin) have been found effective to stimulate capacitation respectively inducing sperm cholesterol efflux and increasing intracellular calcium level (38,39). Adding oviductal fluid in sperm medium increase viability, capacitated and acrosome reacted sperm rates compared the control group (40,41). Addition of late follicular oviductal fluid in sperm medium increases viable capacitated and acrosome reacted sperm rate more than addition of early luteal oviductal fluid in sperm medium (40). It has been also reported that addition of oviductal fluid in sperm medium increased the stabilization of sperm DNA integrity (40,42). It has been speculated that antioxidant molecules in oviductal fluid might be important to maintain sperm DNA integrity until fertilization (40,42). The incubation of bull sperm for a short time with bovine oviductal fluid before IVF is not enough to improve bovine embryo quality and development (40). The content of sperm medium (capacitating or non-capacitating) alter the effect of oviductal fluid on some sperm parameters such as motility and sperm DNA integrity (40).

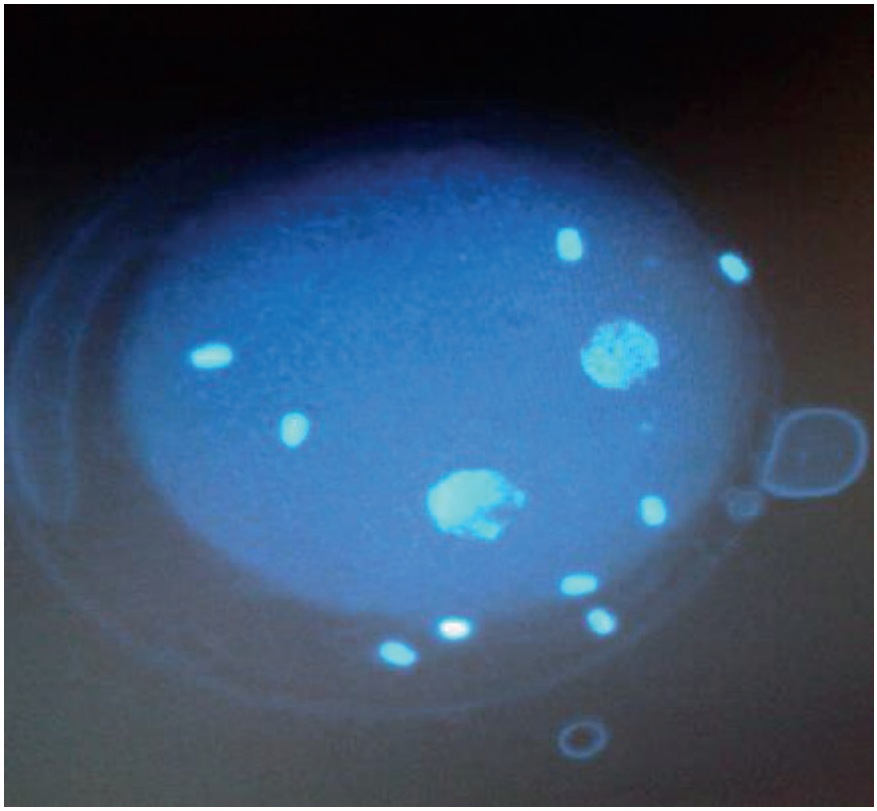


Picture 2. Bull Sperm Comet Images Stained with DAPI to detect sperm DNA integrity.

2.2. Oviductal environment and oocyte

After the ovulation, oocytes are caught by the fimbria in the infundubulum and directed into the oviduct (10). Since, oocytes and embryos are immotile; Oocytes are transported to the fertilization zone within the oviduct fluid by the contractions of the oviduct smooth muscles and the hit of the cilia (1). The transport of oocytes is accelerated by the presence of estrogen and decelerated by the presence of progesterone if these hormones are administrated at the appropriate time and dose (43).

It has been reported that the zona pellucida of oocytes, which collected from oviducts and ovaries show structural differences, and it is believed that some oviduct-derived glycoproteins contribute to oocyte maturation, hardening of zona pellucida and regulation of sperm penetration (1,6,44). It has been reported that the development and quality of porcine embryos obtained from oocytes treated with oviductal fluid before the fertilization is better compared to control embryos (5). However, bovine embryos that were obtained from oocytes that exposed to oviduct fluid before the fertilization were found similar to the control group in terms of the developmental and morphological evaluation (45). In addition, these embryos have been found to differ in terms of the gene expression (45).

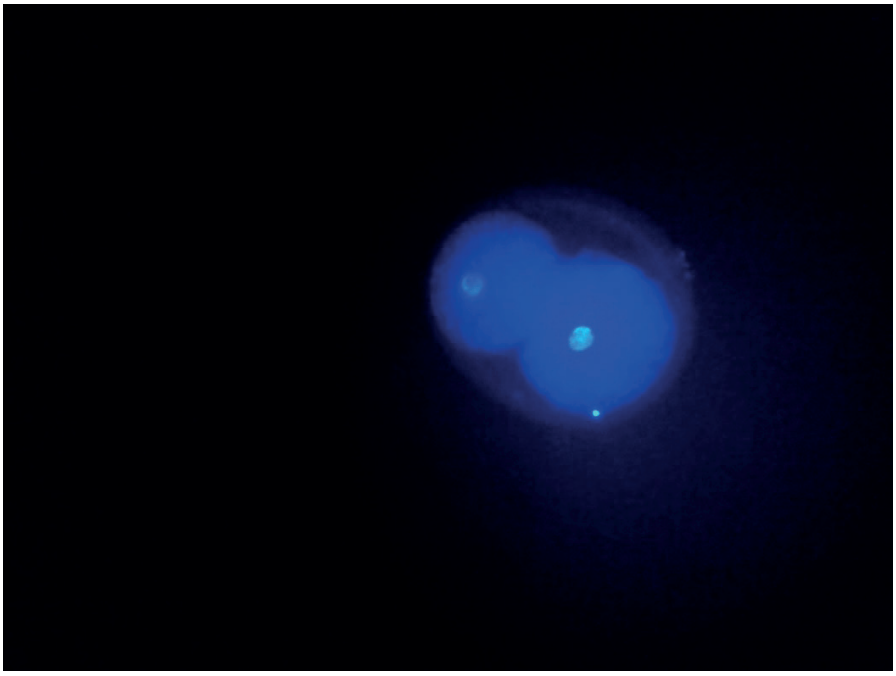


Picture 3. Putative bovine zygote assessed at 20 h post insemination showing two pronuclei and sperm bound to zona pellucida.

2.3.Oviductal environment and fertilization

In the vast majority of mammals, fertilization occurs in the ampulla region of the oviduct. Recent evidence suggests that the oviductal environment has an important effect on fertilization. Studies have shown that sperm-oocyte binding results in a variety of complex processes mediated by proteins present in the sperm plasma membrane and carbohydrates present in the extracellular matrix of oocytes (46,47,48). Additionally, different carbohydrates originating from different glycoproteins may be responsible for independent sperm binding sites with low and high sperm affinities in the zona pellucida (49,50). There are findings that sperm-oocyte binding is affected by certain molecules such as OVGP1, heparin-like glycosaminoglycans, glycosidases, and plasmin-plasminogen system components of the oviduct fluid (6,16,51). The OVGP1 and heparin-like glycosaminoglycans cause hardening of zona pellucida before the fertilization and zona pellucida become more resistant to proteolytic enzymes and sperm penetration (44). Furthermore, especially OVGP1 and heparin-like

glycosaminoglycans contribute to the control of polyspermi which is a major problem in pig in vitro fertilization (1,7,44). It is also speculated that the levels of some glycosidase enzymes found in the oviduct fluid change dynamically during the estrous cycle and these changes affect the sperm-oocyte binding (19,20,52). Plasminogen-plasmin system components detected in the oviductal environment and plasminogen detected in the oviduct fluid contribute to the regulation of oocyte sperm interaction (51). Finally, addition of oviductal epithelial cells in vitro fertilization medium effects some IVF parameters and consequently sperm-oocyte binding process (53).

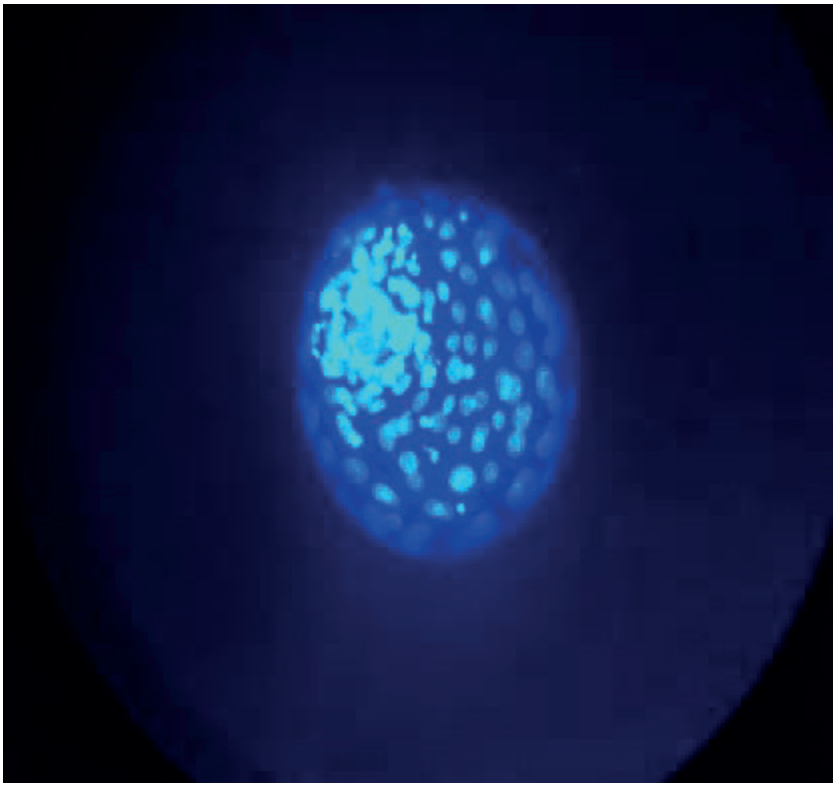


Picture 3. Bovine Zygote Stained with Hoechst.

2.4. Oviductal environment and early embryonic development

Embryos directly interact with oviductal environment after fertilization and spend the first 2-6 days in the oviduct (10). Recent evidences have suggested that oviductal fluid and its some components have a potential to support in vitro embryo development and quality. In this respect, addition of bovine oviductal fluid in embryo culture medium was found beneficial to enhance the quality and development of bovine embryos (8). Furthermore, these embryos are more resistant to cryopreservation and have higher expression values in some genes related to the embryo development (8). These results were supported by another study in which bovine embryos were treated with ovi-

ductal fluid and/or uterine fluid (54). Previous studies have been also shown that OVGP1 component oviductal fluid has a function to overcome two-cell embryo block in rabbits (55) and it is efficient to enhance in vitro embryo development in pig (56) and goat (57).



Picture 4. Bovine Blastocyst Stained with Hoechst.

3. Conclusion

The studies focus on the effects of oviductal environment on gamete maturation, gamete functionality, fertilization, and early embryonic development have great potential to understand reproductive events in oviduct and to advance sperm, oocyte and embryo biotechnologies.

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Chapter 5

TRACING THE CONNECTION: MYSTERIOUS INTERACTIONS BETWEEN HELICOBACTER PYLORI ATROPHIC GASTRITIS AND THYROID FUNCTIONS

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Introduction

Diabetes mellitus, characterized by chronic hyperglycemia resulting from insufficient insulin production or impaired insulin sensitivity, has become a global health challenge, with its prevalence steadily rising. If left untreated, diabetes poses significant morbidity and mortality risks (1). The primary goal of diabetes management is to mitigate the adverse effects of prolonged hyperglycemia, which can lead to various complications affecting multiple organ systems (1).

Simultaneously, thyroid disorders, including autoimmune thyroiditis, hyperthyroidism, and hypothyroidism, stand as prevalent endocrine conditions with substantial implications for overall health and well-being. The intricate interplay between thyroid hormones and metabolic homeostasis underscores the importance of understanding the connections between diabetes and thyroid function.

Helicobacter pylori (Hp), a bacterium residing in the gastric mucosa, has been extensively studied in the context of gastric diseases, including gastritis, peptic ulcers, and gastric malignancies (2). The pathophysiological influence of Hp is noteworthy, as it triggers chronic inflammation, gastric gland atrophy, and potentially premalignant changes (2). The progression of Hp infection, if untreated, can lead to atrophic gastritis—a condition characterized by the gradual loss of gastric glands. Beyond its association with gastric disorders, Hp has also garnered attention for its potential involvement in various systemic diseases, including metabolic and autoimmune conditions (3).

Remarkably, recent research has highlighted potential links between Hp infection, atrophic gastritis, and thyroid dysfunction. The intricate interactions between these seemingly disparate systems raise compelling questions regarding their underlying mechanisms and clinical implications. Observations suggest that diabetic individuals might have an elevated susceptibility to Hp infection due to compromised immune responses (3). Furthermore, the association between Hp prevalence and increased body mass index (BMI) introduces a multifaceted dimension to this relationship (4). Notably, Hp infection has demonstrated connections not only with metabolic disorders but also with autoimmune diseases, hinting at a broader impact on immune homeostasis (3).

Significantly, emerging evidence points towards a potential correlation between Hp infection, atrophic gastritis, and autoimmune thyroiditis—an autoimmune condition affecting the thyroid gland (5). Shared immunological mechanisms between atrophic gastritis and autoimmune thyroiditis could provide a basis for this intriguing connection. Specifically, the release of specific autoantibodies in response to Hp infection might contribute to the development of atrophic gastritis in individuals with autoimmune thyroid disease (6).

Investigating the intricate links between these conditions holds the promise of unveiling novel insights into both thyroid and gastric pathophysiology.

In light of the growing prevalence of diabetes, thyroid disorders, and Hp-related conditions, this research aims to delve into the complex relationships between Helicobacter pylori-induced atrophic gastritis and thyroid functions, shedding light on the potential shared mechanisms, clinical implications, and avenues for therapeutic interventions. By exploring these connections, we seek to contribute to a deeper understanding of the intricate interplay between metabolic and endocrine systems, potentially paving the way for innovative approaches in the management of these prevalent and impactful health conditions.

General Information

Diabetes Mellitus (DM)

Diabetes, a chronic metabolic disorder characterized by chronic hyperglycemia resulting from insulin deficiency, inefficiency, or a combination of both (1). Common symptoms of diabetes include fatigue, polyuria, polyphagia, polydipsia, and less frequently observed symptoms such as weight loss, opportunistic infections, recurrent fungal infections, and blurred vision (1).

Epidemiology

Diabetes Mellitus is a rapidly growing global health concern, with an estimated 463 million individuals worldwide being affected by diabetes in 2019, projected to reach 578 million by 2030 (7). Unfortunately, Turkey ranks among the top five countries in Europe in terms of diabetes prevalence and the density of diabetic population (8).

Pathogenesis and Diagnostic Criteria for Diabetes Mellitus

Factors contributing to the pathogenesis of Type 2 DM include reduced insulin secretion, insulin resistance, and insufficient incretin hormone function. Additionally, increased lipolysis, enhanced glucagon secretion in pancreatic islet cells, and disruptions in neurotransmitter release mechanisms have been observed (9).

The presence of one of the criteria mentioned below is diagnostic for overt Diabetes Mellitus (10). Prediabetes refers to cases where blood glucose levels are higher than the normal range yet do not fully meet the criteria for a diabetes diagnosis (11).

Classification of Diabetes Mellitus

Diabetes Mellitus is classified into primary and secondary forms. Type 1 DM, Type 2 DM, and Gestational DM are considered primary, while other forms are evaluated as secondary (8,12).

Treatment Options for Type 2 Diabetes

For Type 2 Diabetes Mellitus, non-pharmacological approaches including diet, exercise, along with oral antidiabetic medications and insulin therapy regimens are employed.

Non-pharmacological Treatment

Effective management of plasma glucose levels in Type 2 diabetes can prevent the development of microvascular and macrovascular complications. Regular exercise and a balanced diet are beneficial for diabetes control and play a significant role in preventing the progression from prediabetes to diabetes (10).

Antidiabetic Drug Treatment

Type 2 diabetes is managed through a combination of diet, exercise, oral antidiabetic medications, and insulin therapy (10).

Oral Antidiabetic Medications

1-Insulin Secretagogues Meglitinides Sulfonylureas

2-Insulin Sensitizers Thiazolidinediones Biguanides

3-Alpha-Glucosidase Inhibitors

4-Incretin-Based Drugs Glucagon-Like Peptide 1 Agonists Dipeptidyl Peptidase 4 Inhibitors

5-Sodium-Glucose Co-Transporter 2 Inhibitors (SGLT-2 Inhibitors)

Insulin therapy is primarily used in classic Type 1 Diabetes and specific cases of Type 2 diabetes. Special situations for Type 2 diabetes include the inability to achieve target glycemic control with non-insulin medications (HbA1C >10/86 mmol/mol) and/or a blood glucose level ≥300 mg/dl. Acute myocardial infarction, severe systemic/febrile illnesses, diabetic ketoacidosis, hyperosmolar conditions, major surgeries, pregnancy and lactation, advanced liver and kidney failure, allergic reactions to non-insulin drugs, and prolonged use of high-dose steroid treatment are among the indications for insulin therapy (10).

Helicobacter Pylori

Hp was first identified in the human gastric mucosa in 1983 by Robin Warren and Barry Marshall as a spiral microorganism resembling campylobacter (14). In 1989, Goodwin et al. named it Helicobacter pylori due to its helical shape and its greater presence in the gastric mucosa (15).

Bacteriological Characteristics

Hp belongs to the Helicobacter genus within the campylobacter group. It is classified into two groups: gastric helicobacters and enterohepatic helico-

bacters. Hp is a gastric helicobacter (16). It is a gram-negative, unipolar flagellated, spiral bacillus. Hp grows in a microaerophilic environment and exhibits positive biochemical features for oxidase, urease, catalase, and protease. This enhances its ability to adhere to gastric epithelium. It colonizes the gastric mucosa via receptor-mediated adhesion to the gastric type mucosa. However, it does not adhere to intestinal epithelial cells, allowing it to settle in the stomach and duodenal epithelium (17).

Epidemiology and Routes of Transmission

Around 50% of the world population is colonized with Hp in the gastric mucosa (18). Hp is more commonly observed in developing countries (80%) compared to developed countries (50%). In the TURHEP (Turkey Helicobacter Pylori Prevalence Study 2003), the prevalence in our country was found to be close to 82%, similar to developing countries. Factors contributing to the frequency of Hp include low socioeconomic status, low educational level, a larger number of family members sharing the same house, limited access to clean water, and living in crowded cities (19). Some studies have shown an increased frequency of Hp with consumption of salty foods (20). Although the exact route of transmission of Hp is not known, it has been shown that it can be transmitted through gastric secretion via fecal-oral and oral-oral routes (21).

Pathogenesis

Hp lives by invading the tissue it is in, residing beneath and within the mucous layer. Only Hp that settles in the gastric epithelium causes acute gastritis, chronic gastritis, and peptic ulcers due to mucosal changes it induces (18). It mainly affects the antrum of the stomach. Over time, its presence in the corpus leads to pangastritis.

The main virulence factors of Hp are its ciliated structure, adhesive strength, urease activity, catalase, mucinase, antioxidant release, and genes that regulate vacuolization. The OipA adhesion molecule, apart from binding, enhances the severity of inflammation by increasing IL-8 secretion. Hp leaves the gastric mucosal layer vulnerable to the effects of the acidic environment by secreting enzymes and toxins, thus causing damage (18, 23).

The catalase enzyme produced in large quantities by the bacteria acts as an antioxidant. It protects Hp from oxygen radicals and allows it to multiply within the gastric mucosa (25).

The vacuolization-associated cytotoxin antigen released by Hp (VacA) is a mucin-dissolving protease that induces vacuolization in epithelial cells. Additionally, the cytotoxin-associated antigen (CagA) produced is effective in adhering to the gastric epithelium. The antibodies produced against CagA proteins indicate that infection occurs with more virulent strains. With its high virulence effect, CagA+ Hp infections are more likely to lead to secondary gas-

tric adenocarcinoma and duodenal ulcers (26). Moreover, the Ice A (induced by contact with epithelium gene) variants A1 and A2 are present, and A1 has been shown to contribute to the development of peptic ulcers (27, 28).

Diagnosis Methods for Hp Infection

For Hp diagnosis, both invasive tests that require endoscopic procedures and non-invasive tests that do not require intervention are used. Invasive tests include histological examination, isolation in culture, biopsy urease test, rapid urease test, and molecular diagnostic tests.

Non-invasive tests include urea breath test, serological tests, Hp antigen tests, saliva and urine antibody tests, and string (enterotest). Although the use of multiple tests is recommended for a definitive Hp diagnosis, the gold standard for diagnosis is to culture the bacteria or demonstrate it histopathologically after biopsy (30).

In developed countries, since Hp is sporadically observed, diagnosis is primarily recommended through invasive tests involving biopsies. In developing countries, due to the endemic prevalence of Hp, invasive tests should be performed in patients with alarm symptoms, while non-invasive tests are preferred for other patients (31). Alarm symptoms recommended for invasive testing include the presence of unexplained dyspepsia in individuals under 55 years of age, the presence of chronic disease in individuals over 45 years of age, persistent vomiting, gastrointestinal bleeding, weight loss, dysphagia, odynophagia, anemia, and a family history of gastrointestinal system cancer (32).

Invasive Tests

• Molecular Methods: Hp diagnosis, determination of virulence, identification of eradication factors in recurrent infections, and assessment of drug resistance are achieved using molecular methods. They have high sensitivity and specificity (33).

• Histological Examination: Hp is more commonly found in the mucosa of the gastric antrum. Therefore, it is recommended to obtain biopsy samples from two antrums and one each from the corpus and angularis incisura during biopsies. The Sydney classification is used for histopathological evaluation of Hp gastritis. The Sydney classification is a visual classification that can show gastritis from gastritis to metaplasia (33).

• Bacterial Culture: Bacterial culture is the gold standard for Hp diagnosis. Its sensitivity is considered between 70-90%. The use of proton pump inhibitors or antibiotics before endoscopy reduces sensitivity. Therefore, bacterial culture is not routinely recommended (33).

• Biopsy Urease Test: Despite being cheap and simple, this test is not practical due to the need for biopsies and endoscopy (33).

• Rapid Urease Test: Thanks to the kit used, this test provides results in 1 hour, which is advantageous over gel agar tests. It is easier to use among invasive tests, but its sensitivity and specificity are similar (33).

Non-Invasive Tests

• Serological Test: The most commonly used serological method is the Enzyme-Linked Immunosorbent Assay (ELISA). It can be helpful in treatment follow-up. The most commonly used tests are those that contain Hp-specific IgG antigen in the serum (33).

• Fecal Antigen Test: This is the direct demonstration of Hp antigen in the stool sample using an immunassay method. This method shows the presence of Hp in the gastric mucosa and is quite sensitive and specific. However, it is not affected by Hp eradication and therefore can be used in treatment follow-up (33).

• Urea Breath Test: The urea breath test is based on the fact that Hp breaks down urea to produce ammonia and carbon dioxide. The test is performed by administering urea labeled with a radioactive or stable isotope to the patient. If Hp is present, labeled carbon dioxide is released with the breath and can be detected. It is recommended in the diagnosis, follow-up, and eradication control of Hp (33).

• Urine and Saliva Antibody Tests: Testing for Hp-specific IgG in urine or saliva is a non-invasive method. Tests conducted in saliva have lower sensitivity and specificity compared to those performed in urine (32).

Diseases Associated with Helicobacter Pylori

Hp can cause a wide range of diseases in the body.

Gastrointestinal Diseases

Studies have shown that Hp is associated with various gastrointestinal diseases, including functional non-ulcer dyspepsia, gastroesophageal reflux disease (37), Barrett's esophagus, esophageal adenocarcinoma, acute erosive gastritis, acute non-erosive gastritis (38), peptic ulcers, chronic gastritis (especially type B), gastric carcinoma, and gastric MALT lymphoma (39).

Extragastrointestinal Diseases

Hp has been linked to numerous diseases outside the gastrointestinal system. Ischemic heart disease is the most notable disease associated with Hp. Other diseases that have been linked to Hp include iron deficiency anemia, pernicious anemia, idiopathic thrombocytopenic purpura, systemic sclerosis, Alzheimer's disease, inflammatory demyelinating neuropathy, diabetes mellitus, metabolic syndrome, autoimmune thyroiditis, idiopathic chronic urticar-

ia, and a potential association with scleroderma. These diseases develop on a multifactorial basis, making it challenging to directly attribute them solely to Hp (40-47).

Treatment of Helicobacter Pylori

Multiple antibiotic combinations are recommended for Hp eradication. In terms of treatment, the American College of Gastroenterology Guidelines divides patients into two main groups. The first group consists of patients who require absolute treatment. These include individuals with active gastric ulcers or active duodenal ulcers, those with refractory peptic ulcers who have previously received treatment, those with gastric MALT lymphoma, and those with gastric cancer after early resection. The second group includes patients for whom treatment is not mandatory but is recommended. This group comprises individuals with non-ulcer dyspepsia, NSAID users, those with unexplained iron deficiency anemia, those at high risk of stomach cancer, individuals with gastroesophageal reflux disease, and patients with idiopathic thrombocytopenic purpura (18,48). Initial step therapy is appropriate for patients receiving treatment for the first time, while step therapy is recommended for patients with recurrent infections (47,49). The general treatment regimen, as specified in the consensus report, involves a seven to fourteen-day course of triple therapy containing fluoroquinolone/bismuth or a ten-day quadruple therapy. Drug resistance remains a determining factor in treatment (50).

Thyroid Gland

The thyroid gland develops in the first three months of embryonic development and is the first endocrine gland to form. The thyroid gland is located in the front-lower region of the neck, in front of the trachea, with the right and left lobes on either side of the trachea. In adults, the thyroid gland is expected to weigh around 15-20 grams. This weight can vary based on gland growth (51).

Synthesis and Metabolism of Thyroid Hormones

The first step in thyroid hormone synthesis is iodine uptake. For adults, the daily iodine requirement is 150 mcg/day. The sodium/iodine symporter (NIS), expressed by follicular cells of the thyroid gland, facilitates iodine uptake. While NIS is present in other cells as well, its selective functioning in the thyroid allows it to aid in imaging the thyroid gland, ablation for thyroid malignancies, and hyperthyroidism treatment (52).

The iodide captured by the thyroid gland is oxidized, and iodination occurs within the thyroglobulin with tyrosyl residues. The iodotyrosines within thyroglobulin bind to iodine using the thyroid peroxidase enzyme. This process results in the formation of the active hormones T3 and T4 from iodotyronines. After synthesis, T3 and T4 are released into the circulation as free forms after proteolysis of thyroglobulin, i.e., iodotyronine and iodotyrosine.

Within the thyroid cell, iodotyrosine molecules are deiodinated. The conversion of T4 to T3 occurs through 5'-deiodination within the thyroid cell (53,54).

Thyroid hormones primarily increase basal metabolism by enhancing mitochondrial activity. They increase oxygen consumption in various tissues such as skeletal muscles, the heart, and kidneys. However, thyroid hormones do not always increase basal metabolism; they can also have inhibitory effects on metabolic activity in specific areas of the pituitary and brain (55,56).

Evaluation of Thyroid Function Tests

Thyrotropin-releasing hormone (TRH) released from the hypothalamus stimulates the release of thyroid-stimulating hormone (TSH) from the anterior pituitary. Circulating thyroid hormones suppress TRH and TSH release through negative feedback. Elevated iodine levels suppress organification of iodine in the thyroid. This phenomenon is known as the Wolf-Chaikoff effect. In individuals with autoimmune thyroid disease, this suppressive effect can become permanent (56).

TSH is measured using immunochemiluminescence (ICMA) or immunoradiometric (IRMA) methods. It follows a circadian rhythm. It is the primary hormone used for evaluating thyroid function. In young, healthy adults, the average upper limit is 4 mIU/L. The upper limit varies with age and pregnancy status (55, 57,58).

Triiodothyronine (T3) - Thyroxine (T4)

Different hormone forms are created based on the number of iodine molecules bound to tyrosine. T3 is formed by the combination of one monoiodotyrosine (MIT) and one diiodotyrosine (DIT), while T4 is formed by the combination of two diiodotyrosines (DIT). Although thyroxine is synthesized more, triiodothyronine is more active (59).

Evaluation of Thyroid Autoantibodies

In autoimmune thyroid diseases, serum thyroid peroxidase antibodies (Anti-TPO) are the most important serum marker, but anti-thyroglobulin (Tg) and thyroid-stimulating hormone receptor antibodies (TRAb) can also be present. The prevalence of antibody positivity increases with age in the general population. Anti-TPO antibodies are detected in approximately 95% of Hashimoto's thyroiditis cases and around 85% of Graves' disease cases (59). Thyroid receptor antibody (TRAb) is particularly specific, especially in Graves' disease. TRAb can rarely exhibit inhibitory effects, preventing the function of TSH-R and leading to hypothyroidism. Its primary effect is thyroid-stimulating, causing hyperthyroidism in Graves' disease (59).

Autoimmune Thyroid Disease

Autoimmune thyroiditis is often asymptomatic. It can manifest as euthyroidism, subclinical hypothyroidism, hyperthyroidism, and goiter symptoms. The most common clinical presentation includes goiter, positive Anti-TPO or anti-TG antibodies, and the occurrence of hypothyroidism/euthyroidism (59).

In terms of etiology, environmental factors, genetic factors, and existing factors play a role. Environmental factors include alcohol, smoking, iodine, vitamin D, stress, and infections. Genetic factors are associated with TSH-R, TG, HLA, CD40, FCRL3, FOXP3. Existing factors such as female gender and giving birth can also contribute (59).

Hashimoto's thyroiditis is the most common autoimmune thyroid disease. Hashimoto's thyroiditis (chronic autoimmune thyroiditis) is the most common cause of hypothyroidism in regions without iodine deficiency. It is characterized by autoimmune destruction of the thyroid gland secondary to thyroid epithelial cell apoptosis. Widespread lymphocytic infiltration is observed. Hashimoto's thyroiditis is categorized as primary and secondary. In primary forms, there are six subtypes: classic, fibrous, IgG4-related, juvenile hashitoxicosis, and silent form. Goiter is the most common sign. Primary forms can also be associated with other autoimmune diseases. Secondary Hashimoto's thyroiditis is often iatrogenic or secondary to immunomodulatory agents, such as after interferon-alpha treatment for HCV. While the primary feature of Hashimoto's thyroiditis is hypothyroidism, an increase in hormone release due to inflammatory states can lead to hyperthyroidism initially. Local symptoms can also occur clinically, such as the development of dysphonia, dyspnea, and dysphagia in locations near the gland (60).

Common clinical symptoms include constipation, formation of gallstones, dry and coarse skin, myxedema, nail and hair loss, muscle pain and stiffness, bradycardia, cardiomegaly, pericardial effusion, anemia, oligomenorrhea, infertility, menometrorrhagia, and depression (60).

Relationship between Helicobacter Pylori Atrophic Gastritis and Thyroid

Hp can cause atrophic gastritis following long-term damage. CagA is particularly implicated in the pathogenesis. Hp and CagA antibodies cause atrophic antral gastritis more than atrophic corpus gastritis (62).

About 10-40% of patients with the most common thyroid disease, Hashimoto's thyroiditis, also have stomach issues. Similarly, around 40% of patients with autoimmune atrophic gastritis have Hashimoto's thyroiditis. This relationship is referred to as the thyrogastric syndrome (polyglandular syndrome type IIIb). Embryological and biochemical similarities between thyroid and stomach tissue reinforce the thyrogastric syndrome concept (6). Both the thyroid and stomach tissues develop from the primitive gut. Both cell types

are polarized and have apical microvilli. The thyroid mucosa and thyroid follicular cells both have the ability to concentrate and transport iodine using the sodium/iodine symporter (NIS). These cells also contain similar enzymes with peroxidase activity (6).

In atrophic gastritis, the loss of parietal cells results in impaired hydrochloric acid and intrinsic factor production. This impairment, in turn, affects the solubilization process of levothyroxine by altering iron ionization. Consequently, levothyroxine malabsorption occurs. Th1 lymphocytes help cytotoxic T lymphocytes and produce specific cytokines (TNF-a and IFN-y) that can induce cellular apoptosis in thyroid cells (6).

Similarly, in autoimmune atrophic gastritis, CD4 proliferation is activated through the absence of peripheral tolerance, leading to the activation of Th1 lymphocytes (6). The mechanisms of cellular immunity in autoimmune thyroiditis show similarities to atrophic gastritis. Both cellular and humoral immune collaboration characterize both autoimmune thyroiditis and gastritis and contribute to the production of specific autoantibodies (anti-thyroid peroxidase, anti-thyroglobulin, and anti-parietal cell antibodies). The presence of these antibodies can serve as an alert signal for the presence of stomach disorders in individuals with thyroid autoimmunity and is significant for diagnosis. All these mechanisms are meaningful in explaining the coexistence of these two autoimmune diseases (6).

Conclusions

In the intricate world of medical research, the intriguing interplay between seemingly disparate systems continues to captivate scientists. One such enigmatic connection that has piqued the interest of researchers is the intricate relationship between Helicobacter pylori (Hp) atrophic gastritis and thyroid functions. While traditionally viewed as separate entities, recent studies have unveiled a complex web of interactions that bridge the gap between these two seemingly unrelated systems.

Hp, a notorious bacterium infamous for its role in gastric ulcers and gastritis, has now revealed its impact beyond the confines of the stomach. Studies have shown that Hp infection can trigger atrophic gastritis, leading to the degradation of parietal cells and a subsequent reduction in hydrochloric acid and intrinsic factor production. This, in turn, influences the absorption of essential nutrients like iron and, intriguingly, also impacts the absorption of thyroid hormones. The presence of Hp and its virulence factor, CagA, prompts not only gastric damage but potentially extends its influence to the neighboring thyroid gland.

Emerging evidence suggests that Hp and CagA antibodies, while significantly impacting gastric health, may also induce a cascade of immunological

events that inadvertently affect thyroid tissue. This intriguing crosstalk between the immune systems of the stomach and thyroid appears to be a result of molecular mimicry, where antigens produced by Hp and thyroid tissues share structural similarities. As a consequence, autoantibodies that target thyroid tissue can be inadvertently produced in response to Hp infection, contributing to autoimmune thyroid diseases like Hashimoto's thyroiditis.

The thyrogastric syndrome, a term coined to describe the co-occurrence of Hashimoto's thyroiditis and atrophic gastritis, points to the existence of a deeper connection. The shared embryological origins and the presence of common enzymatic activities in both thyroid and stomach tissues further underscore the potential link between these two systems. The thyrogastric syndrome represents a unique clinical manifestation of this intricate interplay, where patients with one autoimmune disorder are at an increased risk of developing the other.

This newfound understanding of the relationship between Hp atrophic gastritis and thyroid functions carries significant clinical implications. Patients presenting with one of these conditions may benefit from a comprehensive evaluation to detect the presence of the other, thereby enabling early diagnosis and intervention. Additionally, the identification of shared pathways and mechanisms could open up new avenues for therapeutic interventions targeting both Hp infection and autoimmune thyroid disorders.

In conclusion, the mysterious interactions between Hp atrophic gastritis and thyroid functions shed light on the complexity of human physiology. This intriguing connection challenges the traditional boundaries of medical specialties and emphasizes the importance of a holistic approach to patient care. As researchers delve deeper into this intricate web of interactions, they may uncover novel insights into both gastric and thyroid diseases, ultimately leading to improved diagnostic strategies and therapeutic interventions for patients grappling with these enigmatic conditions.

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Chapter 6

METAVERSE INTEGRATION IN DERMATOLOGY: AN ELABORATE SYSTEMATIC REVIEW

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Introduction

The dermatology domain has, in the contemporary era, embraced cutting-edge technological advancements to elevate both patient treatment and pedagogical practices. Digital tools and online platforms have brought about a transformative shift in service provision, with AI-driven methodologies on the verge of integrating into the daily grind of dermatologists. A significant volume of research delves into the deployment of the state-of-the-art conversational AI, Metaverse, within the realm of dermatology. This systematic review intends to collate and evaluate these findings. To methodically scrutinize and synthesize the accumulated knowledge regarding Metaverse's utilization within dermatological settings and its associated domains. A myriad of databases, including PubMed, Cochrane Library, EuropePMC, medRxiv, arXiv, bioRxiv, Dimensions AI, Semantic Scholar, and Google Scholar, were scoured to gather articles until the cut-off date of August 17, 2023. The focus of selection hinged on research that investigated Metaverse's role in dermatological contexts. To mitigate bias, a rigorous filtration process was applied, pulling data from a gamut of resources, ranging from preprints to multilingual articles. Apart from primary articles, supplementary data and acknowledgments were also perused for a holistic overview. The assimilation of the outcomes harnessed both network analysis and thematic synthesis techniques. 87 papers met the stipulated selection criteria. A notable fraction (36%) extended credit to Metaverse for its role in manuscript drafting, computational analysis, or software crafting. Roughly one-fourth (24%) took the form of case studies, highlighting specific dermatological patterns and repercussions. In its capacity to respond to dermatological queries, Metaverse's efficacy spanned from exemplary in oncological contexts to mediocre in niche and esoteric dermatological topics. Nevertheless, an uptick was observed with the introduction of metaverse. There are evident strides in adaptive learning, amalgamating image-centric AI, and refining linguistic algorithms tailored for dermatological applications.

The uptake of Metaverse in dermatologically aligned disciplines has surged notably, predominantly in the realm of case study composition. Given the inherent uncertainties and the premium on safety, an iterative feedback mechanism for error logging is pivotal. This ensures the AI models undergo refinement and remain robust and adept in their operational environment.

General Information

Recent strides in dermatology can be credited to the significant penetration of digital tools and artificial intelligence (AI), transforming visual diagnostics and evaluations [1]. Advancements in mobile devices, specifically smartphones and real-time messaging, have redefined the contours of dermatology service delivery, offering streamlined and cost-efficient platforms for distant consultations and diagnosis [2]. Platforms like TikTok™ have ascended as pivotal

hubs for individuals exploring dermatological insights and for the budding generation of med students who are woven into the fabric of digital learning [3, 4]. The integration rate of social media in private practices, academic residencies, and scholarly periodicals has witnessed an uptick lately [5].

Intriguing methodologies like gamification and serious games are being blended into dermatological pedagogy, aiming to amplify learner engagement and efficacy [6]. AI stands to refurbish the distribution of premium medical services and foster medical consortiums, thereby enhancing the healthcare model's proficiency. Metaverse, representing the vanguard in AI, has piqued interests across healthcare and biomedical landscapes [7, 8] due to its capabilities to augment medical pedagogy, academic inquiry, and clinical governance. Although dermatology's dance with Metaverse is nascent, a multitude of research pieces suggest its relevance. Dissecting these studies systematically can offer rich perspectives into the boons and potential pitfalls of Large Language Models (LLMs) in this domain.

This study's mission is to sift through academic pieces concerning Metaverse's roles and decipher its possible imprints across various dermatological niches. Achieved via a detailed survey and bibliometric analysis of pertinent papers, the outcomes are poised to shed light on Metaverse's varied influences, spotlight potential future trajectories, and underline domains yearning for deeper inquiry.

Methodology

Data Procurement and Examination Strategy

An exhaustive scan was executed over a spectrum of databases employing dermatology-centric terms, capturing facets like skin, hair, and nails. Conditions including psoriasis, dermatitis, acne, vitiligo, alopecia, lichen planus, and themes such as cosmetics, eczema, malodor, hyperhidrosis, bromhidrosis, olfactory reference syndrome, rosacea, and sunburn punctuated the search. The interface of these terms with the keyword "Metaverse" was also scrutinized.

Data wells tapped encompassed EuropePMC, Semantic Scholar, Pubmed, Dimensions AI, Medrxiv, Biorxiv, and Google Scholar.

Protocol Enrollment

The protocol aligned with the health-related outcomes of systematic reviews was cataloged on PROSPERO on August 17, 2023. The search trajectory was recalibrated to better fit the stipulated review blueprint.

Research Inclusion

The filtration parameters embraced research in any tongue, given that ample data (both abstract and full narrative) was on hand to validate their

inclusion. Grounds for exclusion entailed (1) pieces where dermatology-centric terms were leveraged outside their intended scope, (2) mentions of Metaverse devoid of its dermatological applications, (3) concise abstracts from unrelated dermatology conventions, (4) press releases, blogs, correctional pieces, and (5) rescinded publications.

All language publications were on the table. Google and OpenAI's translation arsenal catered to non-Anglicized pieces.

Our selection compass incorporated a collective strategy weaving in human authors, Metaverse, and Bard. This was paired with a rigorous database-spanning search blueprint, thus ensuring coverage depth. Additional filters and validation mechanisms were wielded to encapsulate a broad spectrum of relevant studies, minimizing potential bias.

Initial forays involved title scrutiny for relevance and repetitiveness. Iterations of the same study were spotted, with precedence given to the latest, enriched version.

Manual and API-enabled full-text scans were conducted by the human reviewer. Subsequently, Metaverse screened these against pre-defined criteria, and if fitting, earmarked them under dermatological sub-categories. Contentious points were resolved via iterative reviews, honing prompts, and fine-tuning text preprocessing.

Data Mining & Aggregation

Enumerated data from the surveyed articles involved External IDs like DOI, PMID, PMCID, coupled with Title, Abstract, Acknowledgments, Funding avenues, MeSH terms, Fields of Research, Publication Date, and Type. Further details encompassed the author's identities and affiliations, citation statistics, recent citation data, RCR metrics, the journal's Impact Factor, and the originating source link.

Beyond pure text processing, a quality appraisal was undertaken covering aspects from review selection to primary outcomes.

Python codes, tweaked from [7] for the Semantic Scholar API, were the bedrock of data integration and analysis.

VOS Viewer software [9], in its Version 1.6.18, stood as the analysis and visualization cornerstone. It rendered both descriptive stats and scientific mappings, revealing authorship collaborations, terminologies, and thematic interconnections.

Thematic synthesis took precedence post network analysis, identifying recurrent themes or motifs across surveyed studies. Data coding, extraction, and theme clustering facilitated a layered comprehension of the core research motif.

The human reviewer, aided by Metaverse and Bard, played a pivotal role in data extraction. Any discord was mediated by the human reviewer, revisiting data, and refining prompts until harmony was achieved. Once consistent themes surfaced, they were meticulously organized, offering a panoramic view of individual research pieces' commonalities and contrasts. The culmination saw the emergence of interpretive themes, crafted from the wisdom accumulated across prior stages.

Results

Assessment of Inspected Studies

Figure 1 provides a visual representation of our analytical progression in selecting relevant studies. From a myriad of databases, we sourced a considerable 835 studies encompassing languages like English, German, French, Japanese, Polish, Portuguese, and Slovenian. Of this assortment, 263 were repetitious and consequently omitted. Our introductory evaluation led to the exclusion of 91, primarily owing to their informal nature, such as blog entries or journalistic articles. Further scrutinization in terms of publication title, type, and source/journal led to the removal of an additional 106 studies, mainly conference notes or literary volumes unrelated to our focus. Additionally, two studies were discounted due to their inoperative DOIs.

The resultant 373 studies predominantly included arXiv drafts, in-depth analyses from Cureus, and articles from dermatological publications, primarily traced back to Dimensions and Google Scholar. Beyond arXiv, we expanded our selection to feature drafts from platforms like medrXiv, bioRxiv, psyarxiv, edarxiv, ResearchGate, osf, the SSRN e-journal, and JMIR. Of these, dedicated dermatology publications provided 21 articles.

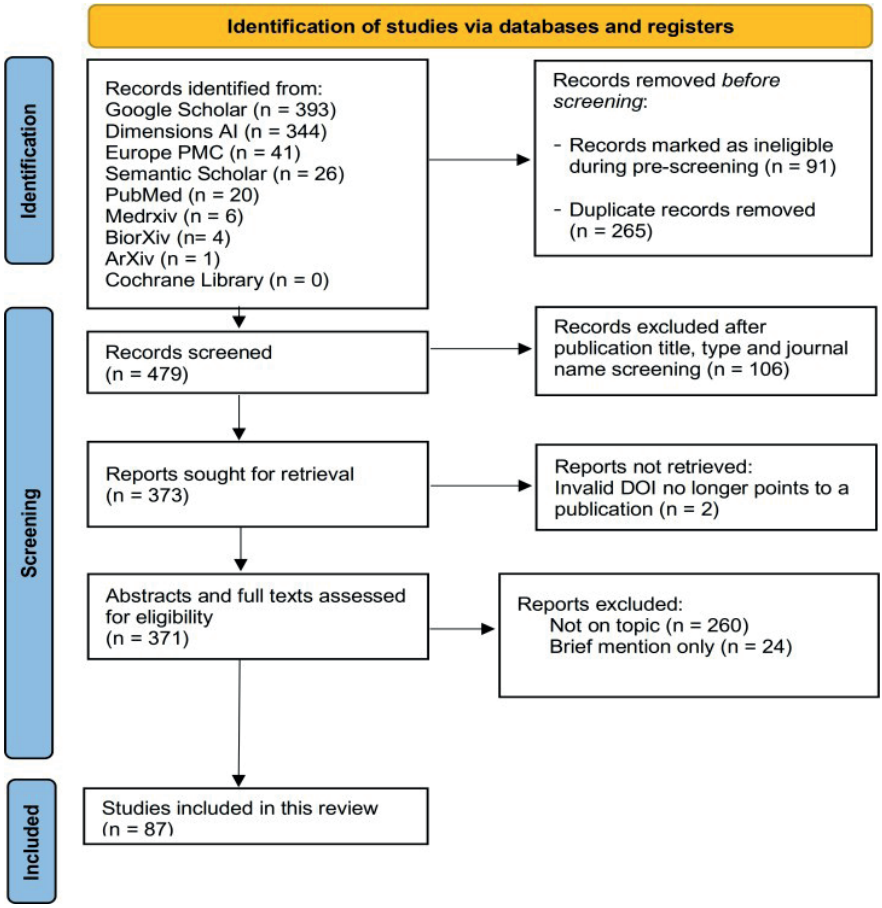

Figure 1 Prisma (2020) diagram.

Upon further exploration, we sidelined another 284 studies. Many were tangential in addressing Metaverse or its dermatological implications. Some ambiguously utilized dermatological terminology in divergent contexts. This ranged from the philosophical musing on skin tones in logical reasoning to psychological discourses on skin hue, to introspective tales of lost skin sensitivity. References to "ivory-skinned with flowing locks" in Metaverse or the allusion to its "surface-level appeal" were also noted. Notably, some articles, while not entirely congruent with our outlined criteria, offered intriguing insights into the juxtaposition of Metaverse and expansive linguistic models in contexts peripherally related to dermatology. Examples encompassed thermal modulation or the creative use of dermatological knowledge in the Metaverse or in crafting speculative narratives. Opting for inclusivity, we retained four articles under the tag "Brief mention only", deeming them complementary to the remaining 83 congruent studies. Hence, our review comprises 87 articles:

84 in English, with single representations in Japanese, German, and Spanish; with nearly two-fifths being preliminary drafts.

Taxonomic Structures

Our curated set of articles demonstrated a spectrum of methodologies, echoing sentiments from previous comprehensive reviews on Metaverse's medical implications [10-13]. The trailblazing study [10], refreshed on August 17, 2023, highlighted "academic/scientific literature" as the overarching theme in over half the entries. Benefits of scientific inquiry dominated a third, with emphases on dissecting colossal datasets like electronic health diaries and genomic profiles. Healthcare advantages spanned tailored treatments, foresight into ailment risks, clinical procedure enhancement, diagnostic refinement, fiscal prudence, and heightened health consciousness. A fraction exceeding 10% underlined the didactic advantages in medicinal disciplines, spotlighting the crafting of accurate clinical scenarios.

Li et al. [11], comprehensive up to March 20, 2023, suggested a dual-tiered - application-driven and consumer-driven - classification for the enlisted articles. For instance, articles revolving around academic prose, literary critiques, and research conceptualization were grouped under "medical research". The "advisory" category encapsulated Metaverse's medicinal counsel in both institutional and individual domains. "Clinical functions" embraced tasks like diagnostic conclusions, therapeutic proposals, and clinical dossier creation. Depth was another suggested criterion, ranging from overarching opinions on potential applications (Level 1), to detailed expositions of particular Metaverse instances (Level 2), and extending to both subjective and empirical evaluations of Metaverse responses (Level 3). Muftić et al. [12] blended Level 1 with specific Level 2 entries, spotlighting 21 flagship studies, encompassing segmental observational studies and performance evaluations.

Parallelly, Goedde et al. ([13], accounting for publications up to March 2023) discerned that close to half the articles were concise commentaries like editorials or epistolary responses, whereas a third consisted of evaluative articles or reflections. Original research pieces, analytical summations/meta-analyses, and clinical logs were less frequent.

Our chosen categorization paradigm drew inspiration from the preceding studies and was augmented with insights regarding the prospective utility of Metaverse by healthcare experts, support staff, and clientele, as articulated by a dermatological expert [14]. We segmented these into operational dermatology (patient communications, standardized documentation), educational initiatives (patient-centric informative brochures), therapeutic consultation (ailment recognition, treatment blueprints), and academic writing.

Discussion and Conclusion

In our comprehensive assessment, nearly half (47%) of the articles we reviewed primarily examined Metaverse's prowess in writing, particularly in medical domains like dermatology. This review included tasks like drafting clinical reports, crafting persuasive content, formulating medical documents, and offering medical solutions, especially related to dermatology. Interestingly, 54% of the 41 papers assessed offered quantitative data and observational cross-selection studies compared to those grounded in less robust evidence (referenced in prior reviews on health applications).[11,12]

Our analysis brought to light some key observations. For instance, Metaverse-generated dermatology reports surpassed human-written counterparts in terms of quality and clarity [15]. The synergy between AI and human professionals notably enhanced document clarity, such as in medical consent forms, without skimping on vital clinical details. Metaverse also showed potential in summarizing medical evidence and crafting compelling narratives. All in all, 37 out of the 86 studied (43%) either harnessed Metaverse for its writing capabilities or put them under scrutiny.

It's noteworthy that Metaverse excelled in medical tests in multiple languages, with significant accomplishments in exams like the Spanish MIR 2022, the USMLE, and the Japanese NMLE, among others.

While Metaverse (GPT-4) performed admirably in radiation oncology physics tests, it faltered in improving scores when determined by consensus across attempts. Conversely, a group of medical physicists outdid Metaverse when using a consensus-based approach, underlining the power of collective human knowledge, especially when integrated with AI tools.

A Reddit study comparing Metaverse and actual physicians revealed Metaverse often provided higher quality, more empathetic feedback [16]. However, there were instances where Metaverse generated misleading references, most notably in specialized topics such as PATM's microbial basis. While some questions saw exemplary outcomes, especially around skin cancer [19], niche subjects like eczema proved more challenging [20]. A more holistic assessment of these models in healthcare contexts is crucial, emphasizing model customization for specific medical needs. Another study displayed the potential of the Metaverse-DALL-E integration in crafting datasets for thoracic ailments, highlighting the need for varied, meticulously annotated data for better image-based diagnostics.

Usage: Of the reviewed articles, 31 (36%) recognized Metaverse's role in writing, analyzing data, and tool development. A significant number of these (24%) were case reports detailing dermatological conditions. Particularly, pediatricians found Metaverse immensely beneficial when specialized

consultants, like infectious disease experts, were unavailable [22]. Moreover, Metaverse was even utilized in drafting a thesis, underscoring its academic value.

Innovations: To counter the shortcomings of traditional models in providing precise medical answers, the ChatDoctor model was devised. It displayed enhanced performance, particularly in skin lesion evaluations [23]. Similarly, other models like HuaTuo [24] and SkinGPT [25] were introduced, emphasizing domain-specific expertise.

A few other notable advancements were the incorporation of Metaverse into a 3D metaverse for interactive dermatology learning and CHIE [26], which was crafted for queries related to cosmetic dermatology.

Our review underscores Metaverse's potential as a pivotal asset for dermatological tasks. Its prowess in documentation, academic writing, decision support, and education holds the promise of transforming patient interactions, treatment strategies, and aftercare. Our thematic analysis unearthed rich insights into Metaverse's multifaceted applications in dermatology, from evaluation to utilization and development. An exciting development trajectory is Metaverse's merger with AI-powered dermatological image diagnostics, which could be a game-changer for skin research. Its significant impact on case report writing, a vital component of medical literature, cannot be overstated. With Metaverse's help, doctors can bridge the communication gap in presenting their findings, thereby enriching the dermatological knowledge base. Nevertheless, while Metaverse's general utility is undeniable, it's crucial to be cognizant of its limitations, especially in specialized areas. The disparity in accuracy across various dermatological subfields accentuates the need for more specialized tools tailored to the specific challenges of dermatology.

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Chapter 7

PRESERVATION RHINOPLASTY: MODERN TECHNIQUES, ADVANTAGES, AND DISADVANTAGES

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Nasal Anatomy:

The nose is a respiratory and olfactory organ. Its composition consists of bone and cartilage, with a covering of muscles and skin. Its structure is examined in two parts:

1. Nasus externus (External Nasal Structure)

2. Cavitas nasi (Nasal Cavity)

External Nasal Structure

It is a pyramid-shaped structure situated in the frontal midsection of the face. The symmetrical lateral surfaces of the pyramid come together at the front to form the dorsum nasi (nasal bridge). The part of the dorsum nasi continuing upwards to the forehead is called the radix nasi (nasal root). The freely ending front-lower tip is referred to as the apex nasi (nasal apex). On the undersurface of the pyramid, there are nostrils or nares. The protruding lower portions surrounding the nares on the lateral surfaces are called alae nasi (nasal wings). The mobile part of the nasal septum, referred to as the pars mobilis septi nasi, separates the nares from each other in the middle.

Nasus externus is composed of bone, cartilage, and muscles. The skin in this area is thin and loosely attached to the bone. On the other hand, the skin covering the cartilages is thick and tightly adhered to the perichondrium. The skin contains numerous sebaceous glands. Beneath the skin, there are facial muscles including the Nasalis muscle, Procerus muscle, and Depressor septi muscle. The components constituting the external nose include the nasal bone, the maxillary nasal process, and the frontal bone's nasal section. The outwardly opening portion of this bony structure is referred to as the apertura piriformis. This opening is defined superiorly by the lower border of the nasal bone, laterally by the nasal notches of the maxilla, and anteriorly by the anterior nasal spine. (1).

Nasal Cartilages:

The nasal cartilages include the cartilago septi nasi, cartilago alaris major, cartilagines alares minores, and cartilagines nasales accessoriae.

1. Cartilago septi nasi (Septal Cartilage): This cartilage is divided into two parts: the posterior (sphenoidal) process and the lateral process. The cartilago septi nasi is involved in shaping the nasal septum, which divides the nasal cavity into two sections. The posterior-upper edge of the cartilage attaches to the lamina perpendicularis ossis ethmoidalis, the posterior-lower edge attaches to the vomer and the anterior nasal spine, and the anterior-lower edge attaches to the medial crus of the cartilago alaris major. The area within the nasal cavity that stretches from the perpendicular plate of the ethmoid bone to the vomer is referred to as the posterior process.

The lateral processes are paired and contribute to the structure of the dorsum nasi. Triangular in shape, the upper edge attaches to the nasal bone and the frontal process of the maxilla, while the lower edge is connected to the lateral crus of the cartilago alaris major through fibrous tissue.

2. Cartilago alaris major (Major Alar Cartilage): It is comprised of two parts: the lateral crus and the medial crus. Both of these cartilaginous structures are situated around the nostrils. The lateral crus is situated within the upper lateral wall of the nares, while the medial crus is located in the medial wall of the nares. The two medial crura and the anterior-lower part of the cartilago septi nasi come together, connected by fibrous tissue, to form the pars mobilis septi nasi, which separates the nostrils from each other (1).

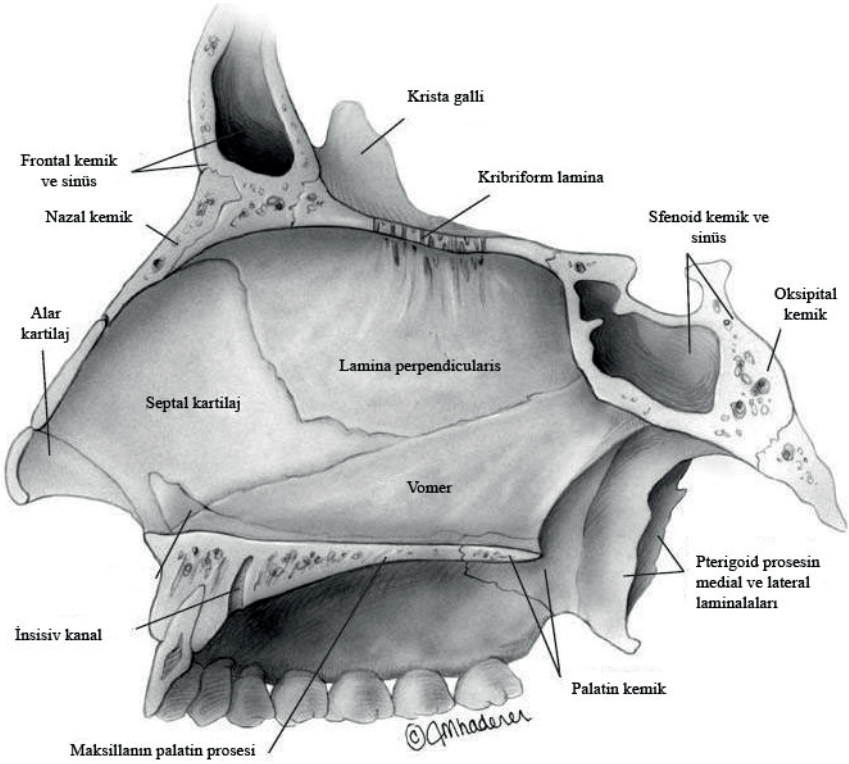

Figure 1. Nasal Septal Anatomical Structures (Sinus Surgery Endoscopic and Microscopic Approaches.Levine H.L. , Clemente M.P. Authors.Thieme Medical Publishers Newyork. 2005;13)

Cartilagines Alares Minores (Minor Alar Cartilages): These cartilages are positioned adjacent to the lateral process of the cartilago septi nasi and the lateral crus, near the frontal process of the maxilla. They play a role in forming the alae nasi (nasal wings), and their quantity usually ranges from 3 to 4. The

lower part of the structure surrounding the nostrils of the cartilagines alares minores contains fat and fibrous connective tissue.

Accessory Nasal Cartilages (Cartilagines Nasales Accessoriae): These small cartilages exhibit variability in both number and shape among individuals. Their purpose is to help maintain the stability of the nasal walls during the process of breathing.

The dorsum nasi is covered by the Nasalis muscle (1).

BLOOD VESSELS AND NERVES OF NASUS EXTERNUS (EXTERNAL NOSE):

Arteries: The arteries include the R. lateralis nasi (branch of the A. facialis), R. septi nasi (branch of the A. labialis superior), A. dorsalis nasi (branch of the A. ophthalmica), and A. infraorbitalis (branch of the A. maxillaris).

Veins: Venous drainage is directed towards V. facialis and V. ophthalmica.

Nerves: Sensory innervation of the skin is supplied by the N. infratrochlearis (a branch of N. ophthalmicus), R. nasalis externi (a branch of N. nasociliaris), and N. infraorbitalis (a branch of N. maxillaris). The Nasalis muscle is innervated by the N. Facialis (1).

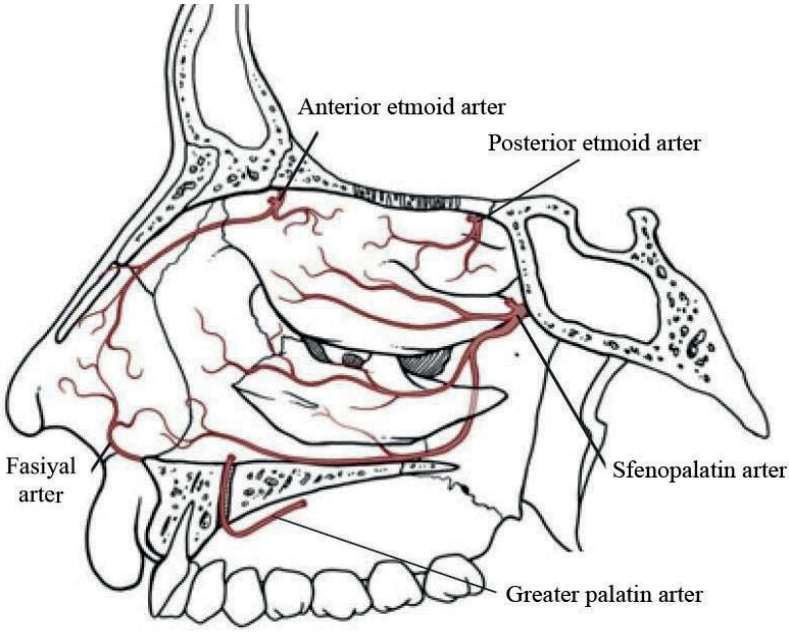

Figure 2: Arterial blood supply to the lateral nasal wall (Sinus Surgery Endoscopic and Microscopic Approaches.Levine H.L. , Clemente M.P. Authors .Thieme Medical Publishers Newyork. 2005; 26)

Nasal Cavity:

The nasal septum divides the nasal cavity into two parts. The front of the nasal cavity opens outward through the nostrils (nares), while the rear connects to the nasopharynx via the choanae. The boundaries of the choanae are defined by the vomer on the medial side, the medial lamina of the pterygoid process on the lateral side, the posterior edge of the horizontal lamina of the palatine bone on the inferior side, and the sphenoid bone on the superior side. Each nasal cavity has four walls:

1. Superior Wall (Roof): From front to back, it consists of the nasal bone, frontal bone, the cribriform plate of the ethmoid bone, and the body of the sphenoid bone. The anterior part of these bony structures contains nasal cartilages.

2.The inferior wall, known as the floor, is created by the palatine process of the maxilla and the horizontal lamina of the palatine bone. This structure is commonly referred to as the hard palate, serving as a barrier that separates the nasal cavity from the oral cavity. Approximately 2 cm behind the anterior edge of the floor, there is a minor depression where the incisive duct opens.

3. Medial Wall: It is created by the nasal septum, composed of the perpendicular plate of the ethmoid bone, the vomer, and the septal cartilage.

4. Lateral Wall: From anterior to posterior, it consists of the frontal process of the maxilla, lacrimal bone, the superior and middle nasal conchae of the ethmoid bone, and, lower down, the inferior nasal concha. Additionally, it includes the perpendicular plate of the palatine bone and the medial lamina of the pterygoid process (1).

On the outer side of the nasal cavity's lateral wall lie the superior nasal concha, middle nasal concha, and inferior nasal concha. The gaps connecting these three conchae to the outer nasal wall are known as the superior nasal meatus, middle nasal meatus, and inferior nasal meatus. The paranasal sinuses open into these openings. The hollow area on the posterior and upper aspect of the superior nasal concha is known as the sphenoethmoidal recess. Occasionally, above the superior nasal concha, there can also be a supreme nasal concha. When the middle nasal concha is removed, a rounded elevation is visible on the lateral wall. This elevation is called the ethmoidal bulla. Below the ethmoidal bulla, extending from the front to the back, is a projection belonging to the ethmoid bone called the uncinate process. The opening between the uncinate process and the ethmoidal bulla is referred to as the hiatus semilunaris. (1).

The nasal cavity is functionally divided into three parts: vestibulum nasi, regio respiratoria and regio olfactoria:

Vestibulum Nasi: It is the slightly wider entrance part of the nasal passage on the inner side of the nares. The skin in this area folds inward towards the septum nasi and lateral walls. The vestibulum nasi narrows towards the apex nasi, and it is bounded upwards and backwards by the limen nasi. The limen nasi is formed by the lower edge of the cartilago septi nasi. The vestibulum nasi is bounded externally by the cartilago alaris major and alae nasi, and internally by the crus mediale. The vestibulum nasi is covered with skin. Sweat and sebaceous glands are present in the skin. The hairs found here are called vibrissae. These hairs prevent foreign particles carried by the respiratory air from reaching deeper parts of the nasal cavity. Above the limen nasi, the area in front of the meatus nasi medius is referred to as the atrium meatus medii. Above the atrium and in front of the concha nasalis medius, there is a prominence known as the agger nasi.

Regio Respiratoria: It is the richly vascularized, shiny, dark, pink-colored area located at the back of the vestibulum of the cavitas nasi. It is related to respiration. In this region, the air coming into the nose is warmed, humidified to some extent, and passes into the lower respiratory passages. The mucosa in this area is covered with multi-layered ciliated columnar epithelial cells.

Regio Olfactoria: The parts above the concha nasalis superior and the septum nasi facing this concha are referred to as the regio olfactoria. It is associated with the sense of smell. The color of the mucosa in this region is gray-yellow. In this area, there are olfactory cells, their extensions called olfactory fila, and supporting cells (1).

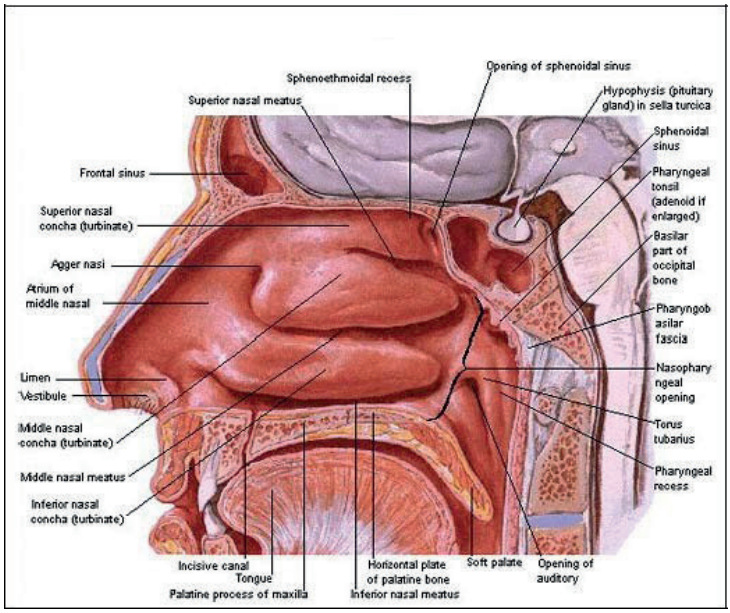

Figure 3. Nasal Lateral Wall (Atlas of Human Anatomy. Netter F.H. Author. Elsevier Medical Publishers. 2005; 66)

Nerves and Vessels of the Nasal Cavity:

Arteries: The nasal cavity receives its blood supply from several arteries. The A. Ethmoidalis anterior and A. Ethmoidalis posterior, both branches of the ophthalmic artery, provide vascular support to the nasal cavity's roof, ethmoidal cells, and frontal sinus. The A. Sphenopalatina, a branch of the Maxillary artery, nourishes the turbinates, meatuses, nasal septum, and sphenoidal sinus. The R. Septi nasi, a branch of the superior labial artery, delivers blood to the nasal septum in the vestibular region. The A. Alveolaris superior posterior and A. Infraorbitalis, branches of the Maxillary artery, supply blood to the maxillary sinus. Lastly, the R. Pharyngeus, a branch of the Maxillary artery, provides vascular support to the sphenoidal sinus (1).

Veins: Within the submucosal layer, there is an extensive venous plexus, primarily situated beneath the middle nasal concha, inferior nasal concha, and the lower portion of the nasal septum. These veins eventually drain into the V. facial vein, V. sphenopalatine vein, and V. ophthalmic vein. In instances where the foramen caecum remains open, one of the nasal cavity veins may establish a connection with the superior sagittal sinus (1).

Nerves: The N. ethmoidalis anterior (a branch of the N. nasociliaris) receives sensory input from the nasal septum and the front portion of the lateral wall. The N. alveolaris anterior superior (a branch of the N. maxillaris) transmits sensations from the anterior part of the inferior nasal concha and the inferior meatus. The N. nasopalatinus (a branch of the N. maxillaris) conveys sensory signals from the middle section of the nasal septum. The N. palatinus major (a branch of the N. maxillaris) is responsible for sensory perception in the posterior part of the lateral wall. The N. olfactorius spreads across the mucosa of the olfactory region and plays a role in the sense of smell. Additionally, branches from the pterygopalatine ganglion provide sensory input from the posterior and upper regions of the nasal cavity's roof and septum (1).

Lymphatics of the External Nose and Nasal Cavity: They drain into the submandibular lymph nodes, parotid lymph nodes, buccal lymph nodes, and deep cervical lymph nodes (1).

Nasal Histology:

A substantial portion of the rear two-thirds of the nasal cavity is covered by pseudostratified ciliated columnar epithelium that includes goblet cells. The mucous membrane, housing numerous mucous and serous glands, is tightly anchored to the underlying periosteum or perichondrium within the lamina propria. Notably, the basal membrane dividing the lamina propria from the respiratory epithelium is thicker in this area compared to other regions. The lamina propria of the mucous membrane is naturally richly supplied with blood vessels.

In light microscopic examinations of the inferior nasal concha, a dense and uniform basal membrane measuring 10-15 nm in thickness is observable just beneath the intact epithelium. When examined using electron microscopy, this basal membrane is composed of a subepithelial basal lamina and thick collagen fibers. The basal lamina runs parallel to the basal cell membrane of the epithelial cells and is comprised of a 100 nm thick lamina densa, along with a 60 nm thick lamina rara. The lamina rara extends between the lamina densa and the basal epithelial membrane. In cases of concha hypertrophy or inflammation, inflammatory cells such as granulocytes, monocytes, and lymphocytes can infiltrate the space between the basal membrane fibers.

In the anterior section, the lining of the vestibule consists of keratinized stratified squamous epithelium. Moving towards the caudal part of the vestibule, you'll find hair follicles, sweat glands, and sebaceous glands. Deeper within, the keratinized epithelium transitions into non-keratinized stratified squamous epithelium. As you progress further backward, the epithelial layer becomes progressively flatter, eventually giving way to pseudostratified ciliated columnar epithelium. The upper portion of the superior nasal concha is covered by non-ciliated columnar epithelium, known as olfactory epithelium. The superficial subepithelial layer is closely associated with an extensive capillary network.

Deeper arterioles nourish the periglandular capillaries. There are numerous venous sinuses and spaces between capillaries and venules. These sinuses and spaces are interconnected by capillaries and lead to two rows of arterioles. Their walls are supported by elastic fibers and contain smooth muscle fibers that run in a circular and spiral manner, controlled by the autonomic nervous system. These muscle fibers regulate vasoconstriction and vasodilation according to the body's physiological needs. At the distal ends of the sinuses, there are circular muscle bundles acting as sphincters. The engorgement of these sinuses forms an erectile structure. Hence, the term "erectile tissue" is often used to describe this vascular structure that surrounds the inferior and middle nasal conchae and, to a lesser extent, the superior nasal concha and septum (2, 3).

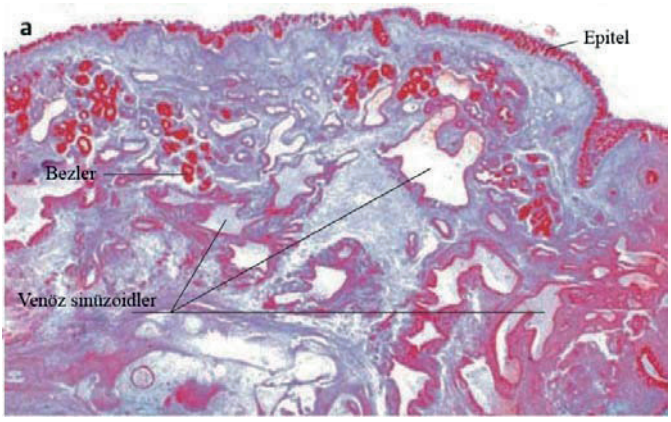

Figure 4: Histological section showing the structure of the lamina propria in the nasal mucosa (Basic Otorhinolaryngology. Probst R., Grevers G., Iro W. Authors. Thieme Medical Publishers Newyork. 2006;13)

The nasal conchae are typically lined with pseudostratified ciliated columnar epithelium that includes goblet cells. There are exceptions to this pattern, notably at the anterior tip of the inferior concha and on the lateral surface of the superior concha. At the anterior tip of the inferior concha, the lining consists of non-keratinized squamous epithelium, resembling the nasal vestibule. Conversely, the lateral surface of the superior concha is covered with olfactory mucosa (4,5).

An important distinguishing feature in conchal histology is the presence of venous sinuses surrounded by numerous thin-walled smooth muscles within the mucosa. These venous sinuses, enveloped by these delicate smooth muscles, contribute to the increased thickness of the mucosa in the conchae compared to the norm. In the inferior concha, there are a greater number of venous sinuses, whereas in the middle concha, more submucosal glands are present. When stimulated by parasympathetic innervation and specific neuropeptides, the venous sinuses become engorged with blood, leading to a significant increase in mucosal thickness, and consequently, an enlargement of the conchae (6).

The olfactory region of the nasal cavity is coated with specialized olfactory epithelium, responsible for our sense of smell. This olfactory neuroepithelium blankets the cribriform lamina, the superior concha, and the upper part of the septum. It occupies approximately 200-400 mm2 within each nasal passage, making up around 2.5-3% of the nasal mucosa. Within the lamina propria, you'll find tubuloalveolar Bowman's glands that secrete mucus, as well as blood vessels, connective tissue, and axons of olfactory neurons (7).

Paranasal sinuses are also lined with respiratory epithelium and serve a function in heating and humidifying the inhaled air. Similarly, the nasopharynx,

which is also covered with respiratory epithelium, provides protection against antigens present in the inhaled air through the numerous lymphoid tissues located beneath the epithelium (8).

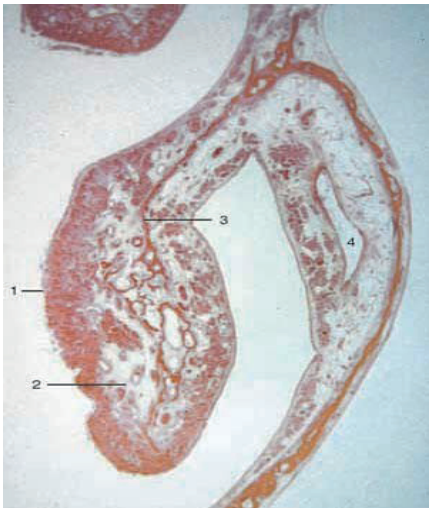

Figure 5: Histological section of the inferior turbinate (Functional Recostructive Nasal Surgery. Huizing E.H. ,de Groot J.A.M. Authors. Thieme Medical Publishers Newyork. 2003;3

Nasal Embryology:

By the conclusion of the fourth week of gestation, facial prominences begin to take shape, primarily composed of neural crest-derived mesenchyme and predominantly originating from the first pair of pharyngeal arches. The maxillary prominences are positioned laterally to the stomodeum, whereas the mandibular prominences become apparent caudally to this structure. The frontonasal prominence, originating from the mesenchyme in front of the developing brain vesicles, constitutes the upper boundary of the stomodeum. Adjacent to the frontonasal prominence on both sides, local surface ectodermal thickenings called nasal (olfactory) placodes form under the inductive influence of the ventral part of the forebrain (9).

During the fifth week of development, the nasal placodes undergo invagination, giving rise to the formation of nasal pits. During this process, tissue swellings appear around each pit, ultimately leading to the development of nasal prominences. The outer edge of these prominences is referred to as lateral nasal prominences, while the inner edge is known as medial nasal prominences (9).

Over the subsequent two weeks, the maxillary prominences undergo continued growth in size. Concurrently, they expand medially, exerting

pressure on the medial nasal prominences towards the midline. As development progresses, the groove that initially separates the medial nasal prominence from the maxillary prominence disappears, leading to the fusion of these two structures. Consequently, the upper lip is ultimately formed by the fusion of two medial nasal prominences and two maxillary prominences. It's important to note that the lateral nasal prominences do not play a role in the formation of the upper lip. Conversely, the lower lip and jaw originate from the mandibular prominences, which unite in the midline (9).

Initially, there exists a deep groove, the nasolacrimal groove, separating the maxillary and lateral nasal prominences. At the base of this groove, an epithelial cord forms from the ectoderm above and subsequently separates from the overlying ectoderm. This cord eventually undergoes canalization, resulting in the formation of the nasolacrimal duct. The upper portion of the nasolacrimal canal expands to give rise to the lacrimal sac. Following the separation of this cord, the maxillary and lateral nasal prominences merge with each other. This fusion causes the nasolacrimal duct to extend from the inner corner of the eye to the inferior nasal meatus. Subsequently, the maxillary prominences continue their growth to eventually form the cheekbones and maxillary bones (9).

The nose consists of five facial prominences: the frontal nasal prominence contributes to the formation of the nasal bridge, while the fused medial nasal prominences give shape to the nasal tip and dorsum. Additionally, the lateral nasal prominences are responsible for the development of the nasal wings, also known as alae (9).

The accurate knowledge of anatomy plays a pivotal role in all surgical operations. With appropriate anatomical understanding, the success of the surgery will be not only evident but also complications will be significantly reduced. Rhinoplasty, both in terms of surgical success and potential complications, is a highly intricate procedure. Surgical success hinges on a multitude of factors, among which a comprehensive grasp of the anatomical structure of the surgical site stands out as critical. Precise anatomical knowledge acts as a guide for the surgical team; understanding the anatomical structure facilitates surgical planning and execution, minimizing the occurrence of complications. This understanding enables the surgeon to comprehend the relationships between different tissues, thus ensuring a safer surgical process and contributing to more successful outcomes.

Rhinoplasty, in this regard, is complex due to its dual impact on both surgical success and the likelihood of complications. As a surgery focused on enhancing nasal aesthetics, it demands an in-depth understanding of nasal anatomy, given its central position on the face. Without accurate anatomical knowledge, interventions that are incomplete or misguided in relation to the

nuances of nasal structure can lead to undesirable outcomes. For instance, a rhinoplasty performed without considering the balance of internal and external nasal tissues can result in serious complications such as breathing difficulties. Consequently, surgeons undertaking rhinoplasty procedures need a profound understanding of nasal anatomy.

In conclusion, anatomical knowledge plays a critical role in achieving surgical success and minimizing the risks of complications. This is particularly evident in the execution of intricate procedures like rhinoplasty. Surgeons' comprehensive understanding of anatomical structures, coupled with proper training and experience, paves the way for safer procedures and the attainment of desired outcomes. Ultimately, this enhances patient satisfaction and elevates the overall success of surgical interventions.

Rhinoplasty is a surgical procedure known as nose reshaping or nose correction surgery. This procedure aims to improve the nasal functions both aesthetically and functionally by altering the cartilage and bone structure of the nose. It can especially correct deformities that are either congenital or result from trauma. It can also be combined with septoplasty to address breathing issues. Rhinoplasty is considered one of the most complex procedures in aesthetic surgery, and therefore, it's recommended to be performed by an experienced surgeon. Results are typically permanent, but the recovery process can vary from individual to individual (10,11).

Dorsum-preserving rhinoplasty is a technique that has gained popularity in rhinoplasty procedures in recent years. In this method, the nasal dorsum is preserved to achieve more natural results. In traditional rhinoplasty techniques, the cartilage and bone on the nasal dorsum are usually removed to achieve a flatter and more aesthetic appearance. However, in inexperienced hands, this approach can impair nasal functions and potentially result in an artificial appearance (12,13).

Dorsum-preserving rhinoplasty, in contrast to classical techniques, maintains a significant portion of the nasal dorsum and makes only minimal changes, ensuring a more natural look. This technique can help patients to be satisfied with both the aesthetic and functional outcomes. Another advantage of the dorsum-preserving approach is that the recovery process is generally faster and less complicated (13).

Considering the importance and growing popularity of this approach, it is crucial to consider this technique when deciding on rhinoplasty. However, selecting an experienced surgeon is vital to achieve the best result.

In rhinoplasty, two primary surgical approaches are commonly employed: open (external) and closed (endonasal). Here's a brief description of both:

Open Approach (External Rhinoplasty):

In the open approach, an incision is made across the columella, the narrow strip of tissue that separates the nostrils. This allows the skin to be lifted off the nasal structures, providing the surgeon with a direct view of the underlying nasal anatomy. The primary advantage of the open approach is the enhanced visibility and accessibility it offers, making it especially useful for more complex cases or when precise modifications are needed. However, it may result in a small, albeit usually inconspicuous, scar on the columella and potentially longer recovery times compared to the closed approach.

Closed Approach (Endonasal Rhinoplasty):

The closed approach involves making incisions inside the nostrils, without any external cuts. This means that all surgical modifications are made through these internal incisions, requiring the surgeon to manipulate and modify the nasal structures without direct visualization. The closed approach has the advantage of leaving no visible external scars and may have a slightly shorter recovery period. However, it might provide less direct access to some nasal structures, making it more challenging for intricate or extensive modifications (14,15).

In rhinoplasty, there are periods when two approaches, closed and open, became popular. Initially, rhinoplasty, which began with the closed approach, shifted to the open technique due to challenges in learning, the ease of mid-vault approaches, a clearer visual perspective, and the ample opportunity for documentation. The easier learning curve for inexperienced surgeons has quickly popularized this among newcomers. No matter how much surgical habits and experience increase, minor revisions and complicated and challenging cases are still encountered in rhinoplasty. Rhinoplasty requires a variable surgical planning. There are multiple techniques to solve the same problem, and when combined, countless surgical plans emerge. Just as each patient requires a different combination of techniques, each surgeon has adopted a different approach and presented their own planning. This has actually made it difficult for rhinoplasty surgeons to explain their techniques amongst themselves. One of the most heard phrases in surgical congresses is "this technique works or doesn't work in my hands." The inability to explain in enough detail during presentations is actually one of the most significant problems here. This is the reason why surgical experience is so essential. No matter how theoretically knowledgeable one is, it takes time for an inexperienced surgeon to learn to use this knowledge in practice (16).

Preservation rhinoplasty is actually a type of closed rhinoplasty. The aim here is to correct the nose without separating the upper lateral cartilages from the septum and without disrupting tissue integrity. This means that the complications

that may arise afterwards will not be very complex. Every technique has its own inherent problems; firstly, not every technique is suitable for every patient. This is because each patient's facial structure, skin texture, and nasal deformities are different. Preserving the dorsum completely can sometimes lead to minor complications. In patients who undergo dorsum preserving surgery with a closed rhinoplasty approach, letdown or pushdown techniques are used. In the letdown technique, a wedge resection is made from both lateral nasal bones, lowering the dorsum level; and an inferior or superior strip is removed from the septum to perform this procedure. In the pushdown technique, no bone resection is made, and the bone is placed inside (17,18). (Figure 1)

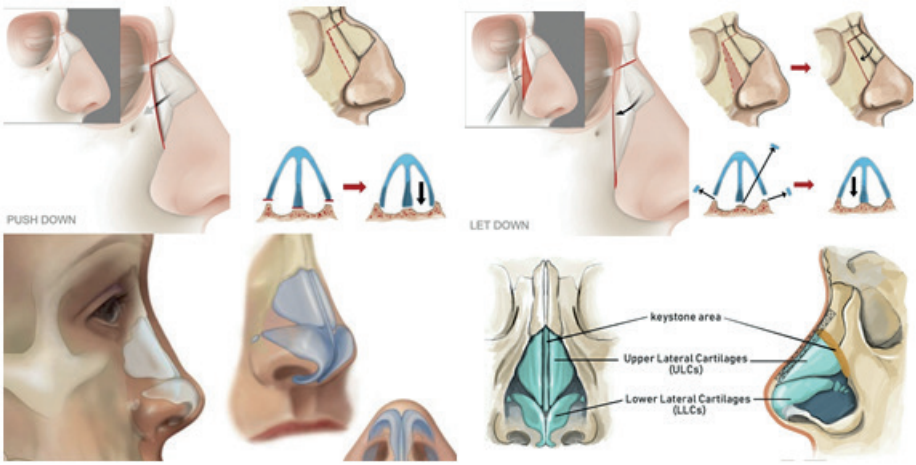

Figure 6. Letdown and Pushdown Technique

"Letdown Preservation Rhinoplasty" is advantageous especially for patients with a high radix, referring to those with a high nasal root. This method allows for smoother and more natural transitions between the forehead and the nose, resulting in a more harmonious profile. Additionally, for those with crooked noses, the letdown technique, when correctly applied, can yield effective results (19).

The utilization of the Piezoelectric device ensures surgical osteotomies are more precise, smooth, and controlled. This can enhance the success rate of the letdown technique and reduce post-operative complication risks. Particularly in crooked noses, addressing bone irregularities such as C or reverse C deformities is challenging. However, the Piezoelectric device, in conjunction with surgical burr, proves effective in overcoming these challenges.

In summary, the advantages of Letdown Preservation Rhinoplasty are:

Suitability for High Radix Patients: The technique facilitates aesthetic

outcomes by creating more natural transitions between the forehead and the nose for those with a high radix.

Effectiveness in Crooked Noses: Especially in cases with C or reverse C deformities, the letdown technique aids in rectifying asymmetric bone structures.

Use of the Piezoelectric Device: This device allows for controlled, smooth, and atraumatic osteotomies, subsequently decreasing the complication risks.

Application of Surgical Burr Device: Especially when the bone is thinned and its strength reduced, the "tur" method is effective in correcting bone asymmetry. While traditional osteotomies can lead to uncontrollable bone fractures, the Piezoelectric device facilitates more controlled incisions. (20-22).

Modified Letdown Rhinoplasty Assisted by Piezoelectric Device and Surgical Burr: Technical Steps:

1. An inverted v incision is made on the columella, and nasal tip decortication is performed. (Figure 2)

Figure 7. Inverted V Incision

2. Subsmass dissection is performed on the upper lateral and lower lateral cartilages. (Figure 3)

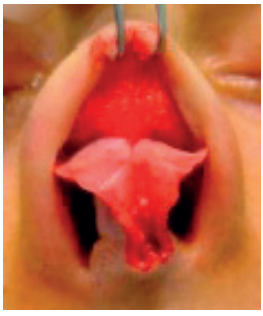

Figure 8. Subsmas Nasal Tip Decortication.

3. Entry is made below the periosteum on the nasal bone, followed by an extensive subperiosteal elevation. (Figure 4)

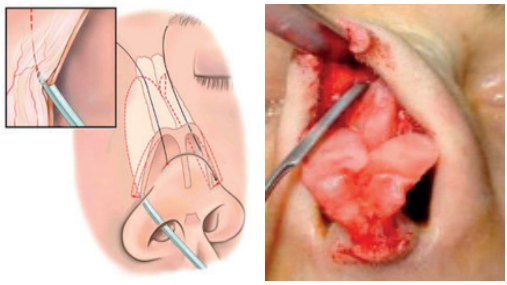

Figure 9. Nasal Bone Dissection with Wide Subperiosteal Elevation

4. If the patient has a high radix, the skin is elevated subperiosteally up to the frontal protrusion. If the radix level of the patient is appropriate, the skin on the radix is preserved and not elevated, as a significant drop in the radix level is not desired.

5. For septal graft retrieval, without opening the upper lateral, the septum is exposed using a subperichondrial dissection; a septal graft is retrieved from a broad area suitable for the septal deviation. A L strut is preserved. (Figure 5)

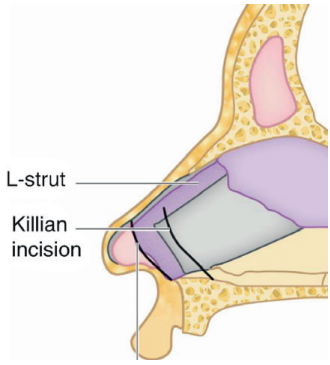

Figure 10. Obtaining a Septal Cartilage Graft

6. A bone wedge is removed from the upper level of the maxilla's perpendicular bone, preparing the septum for the letdown procedure.

7. Moving to the dorsum, excesses on the Upper lateral cartilage are removed. During the removal of the upper lateral cartilages, one must ensure that the inner mucosa of the septum is not separated. If there's a bone hump, it is removed with the help of a surgical burr. Irregularities on the cartilage below the bony cap are also corrected. (Figure 6)

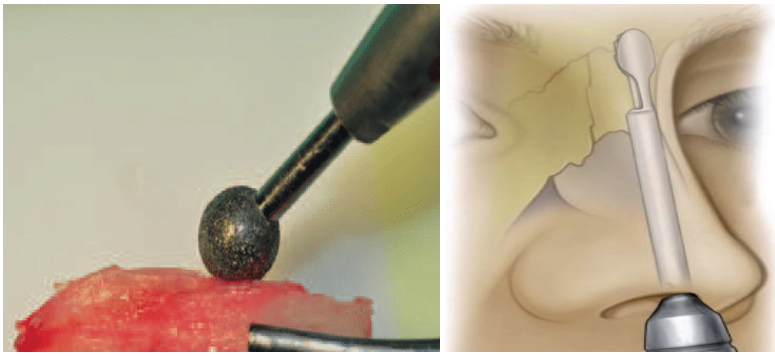

Figure 11. Removal of Nasal Hump with Surgical Burr

8. If asymmetry exists in the nasal bones, the surgical burr is used to adjust the concave and convex surfaces, aiming for symmetry.

9. Approximately 2-3 mm below the junction of the maxillary and nasal bone, a piezo cut is made, followed by bilateral 2 mm bone resections. The osteotomies are extended up to the radix.

10. If the patient has a high radix, lateral osteotomy cuts are joined, and the letdown is performed. (Figure 7)

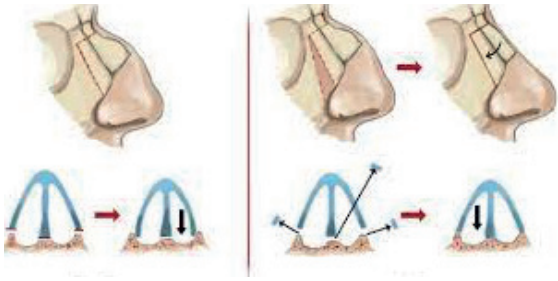

Figure 12. Letdown Osteotomy with Piezo Device

11. If the patient does not have a high radix, the skin on the radix is preserved to prevent excessive projection loss.

12. After performing the letdown procedure on the patient, the height of the dorsum is checked, and an approximate 2-3 mm septal split is removed. The septum is then fixed to the nasal spine, followed by performing the tipplasty procedure.,

In conclusion, while "Letdown Preservation Rhinoplasty" has its advantages, it's important to note that there are various factors to consider in choosing the right technique for each individual patient. The technique's

benefits, such as its suitability for patients with a high radix and effectiveness in correcting asymmetries, are significant. However, it's worth remembering that no single approach fits all patients due to the diverse nature of facial structures, skin types, and nasal deformities.

While the Piezoelectric device and surgical burr offer improved precision and controlled incisions, it's important not to overlook the importance of surgical experience. The success of any rhinoplasty technique depends not only on the tools but also on the surgeon's skill and expertise in evaluating each patient's unique features and tailoring the approach accordingly.

In the dynamic field of rhinoplasty, it's also essential to consider that alternative methods might provide effective solutions as well. The diversity of patient needs, and anatomical variations suggests that maintaining a broad understanding of different approaches and techniques can be beneficial in achieving optimal results. It's important for surgeons to adopt a flexible approach and be open to utilizing alternative methods, when necessary, always prioritizing the safety and satisfaction of the patient.

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Chapter 8

THE RELATIONSHIP BETWEEN SLEEP AND EATING DISORDERS IN CHILDREN

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Introduction

Sleep has an important role for health and is a reversible state in which the threshold of living things to respond to stimuli increases. A healthy adult falls asleep after putting his head on the pillow within 5-18 minutes, and the sleep cycle continues every 5-20 minutes (Algın et al., 2016).

Circadian rhythm refers to the change in the organism's naturally functioning processes that repeat cyclically one after the other in a 24-hour period (Akinci & Orhan, 2016).

The center responsible for the circadian rhythm in humans is the suprachiasmatic nucleus (SKN), located in the anterior hypothalamus, which we know as the biological clock. Sleep cycle, growth hormone and melatonin secretion are under the control of SKN (Selvi et al., 2011).

People generally have the ability to live during the day. However, due to today's conditions, especially shift workers have switched to eating at night, working at night, being more active at night and sleeping during the day. This situation causes disruption in the Circadian Rhythm. As a result of this deterioration, metabolic diseases such as obesity and type 2 diabetes mellitus may occur. Today, obesity is at a frightening level in the world and in Turkey. Changes in people's lifestyles, especially working late at night, negatively affect their diet, disrupting their energy balance and causing weight gain (Akbay, 2020).

Sleep Periods

Approximately 20-25% of the sleep is spent in the Rapid Eye Movement Sleep (REM) period, and the other part is in the Non-Rapid Eye Movement (NREM) period (70-75%). When sleep disorders occur, this system is disrupted and sleep efficiency decreases. People with sleep problems experience impaired learning and memory functions, excessive daytime sleepiness, and cognitive dysfunction (Dere et al., 2010).

NREM Sleep: It constitutes 70-80% of total sleep. It is divided into 4 phases. Stage 1 and 2 superficial sleep; Stages 3 and 4 are defined as deep sleep (Keskin & Tamam, 2018).

REM Sleep: It constitutes 20-30% of total sleep. It is an active sleep phase. The first REM phase occurs approximately 90 minutes after the onset of sleep, and then 3-5 REM phases occur per night at approximately 90 minute intervals (Keskin & Tamam, 2018).

Factors Affectıng Sleep Qualıty

Sleep is a necessity for humans and a need that varies from person to person. Sleep requirement in humans; It varies according to gender, age, health status, nutrition, environmental environment, activity and personal characteristics (Lana et al., 2019).

Age and Gender: The REM phase is more common in men than in women. A study states that women's sleep duration is longer than men and that women experience more sleep problems due to hormonal changes that occur with age (Duman, 2016). As dissatisfaction with sleep quality increases due to differences in normal sleep patterns with age, the likelihood of sleep problems increases. Sleep disorders are common in the elderly. Generally, total sleep time and REM sleep rate decrease as we age (Aktaş et al., 2015).

Chronic Diseases: Some studies have shown that the reason for the prevalence of sleep disorders is existing chronic diseases and lack of physical activity (Aktaş et al., 2015), (Ölmez et al., 2015). Problems in the sleep cycle; Sleep problems such as breathing problems, insomnia, and the use of medications due to these diseases may cause sleep problems. There is a connection between insomnia/sleep disorders and body weight gain. It has been shown that insomnia affects neuroendocrine control, causing an increase in appetite. Insomnia causes a decrease in leptin levels and an increase in ghrelin levels.

Physical Activity: In a study conducted by Vuori et al., participants stated that physical activity; They reported that it made it easier for them to fall asleep, provided deeper sleep, and felt better in the morning (Vuori et al., 1988). It is known that adults who do not have any health problems and are active during the day sleep better in connection with physical activity (Kline, 2013).

Environmental Factors: Factors such as increased ambient light, longer working hours, increased travel time due to traffic congestion, increased overtime and night shifts, television, radio and internet use, ambient ventilation, temperature, odor, noise and lighting are environmental factors that affect sleep.

Nutrition and Sleep

Nutrition; It is used in the body by consuming foods that provide the necessary nutrients and bioactive components to maintain life, grow and develop, improve, protect and develop health, increase the quality of life, and ensure productivity (Peuhkuri et al., 2012). Insufficient sleep can negatively impact learning, memory, cognition, a person's perception of pain, immunity, and inflammation. Additionally, changes in glucose metabolism and neuroendocrine function as a result of chronic or partial sleep deprivation can lead to changes in carbohydrate metabolism, appetite, nutrient intake, and protein synthesis (Halson, 2014).

The relationship between nutrition and sleep is complex. Nutrition varies significantly depending on the digestive and metabolic characteristics of each individual, and nutritional factors vary with different eating patterns (Zhao et al., 2020). Not eating healthy is linked to increased insomnia and disruption of sleep patterns. It has been shown that short sleep duration changes the diet

pattern and may cause obesity, and the diet may also cause changes in sleep patterns. In other words, eating and sleeping are in a two-way interaction (Alim et al., 2021).

The Relationship Between Sleep Hormones And Nutrition

Many neurotransmitters have been described that regulate the sleep cycle. These include neurotransmitters such as serotonin, gammaaminobutyric acid (GABA), orexin, melanin-concentrating hormone, noradrenaline, and histamine. For this reason, sleep can be associated with nutrition types that have an effect on neurotransmitters that have a sleep-regulating role (Helvacı & Ayhan, 2019).

Ghrelin and Leptin: Ghrelin is a sleep-promoting factor in humans. Its most well-known feature is that it enables the emergence of slow wave sleep (Weikel et al., 2003). Ghrelin and leptin work in an opposing relationship in the body. Ghrelin is secreted from the endocrine glands of the stomach. It is also produced in the human body from the duodenum and brain region (Yaprak, 2019). While ghrelin levels are high during sleep, ghrelin levels decrease in the morning hours. Irregularity in ghrelin level increases hunger during sleep (Yaprak, 2019). High ghrelin levels in sleep deprivation lead to increased hunger and food intake (Spiegel et al., 2009).

Seratonin, Melatonin and Dopamine: It was identified by dermatologist Aaron Lerner in 1958 by synthesizing tryptophan (N-acetyl-5-methoxy tryptamine), one of the protein building blocks of melatonin, from serotonin. Melatonin; It is a circadian hormone produced by the pineal gland and regulates endogenous rhythm. It is regulated by the light and dark cycle (Daugaard et al., 2017).

Growth Hormone and Cortisol: Growth hormone is a hormone that comes into play during sleep and is important for cell health, allowing cell growth and renewal. Growth hormone and cortisol are as important as leptin and ghrelin in regulating appetite. They are hormones that are partially secreted depending on sleep duration, quality and sleep timing (Leproult & Cauter, 2010).

Sleep Mechanisms and Nutrition Relationship

Sleep state is a dynamic process regulated by two factors; circadian rhythm and homeostatic drive. This two-process model for sleep regulation was developed to demonstrate the interaction of the homeostatic drive and the circadian system in determining the timing of sleep and total sleep time (Richter et al., 2014). The homeostatic process is associated with sleep and wake function, while the circadian process is controlled by a circadian oscillator. The homeostatic drive increases with prolonged wakefulness and decreases again

with sleep. The circadian system, on the other hand, is independent of sleep and wakefulness time, but is affected by environmental factors such as light and interacts with the homeostatic drive. The suprachiasmatic nucleus in the hypothalamus is at the center of this process (Doherty et al., 2019).

Macro Nutrients and Sleep

Although the number of studies on this subject is small and the study methods are inconsistent, the macronutrient pattern of the dinner eaten in the evening caused significant differences in night sleep times in healthy people without sleep problems (Özdişli, 2017). Carbohydrates: It has been observed that consuming a meal with a high percentage of carbohydrates increases the time spent asleep and shortens the transition to sleep (Karadağ & Aksoy, 2009). An increase in the time spent sleeping was found after a meal high in both calories and carbohydrates (Gezmen Karadağ & Aksoy, 2009).

Proteins: When the relationship between proteins and sleep is examined, tryptophan stands out. Tryptophan is an amino acid that is a precursor to serotonin, which causes drowsiness. L-tryptophan is popular today as an insomnia remedy. It has been concluded that use of 1 g/day is effective in sleep disorders such as insomnia. A study was conducted on 15 insomnia patients, and a 1 g tryptophan supplement was given to the patients. It was observed that there was a decrease in the time it took them to fall asleep (Keskin and Tamam, 2018).

Oils: If we look at a study between sleep and fats, it has been reported that a breakfast meal high in fat increases alertness and attention during the day, while a dinner meal high in fat reduces nighttime sleep and causes early waking (Cao et al., 2016). Long-chain fatty acids play an important role in the pineal gland and melatonin production. In recent years, interest in the biological effects of fatty acids on the sleep/wake process has increased.

Micronutrients, Antioxidants and Sleep

The effect of vitamin D, a fat-soluble vitamin, on sleep has been widely researched (Cao et al., 2018). As a result of studies, it has been observed that vitamin D deficiency causes a decrease in sleep duration, sleep health and quality. When short and long sleep durations were compared, it was observed that vitamin C was included less in the diet in short sleepers. It has been found that dietary magnesium intake reduces daytime sleepiness, especially in women. Main sources of magnesium; legumes, hazelnuts, vegetables, and whole grains (Cao et al., 2018).

The Relationship Between Sleep and Eating Disorders

Sleeping and eating behavior are two behaviors that are related to each other. In healthy, young adult males, severe dietary restrictions, continuous eating

rumination, binge eating, sleep disturbances and decreased need for sleep. It has been determined that it causes physical and psychological problems such as: On the other hand, sleep, restriction/insufficient sleep causes increased appetite, carbohydrate and fatty foods. It has been reported that it is preferred and this leads to weight gain. It has been stated that it can open (Soeres et.al., 2013).

The Relationship between Anorexia Nervosa and Bulimia Nervosa and Sleep

Sleep studies on patients with AN are quite limited. with AN (**Anorexia Nervosa)** It is estimated that 50% of patients experience sleep problems. patients with AN When the sleep problems they experience are evaluated clinically, individuals' hunger condition, type of disease (such as restrictive type AN), and degree of weakness of the patient. It has been reported to be associated with (BMI<17.5kg/m2) (Siebern, and Robinson, 2013). The most common observed in patients. It was determined that the sleep problem was waking up early in the morning, light sleep and night. It has been determined that sleep problems such as waking up more than once throughout the day may also occur. Eating behaviors such as bingeing and purging behaviors of individuals with sleep problems. It has been found that disorders are experienced more frequently (Siebern, and Robinson, 2013; Soeres et.al., 2013).

The Relationship Between Obesity and Sleep

The prevalence of obesity worldwide has increased approximately twice since the 1980s has increased times. According to WHO's 2014 data, 1.9 billion adults are overweight.

(39%), and approximately 600 million individuals are reported to be obese (13%). Obesity is considered a preventable health problem in our age. The main cause of obesity is the imbalance between the energy consumed and the energy spent. Therefore, reducing the consumption of fatty and high-energy foods and increasing physical activity is the most important lifestyle changes (WHO, 2015). This changes, the effects of sleep on energy balance, the increase in obesity. It has become quite remarkable. Short sleep duration, appetite regulator. It disrupts energy metabolism by changing the levels of hormones in the blood (Barot and Barot, 2013)**.** When sleep time is restricted through dietary changes for weight loss fat deposits in adipose tissue, where metabolism is negatively affected the catabolism of non-fat energy stores decreases and the catabolism of non-fat energy stores decreases. It has been reported that it increases (Barot and Barot, 2013)**.**

Depression

Depressed mood, loss of interest or desire, low energy, with feelings of guilt or low self-confidence, sleep, appetite and concentration disorders. It is a

common mental disorder that occurs together. These problems are chronic and it can be repetitive and interfere with the individual's ability to perform daily tasks depression can also cause suicidal tendencies (Kaya et.al., 2007). Today, ıt is estimated that approximately 350 million people experience depression. The level of depression in children varies depending on the individual. In the development of depression genetic structure also plays a very important role. First in society in individuals with a first-degree relative with major depressive disorder, major it has been determined that the risk of depression is 2-3 times higher (O'Connor et.al., 2016). Especially individuals aged 30 and over in first-degree relatives with major depressive disorder it has been determined that the risk (relative risk) of developing recurrent major depressive disorders is highest at young ages, depending on genetic characters. of depression the neurotransmitters serotonin and norepinephrine play an important role in the development of is considered. Levels of these two neurotransmitters in individuals with depression was determined to be low. Serotonin and norepinephrine affect mood apart from regulation, it also has effects on appetite, sleep and attention (O'Connor et.al., 2016).

The Relationship Between Depression, Sleep and Eating Disorders

Eating behavior disorders, depression, anxiety and low self-esteem it is associated with psychopathological components. For depression, eating behavior disorders is an important factor. Depression is common in patients with eating disorders. It has been reported to be seen (Büyükgöze-Kavas, 2007; Casper, 1998). In a study conducted in France, eating the prevalence of disorders is 20.5% and depression among those with eating disorders it was found that the probability of being 8.62 times higher (AOR=8.62, 95%CI 3.37-22.10; p<0.01)(105).

What Herpertz-Dahlmann et al. In the study (Herpertz-Dahlmann et.al., 2014), children between the ages of 11-17 were followed until the ages of 17-23. In this study, it was determined that there was eating disorder behavior between the ages of 11-17. significantly 1.31 times higher in young adulthood than children or adolescents reported to show more eating disorder behaviors. Eating disorder individuals with high symptoms are 1.58 times more likely to be overweight and 1.67 times more likely to be obese. Eating behavior disorders, depression, anxiety and low self-esteem it is associated with psychopathological components. For depression, eating behavior disorders is an important factor.

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of being 8.62 times higher (AOR=8.62, 95%CI,3.37-22.10; p<0.01)(105). What Herpertz-Dahlmann et al. In the study (Herpertz-Dahlmann et.al., 2014) children between the ages of 11-17 were followed until the ages of 17-23. In this study, it was determined that there was eating disorder behavior between the ages of 11-17. significantly 1.31 times higher in young adulthood than children or adolescents reported to show more eating disorder behaviors. Eating disorder it was determined that individuals with high symptoms were 1.58 times more likely to be overweight and 1.67 times more likely to be obese. Extreme underweight with early signs of depression in young adults there is a significant relationship between (OR=1.13; 95% CI:1.01-1.25, p=0.0016) has been determined. Restrictive eating behavior disorder in young people with depression it has been reported that it should be monitored for the risk of development (Herpertz-Dahlmann et.al., 2014)

In another study, depression was observed in students with high waist circumference measurements. The risk of developing symptoms was found to be 1.4 times higher. In the same study the risk of depression is 6.98 times higher in those with eating behavior disorders was found (OR = 6.98) (Lazarevich et.al., 2013).

Overweight and obese children or adolescents are more likely than their non-overweight peers to be usually shows more depressive symptoms. Studies in the literature show that depression supports the relationship between symptoms and being overweight or obese (Mooreville et.al., 2014; Goldfield et.al., 2010).In their first study by Mooreville et al. (Mooreville et.al., 2011), children between the ages of 12 and 17 depressive symptoms were found to be associated with total energy intake in students has been determined. In the second study, only girls were found among students between the ages of 8-17 students' depression symptoms were positively related to total energy intake. Uncontrolled loss of energy in young people with high levels of depressive symptoms. It has been determined that they get most of it from sweet foods (Mooreville et.al., 2014). Eating disorders studies on eating disorders, such as depression and anxiety it has been reported that there is a relationship between other psychiatric diseases (Tromp et.al., 2016; Wandar, 2012).

Researching the relationship between sleep, eating behavior disorders and depression the number of studies is also insufficient. Lombardo et al.'s (154) 1109 female students in their study on insomnia, eating behavior disorders and depression. The relationship between eating disorder and eating disorder was examined as the severity of insomnia increased. It has also been reported that its severity has increased. Both insomnia and eating disorders it has been determined to be associated with depression (Lombardo et.al., 2014).

Conclusion

It is stated that sleep quality and eating disorders are related. However, the relationship between sleep quality and eating disorders has been investigated. The number of studies is quite insufficient. Children's sleep-eating disorders in order to better understand the relationship between there is a need to increase the studies.

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Chapter 9

PLEXUS SACRALIS AND ITS BRANCHES

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Introduction

Plexus; It is a word used to mean knitting and network. Plexus lumbosacralis means wide nerve spread in the lumbar and rump region. This neural network consists of the fusion of the plexus lumbalis and the plexus sacralis (Arifoğlu, 2016).

Plexus lumbosacralis carries the signals coming from both the lumbar and sacral parts of the body to the brain and initiates the voluntary movements coming from the brain. The medulla spinalis, which is the origin of the fibers belonging to the parasympathetic system that goes to some regions, especially the sympathetic system, is located in the columna vertebralis (Tunç, 2003). Although there are 33 segments in the spinal cord, there are 31 pairs of spinal nerves that separate from these segments. It has 33 segments, eight cervical, twelve thoracic, five lumbar, five sacral and one coccygeal pair. Spinal nerve fibers emerging from the plexus lumbosacralis innervate the lower extremities and organs of the body. It ensures the realization of various processes such as movement and excretion by transmitting the messages it receives from the brain. Even the slightest damage to the spinal nerves can lead to fatal consequences (Tunç, 2003).

Plexus sacralis anatomy

It is located on the back wall of the pelvis, in front of the musculus piriformis, behind the arteria iliaca interna and vena iliaca interna, the ureter, the last part of the ileum on the right, and the sigmoid colon on the left. It descends down from the inner edge of the musculus psoas major and in front of the articulatio sacroiliaca. The truncus lumbosacralis, formed by the anterior branch of the lumbar 5 spinal nerve and the nerve branching from the anterior branch of the lumbar 4 spinal nerve, and all of the sacral 1, sacral 2, sacral 3 spinal nerves are formed with the participation of a part of the anterior branch of the sacral 4 spinal nerve. The sacral plexus extends within the pelvis and exits through the foramina sacralia anteriora holes of the sacrum (Bergman, 2009).

In front, it is adjacent to the arteria vena iliaca interna and its branches through the fascia pelvis parietalis. In the back is m. It is adjacent to piriformis. The branches going to the lower extremity exit the pelvis through the foramen ischiadicum majus (Bergman, 2009).

a) Nervus musculi quadrati femoris (L4 – L5 – S1)

b) Nervus musculi obturatorii interni (L5 – S1 – S2)

c) Nervus musculi piriformis (S1 – S2)

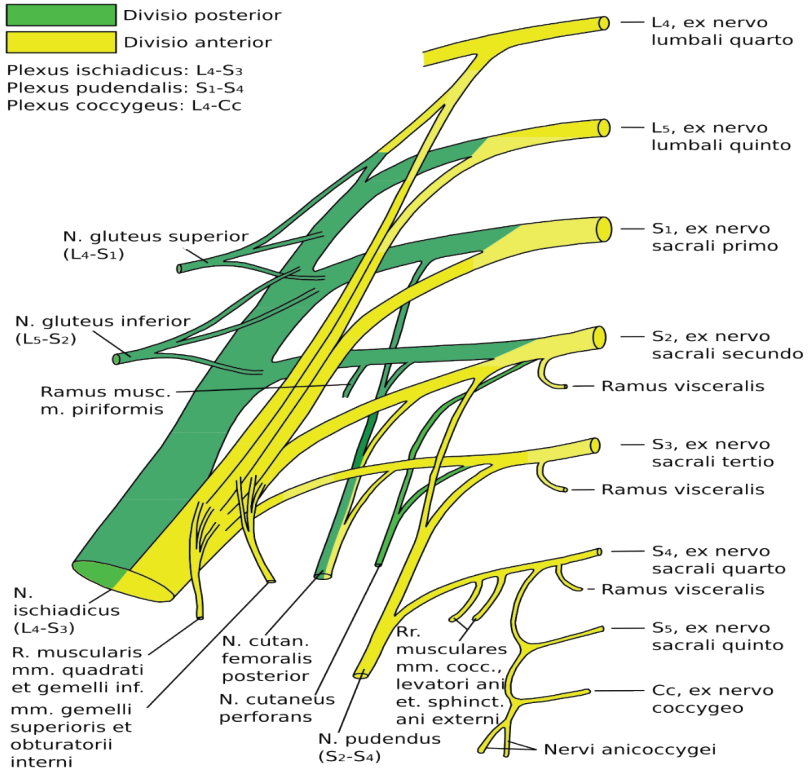
d) Nervus gluteus superior (L4 – L5 – S1)

e) Nervus gluteus inferior (L5 – S1 – S2)

f) Nervus cutaneus femoris posterior (S1 – S2 – S3)

g) Nervus ischiadicus (L4 – L5 – S1 – S2 – S3)

- Nervus tibialis (L4 – L5 – S1 – S2 – S3)
- Nervus fibularis (peroneus) communis (L4 – L5 – S1 -S2)



h)

Nervus pudendus (S2 – S3 – S4)

Figure 1: *sacral nerve branches*

a) Nervus musculi quadrati femoris (L4-L5-S1):

It gives branches to musculus quadratus femoris and musculus gemellus inferior. It exits the foramen infrapiriforme and leaves the pelvis (Büyükmumcu, 2017).

b) Nervus musculi obturatori interni (L5-S1-S2):

It gives branches to musculus obturatorius internus and musculus gemellus superior. It exits the foramen infrapiriforme and leaves the pelvis. It crosses the spina ischiadica and enters the fossa ischioanalis through the foramen ischiadicum minus. It is distributed on the musculus obturatorius internus (Tunç, 2003).

c) Nervus musculi piriformis (S1-S2):

It proceeds on the deep surface of Musculus piriformis and gives branches to this muscle (Tunç, 2003).

d) Nervus gluteus superior (L4-5-S1):

The nerve leaving the pelvis through the foramen suprapiriforme accompanies the branches of the arteria glutea superior and vena glutea superior between the musculus gluteus minimus and divides into two branches. Its upper branch gives branches to musculus gluteus minimus, and its lower branch gives branches to musculus gluteus medius, musculus gluteus minimus and musculus tensor fascia latae. It innervates musculus gluteus medius, musculus gluteus minimus, and musculus tensor fascia lata (Alfrei et al. 2006).

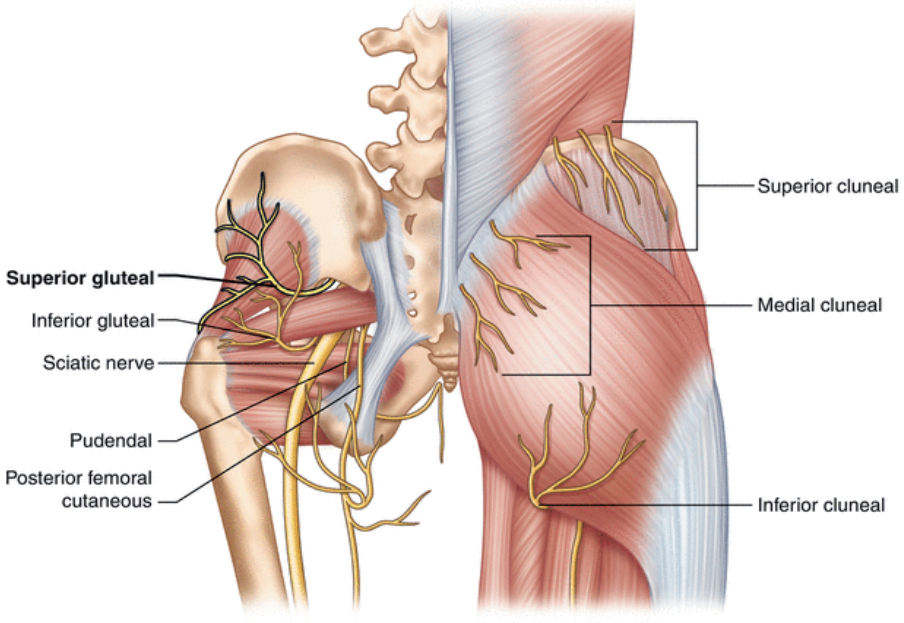
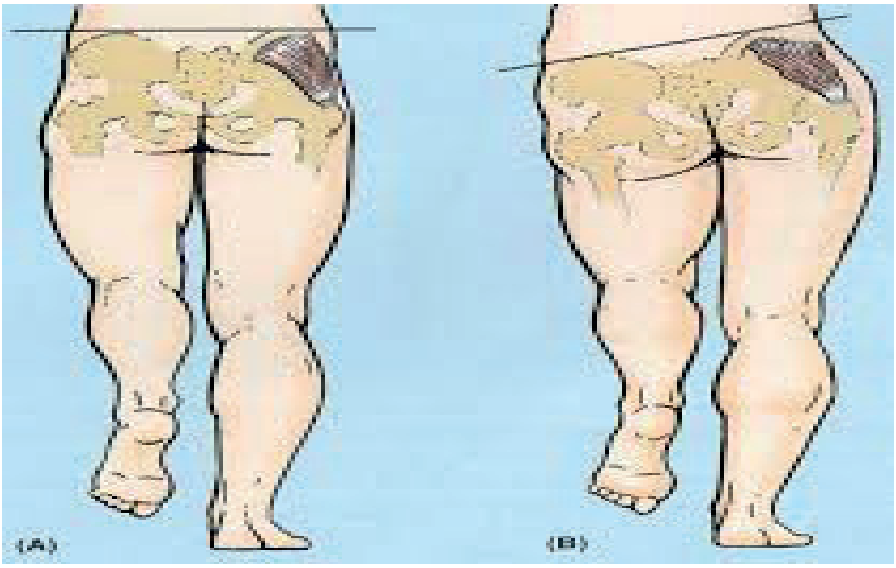


Figure 2: *Sacral nerve branches posterior profile*

In injury to the gluteus superior nerve, the pelvis-supporting function of the gluteus medius musculus is impaired. In this case, the patient leans towards the lesion side every time he takes a step. This symptom is called Trendelenburg sign. In a nerve lesion, for example, if the right gluteus superior nerve does not work, the left side pelvis cannot be pulled up while the right side presses the ground and the left side pelvis falls down. If the right and left gluteus superior nerves are damaged, duck walking is observed (Alfrei et al. 2006).



Resim 3: *Trandelenburg semptom picture (1909)*

e) Nervus gluteus inferior (L5 – S1 – S2):

The nerve leaving the pelvis through the foramen infrapiriforme proceeds on the deep surface of the gluteus maximus musculus and gives branches to the gluteus maximus musculus. Musculus innervates the gluteus maximus (Bergman, 2009).

f) Nervus cutaneus femoris lateralis (S1-S2-S3):

It passes through the foramen imperforate and proceeds downwards adjacent to the ischadicus nerve. It is distributed in the area up to the fossa poplitea on the skin of the posterior surface of the thigh. (Alfrei et al. 2006).

g) Nervus ischiadicus:

It is a continuation of the sacral plexus. It is the thickest nerve of the body, leaving the pelvis through the foramen infrapiriforme. It generally divides into terminal branches called nervus tibialis and nervus fibularis (peroneus) com-

munis in the 1/3rd of the thigh or above. Sometimes, variationally, these two nerves may emerge from the plexus sacralis separately (Alfrei et al. 2006).

Projection of the nerve ischiadicus on the skin: In the regio glutea, the first point between the outer 2/3 and 1/3 of the line connecting the tuber ischiadicum and the trochanter major is accepted as the second point, and the outer 3/5 and 2/5 of the sulcus glutealis. The midpoint of the regio poplitea is known as the third point. By combining these three points, the projection of the ischiadicus nerve on the skin is obtained. In the gluteal region, it passes in front of the musculus gluteus maximus, behind the musculus obturatorius internus, musculus quadratus femoris, musculus gemellus superior and inferior and comes to the thigh. It progresses together with the nerve cutaneus femoris lateralis and arteria glutea inferior in its immediate neighbourhood. In the thigh, it is located behind the musculus adductor magnus and in front of the musculus biceps femoris (Alfrei et al. 2006).

Nervus fibularis (peroneus) communis arises from the posterior part fibers of the sacral plexus, and nervus tibialis arises from the anterior part fibers. These two nerves, wrapped in a sheath, proceed as the ischiadicus nerve to the fossa poplitea. Sometimes, as a variation, these two nerves may arise separately and extend downwards.

Before the nerve ischiadicus divides into its terminal branches in the fossa poplitea, it gives somatomotor and articulatio coxae sensitive fibers from the nervus tibialis section of the nerve to the musculus semitendinosus, musculus semimembranosus, and caput longum of the musculus biceps femoris. Somatomotor branches arise from the nerve fibularis communis section to the caput breves of the musculus biceps femoris (Arıncı, 2014).

While the nerve ischiadicus carries the sensitive sensation of the entire foot skin and most of the leg skin, it gives somatomotor branches to the muscles on the back of the thigh and all muscles of the leg and foot (Atılhan et al., 2000).

A) Nervus tibialis:

It is the thicker terminal branch of the nerve ischiadicus. It crosses the biceps femoris muscle behind the caput longum and reaches the fossa poplitea. As it descends in the fossa poplitea, the arteria crosses the poplitea first from its outer side and then from its back, and proceeds on its inner side. It is located in the lower part of the fossa poplitea, in front of the two heads of the musculus gastrocnemius, behind the musculus popliteus (Bardeen, 1901 and Tunç, 2003).

It passes between the two heads of the gastrocnemius musculus and deep into the arcus tendineus musculi solei and extends between the flexor muscles in the leg. It first runs on the inner side of the arteria tibialis posterior and vena tibialis posterior, then crosses these vessels from behind and is located on the outer side, descending down to the back of the medial malleolus (Tunç, 2003).

At this level, the tibialis nerve is between the medial malleolus and the tendo calcaneus. It innervates musculus biceps femoris, musculus semitendinosus, musculus semimembranosus, musculus gastrocnemius, musculus soleus, musculus plantaris, musculus popliteus, musculus flexor digitorum longus, musculus flexor hallucis longus, musculus tibialis posterior. It is divided into two terminal branches under the retinaculum flexorum: nervus plantaris lateralis and nervus plantaris medialis (Alfrei et al. 2006).

Nervus plantaris medialis:

It is the thickest of the two terminal branches. After passing under the retinaculum flexorum cruris, it courses between the musculus adductor hallucis and the musculus flexor digitorum brevis. It courses on the outside of the arteria plantaris medialis. After giving rise to the nervi digitales plantares proprii, which provides the sensitive innervation of the inner surface of the thumb, it is divided into the nervi digitales plantares communes (Bardeen, 1901).

It is distributed on the sides of the fingers facing each other. It provides sensitive innervation to the plantar and facing surfaces of the three and a half toes, starting from the thumb in the inner half of the sole of the foot, excluding the heel. It gives somatomotor branches to musculus adductor hallucis, musculus flexor digitorum brevis, musculus flexor hallucis brevis and musculus lumbricalis (Tunç, 2003).

Nervus plantaris lateralis:

After passing under the retinaaculum flexorum, it progresses between the musculus quadratus plantaris and the musculus flexor digitorum brevis, together with the arteria plantaris lateralis and on its inner side. It gives somatomotor branches to the musculus quadratus plantaris and musculus abductor digiti minimus (Bardeen, 1901).

It innervates musculus abductor digitorus minimi, musculus quadratus plantaris, musculus lumbricales 2-3-4, musculus adductor hallucis, musculus flexor digiti minimi brevis, musculumembri interossei plantares, musculummbri interossei dorsales. (Bardeen, 1901). The foot is divided into two parts by giving sensitive branches to the outer part of the sole:

1) Ramus superficialis:

It gives two branches called nervi digitales plantares communes. These are divided into two nervi digitales plantares proprii. It gives somatomotor branches to the musculus flexor digiti minimi and both musculuses interrosseus in the fourth metatarsal space. Its branches called rami digitales plantares provide sensitive innervation of the outer half of the sole of the foot, excluding the heel, and the plantar and facing surfaces of the one and a half toes (Alsever, 1996).

2) Ramus profundus:

Together with the arteria plantaris lateralis, it extends inwards at the sole of the foot and distributes only in the muscles. The nerve gives motor branches to the musculus adductor hallucis 2-4 lumbar muscles and all musculumembri interossei except the fourth metatarsal space (Alsever, 1996).

Side branches:

Rami muscularis:

It gives somatomotor branches to all flexor muscles in the back of the leg. These:

Musculus triceps surae,

musculus plantaris,

Musculus popliteus,

Musculus tibialis posterior,

Musculus flexor digitorum longus,

Musculus hallucis longus

Nervus interosseus cruris:

It extends to the membrane together with the arteria tibialis anterior on the interossea cruris (Bardeen, 1901).

Ramus articularis:

They are branches distributed to the knee joint (Bardeen, 1901).

Nervus cutaneus surae medialis:

It separates from the tibialis nerve above the fossapoplitea. It passes between the two heads of the gastrocnemius musculus and descends downwards with the vena saphena parva. It becomes superficial by piercing the fascia cruris in the middle of the leg. Here, it unites with the nervus cutaneus surae lateralis, which is a branch of the nervus fibularis communis, and forms the nervus suralis (Alsever, 1996).

Rami calcanei mediales:

This branch, which becomes superficial by piercing the retinaculum flexorum cruris, separates from the tibialis nerve at the ankle level. It provides sensory innervation to the inner side of the sole of the foot and the heel skin (Alsever, 1996).

B)- Nervus peroneus (fibularis) communis: (L4-L5-S1-S2)

It is the thinner and outermost branch. It extends outward on the outer

side of the fossa poplitea, between the musculus biceps femoris and the caput laterale of the musculus gastrocnemius and reaches the caput fibulae. It wraps around the collum fibulae from back to front and enters the musculus fibularis longus, where it is divided into two branches: nervus fibularis superficialis and nervus fibularis profundus (Anolague et al. 2009).

Before dividing into its terminal branches, it gives sensitive branches to the knee joint as well as the cutaneus surae lateralis branch. Nervus cutaneus surae lateralis combines with Nervus cutaneus surae medialis, the other branch of the tibial nerve, to form nervus suralis (Anolague et al. 2009).

Nervus suralis:

It is formed by the junction of the nerve cutaneus surae lateralis and the nerve cutaneus surae medialis in the middle of the back of the leg. It extends to the outer edge of the calcaneal tendon along with the vena saphena parva. Here, the nervi calcanei lateralis gives off its branch and distributes to the skin on the outer side of the calcaneus (Anolague et al. 2009).

After passing behind the lateral malleolus, it is called the cutaneus dorsalis lateralis nerve, at the level of the outer edge of the foot, and extends to the little finger. It provides sensitive innervation to the outer and back part of the lower 1/3 of the leg and the skin of the outer side of the foot and little toe (Alsever, 1996).

Terminal branches of the nerve fibularis communis:

Nervus fibularis superficialis

It is separated from the nerve fibularis communis within the musculus fibularis longus. Musculus fibularis longus progresses downward and forward between musculus fibularis brevis and musculus extensor digitorum longus and becomes superficial under 1/3 of the leg. The nerve, which gives somatomotor branches to musculus fibularis longus and musculus fibularis brevis, gives skin branches called nervus cutaneus dorsalis medialis and nervus cutaneus dorsalis imtermedius after becoming superficial. Musculus peroneus longus innervates musculus peroneus brevis (Alsever, 1996).

Nervus cutaneus dorsalis medialis:

It passes through the ankle superficial to the retinaculum extensorum and extends from the dorsum of the foot to the inner side. Nervus digitalis is divided into two branches as dorsalis pedis. Of these branches, the medial one extends to the inside of the big toe, and the lateral one extends to the 2-3rd foot. It provides sensitive innervation to the adjacent surfaces of the fingers (Anolague et al. 2009).

Nervus cutaneus dorsalis intermedius:

While it provides sensitive innervation to the majority of the dorsum of

the foot through the cutaneus dorsalis medialis nerve, it also provides sensitive innervation to the 3rd-5th nerves. It is divided into the nerve digitales dorsales pedis, which provides sensitive innervation to the adjacent faces between the fingers. (Atılhan et al. 2000).

The nerve passing between the nerve fibularis longus and the fibula passes to the anterior section by piercing the septum intermusculare anterior. Here, it progresses downward, deep to the musculus extensor digitorum longus, first lateral to the arteria tibialis anterior, then in front of it, and then medially.

Anteriorly, it gives somatomotor branches to the musculus extensor hallucis longus, musculus extensor digitorum longus and musculus fibularis tertius, and to the musculus extensor hallucis longus. It sends sensitive branches to the ankle. It innervates musculus tibialis anterior, musculus extensor digitorum longus, musculus peroneus tertius, musculus extensor hallucis longus, musculus extensor hallucis brevis, musculus extensor digitorum brevis. It passes under the retinaculum extensorum and divides into medial and lateral branches. (Atılhan et al. 2000).

a) Lateral branch:

It passes deep into the musculus extensor digitorum brevis and innervates this muscle and the musculumembri interossei dorsalis (Atılhan et al. 2000).

b) Medial branch:

It extends downwards on the outer side of the arteria dorsalis pedis and divides into two terminal branches as the digitalis dorsalis pedis nerve in the first finger space.

These branches provide sensitive innervation to the adjacent surfaces of the first and second fingers. Before dividing into its terminal branches, it gives somatomotor branches to the musculus interrosseus dorsalis (Atılhan et al. 2000).

Nervus pudendus (S2 – S3 – S4):

It passes between the musculus coccygeus and musculus piriformis, leaves the pelvis through the foramen infrapiriforme, and wraps around the arteria pudenda interna and vena pudenda interna and the spina ischiadica (Anolague et al. 2009).

It passes through the foramen ischiadicum minus and enters the fossa ischioanalis. It extends to the perineal region and external genital organs within the canalis pudendalis (alock canal) located on the outer wall of the fossa ischioanalis. Nervus pudendus first gives rise to the nervi rectales inferiores. In the diaphragma urogenitale, it is divided into terminal branches: nervi perineii and nervus dorsalis penis (Anolague et al. 2009).

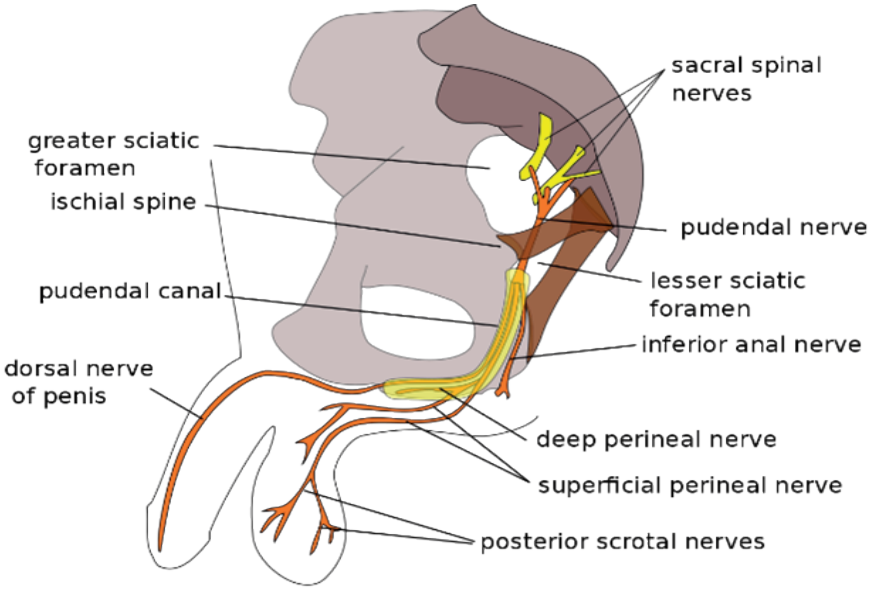


Figure 4: *sacral spinal nerve branches in side profile*

branches:

Nervi rectales informores:

These are the branches of the nerve pudendus where it separates by piercing the inner wall of the canalis pudendalis before giving its terminal branches. Musculus sphincter ani gives somatomotor branches to the externus and carries the sensitive sense of the skin around the anus (Anolague et al. 2009).

Nervi perinealis:

It is the thick and superficial one of the terminal branches of the nerve pudendus. It gives two branches, superficial and deep, in the diaphragma urogenitale. Superficial branches carry sensation from behind the external genital organs, called nervi scrotales (labiales) posteriores. The deep branches are rami musculares, which carry somatomotor fibers to the muscles (Anolague et al. 2009).

a) Nervi scrotales (labiales) posteriores:

They are superficial branches of Plexus sacralis. It provides sensitive innervation to the skin covering the perineum and scrotome in men and the skin covering the labium majus in women (Anolague et al. 2009).

b) Rami musculares:

Rami musculares moves deeper. Musculus transversus perinei superficialis, musculus transversus perinei profundus, musculus bulbospongiosus, musculus ischiocavernosus and musculus sphincter innervate the urethra. (Alsever, 1996).

Nervus dorsalis penis:

It is located deep in the terminal branches. It heads upward along the branch of the ischium pubis together with the arteria pudenda interna. It first progresses between the deep and superficial leaves of the diaphragma urogenitale. By piercing the superficial leaf, the ligamentum suspensorium reaches the dorsum of the penis. Here, the arteria dorsalis extends with the penis to the glans penis. Nervus dorsalis penis is a thinner nerve (Alsever, 1996).

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Chapter 10

MELATONIN RECEPTORS AND MECHANISM OF ACTION: CIRCADIAN RHYTHM AND RELATIONSHIP WITH DISEASES

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Introduction

Melatonin is a hormone and antioxidant that is frequently referred to as biological clock of the body and is crucial in controlling the circadian rhythm. Melatonin, whose chemical formula is expressed as N-acetyl-5-methoxytryptamine, is mainly secreted from the pineal gland and retina, however is also synthesized and secreted from the ovary, bone marrow cells, bile and gastrointestinal tract. Due to melatonin's high lipid solubility in comparison to water and its partial solubilization in aqueous environments, which contributes to its intracellular effects, cells can readily absorb melatonin. Melatonin has a secretion mechanism regulated by a biological clock, which is located in the suprachiasmatic nucleus of the hypothalamus. The suprachiasmatic nucleus is tuned to a 24-hour period that is synchronized with the daily light/dark cycle. This synchronization begins with a light signal transmitted to the suprachiasmatic nucleus via retinal pathways in the eye. Melatonin regulates sleep and the circadian rhythm, which helps treat or lower the risk of developing certain disorders. Sleep disorders, migraine, Alzheimer disease, reduced risk of cancer, immune system support, etc. Thanks to its antioxidant properties, it can protect immune system cells and help fight infections. Although there is some evidence that melatonin is associated with these diseases, more research is needed on its exact effects and use. Side effects and interactions of melatonin supplements should also be considered. People who are considering using melatonin supplements for any health condition or disease should be advised to discuss this with their healthcare professional.

The pineal gland and a few other tissues in the body create the hormone melatonin. Most melatonin production occurs during the night and this helps the body prepare for night sleep. Melatonin is a key component that regulates the body's circadian rhythm. The circadian rhythm functions like an internal clock that determines the body's biological clock. This rhythm regulates our daily activities such as wakefulness and sleep. Furthermore, melatonin has an significant role in sleep regulation. The body prepares for night sleep by increasing melatonin production. Therefore, melatonin supplements can be used to deal with conditions such as sleep irregularity or jet lag. Melatonin is also a powerful antioxidant. It can neutralize free radicals that damage cells and reduce oxidative stress. Therefore, melatonin helps to slow down the aging process and reduce the risk of certain diseases. As well as its sleep regulation and antioxidant properties, it has been associated with many health benefits.

These benefits include migraine treatment, prevention of Alzheimer's disease, reduction of cancer risk and support of the immune system. Maintaining a healthy lifestyle and preventing obesity, cardiovascular disease, diabetes, and other chronic diseases are only a few advantages of regular physical activity and exercise (Karadag et al., 2021). In addition to regular exercise, melatonin supplements are widely used to deal with sleep problems.

However, it is important to talk to a health professional before using these supplements because side effects and interactions may occur.

Melatonin receptors and mechanism of action

The high lipid solubility facilitates melatonin to enter the cells easily. Thanks to this high solubility, the effect of melatonin is not only directed at the membrane but also inside the cell. Furthermore, the partial solubilization of melatonin in aqueous media helps its effects to occur in the intracellular fluid. High amounts of melatonin in the nucleus are indicated by the presence of particular melatonin binding sites. Melatonin's link to molecular activities in the nucleus is thus comparable to that of thyroid and steroid hormones (Penev and Zee, 1997). Radioreceptor determinations with the melatonin agonist 2-(I^{125}) iodomelatonin and in vitro quantitative In autoradiography studies, the presence of melatonin receptors in the brain and peripheral tissues of some vertebral species, including humans, and that these receptors are coupled with G-proteins have been reported. Two types of membrane-dependent receptors of melatonin, known as ML1 and ML2, belonging to different pharmacological families have been identified. In studies using the potent melatonin agonist 2-(I^{125}) iodomelatonin, it was determined that ML1 receptors were high -affinity receptors in chicken and rabbit retinas, whereas ML2 receptors showed low affinity for the same melatonin agonists (Dubocovich et al, 1997). ML1 receptors cerebellum, hippocampus including neuronal location. Also suprachiasmatic the presence of ML1 receptors has been demonstrated in the nucleus, hypothalamus , thalamus , preoptic area, plexiform layer of the retina, and many regions of the cerebral cortex (Reppert et al, 1996). Non- neuronal ML1 receptors have been found in the cerebral and caudal arteries, the hypophyseal pars tuberalis, the ovary, the kidney, and the small intestine. The specific distribution of ML2 receptors in mammalian cells is not yet well elucidated (Reppert et al, 1996). ML1 receptors renal function, sleep, circadian It is responsible for rhythm, reproduction and cerebral artery contractility . These receptors are responsible for Ca^{2+} -dependent dopamine release and retinal detachment in the mammalian retina. It plays a role in light-dependent events such as phagocytosis of photopigment discs. ML1 receptor activation (via GI proteins) adenylate cyclase It causes a decrease in the level of 3'5' c-AMP with its inhibition. Furthermore, only ML1 receptors have been shown to stimulate arachidonate release via phospholipase activation (Song et al, 1997). It is suggested that low- affinity ML2 receptors are also coupled with G-proteins and behave similarly to ML1 receptors in signal transduction. However, unlike ML1s, it has been reported that activation of ML2 receptors is coupled with phosphoinositide (PI) hydrolysis and selective ML2 antagonist administration reverses this hydrolysis (Dubovich, 1995). Interestingly, potent α1-adrenergic antagonist Prazosin has high affinity for ML2 receptors and effectively reduces the hydrolysis of melatonin-induced phosphoinositol.

However, non-selective α- adrenergic antagonist Phentolamine and the relatively non- selective serotonin antagonist metizerjide were found to be ineffective in the hydrolysis of melatonin-induced phosphoinositol. The physiological significance of ML2 receptors has not been fully elucidated. Bioavailability and negative outcomes Melatonin used orally has a variable absorption. 1 hour after oral doses of 1-5 mg, serum melatonin increases 10-100 times the nighttime plasma level and decreases within 4-8 hours. Low doses (0.1-0.3 mg) can give peak concentrations of melatonin at night when taken during the day. Today, melatonin is available in 0.2 mg, 0.3 mg, 0.5 mg, 1 mg, 2.5 mg, 3 mg, 5 mg. preparations are available. There are also preparations in liquid form (1 mg/mL, 1 mg/4 ml), and no serious side effects have been reported for melatonin preparations. Despite its pronounced effects on the endocrine system, high pharmacological doses can increase serum LH and decrease prolactin (Vijayalaxmi et al, 2002).

Melatonin Antioxidant Effects

In some studies, it has been reported that the antioxidant and free radical scavenging effects of melatonin are not receptor-mediated and these effects occur above physiological concentrations. Studies show that antioxidant parameters are important markers for oxidative stress-related diseases (Bolatli et al, 2021). Melatonin is a powerful scavenger of hydroxyl and peroxyl radicals. Its OH radical neutralizing effect is 5 times higher than glutathione and ROO. In its inactivation , it is 2 times more effective than vitamin E. However, the effect of melatonin on peroxyl radical is controversial. Namely, some researchers have stated that melatonin has a lower scavenging effect against peroxyl radicals than vitamin E, so it has less neutralizing effect against lipoperoxyl radicals (Reiter et al, 2009). Melatonin also has a scavenging effect against a toxic oxygen derivative (HOCl), which is formed by the activation of macrophages during inflammation reactions. But melatonin's H_2O_2 and $O_2^{\cdot}$ -direct scavenging effect on radicals is weak. It has been reported that N1-acetyl-N2-formyl-5-methoxy chloramine (AFMK) is formed as a result of the reaction of melatonin with H_2O_2, and AFMK is converted to N1-acetyl-5-methoxy chloramine by catalase, and these metabolites with antioxidant effect increase the scavenging effect of melatonin (Reiter et al, 2007). Free radicals are also indirectly impacted by melatonin. Melatonin, its hydroperoxides by activating the GSH-Px enzyme that metabolizes O_2. It shows an antioxidant effect by increasing the activity of SOD that catalyzes the radical to H_2O_2, preventing the decrease in catalase activity during oxidative stress and inhibiting the nitric oxide synthase (NOS) enzyme responsible for NO formation (Reiter et al, 2009b). Another enzyme associated with the antioxidant defense system is the cytochrome P450 enzyme. Through the metabolism of xenobiotics, this enzyme enhances free radical production. Free radical production and

consequent oxidative damage have both been proven to be decreased by melatonin. P450 activity (Reiter et al, 2009b). By inhibiting NOS activity in the cerebellum at physiological concentrations, melatonin contributes to the physiological regulation of neuronal and cardiovascular functioning. This effect is mediated by the inhibitory effect of melatonin on free radical formation. $NO^{\cdot}$ is a free radical on its own and $O_2^{\cdot-}$ Increases $ONOO^-$ formation in its presence. The suppression of NOS activity with melatonin reduces the formation of NO and reduces oxidative damage through this pathway (Gilad et al, 1997). The scavenging effect of melatonin on free radicals also explains that it is a powerful anti-inflammatory agent. Blockade with melatonin at various levels on the pathway leading to tissue damage by stimulation of inflammation provides this agent with an anti-inflammatory and tissue protective effect. Inflammation-induced adhesion molecule expression, the role of NF-kB, iNOS, PG, superoxide and peroxy nitrite radicals in tissue damage and the suppression of the formation of these factors and the anti-inflammatory effect of melatonin are known. Melatonin significantly reversed oxidant damage to tissues caused by neutrophil activation in various inflammation models (such as burn injury, sepsis, ischemia/reperfusion) (Kaçmaz et al, 2005).

Immunological Effects of Melatonin

Maestroni et al. reached the first remarkable findings on the immunological role of melatonin in their study (Maestroni et al, 1987). In an environment where mice in which melatonin formation is inhibited are constantly exposed to light, it has been found that immune functions are suppressed under conditions such as the administration of β-adrenergic receptor blockers. Recent studies have revealed that melatonin increases humoral and cellular immune response. These effects of melatonin become evident in conditions where the immune system is suppressed such as corticosteroid use, aging, acute stress or viral diseases. In humans, continuous melatonin administration boosted leukocyte natural killer activity, according to research by Wichmann et al. In mice, melatonin restored the suppression of immunological activities and soft tissue damage brought on by hemorrhagic shock (Wichmann et al, 1996). The effects of melatonin against immunosuppression, such as its immune function enhancing effects or its binding to specific receptors on T-helper lymphocytes are very important. The binding of melatonin to these receptors leads to the secretion of opioid peptides and increased levels of IL-2 or gamma-interferon. Melatonin-induced opioids are said to lead to the hemotopoietic effect of melatonin. It has been reported that melatonin protects the blood cells of melatonin-treated tumor-bearing mice from the toxic effects of chemotherapeutic drugs (Maestroni, 1995).

Organ and cell transplantation success largely depends on immunosuppressive therapy aimed at preventing graft rejection. However,

immunosuppressant therapy has infectious and malignant effects. Melatonin has been shown to be protective against dysfunctions in various organs in viral and bacterial infections (Reiter and Maestroni, 1999). In addition, melatonin also shows an immunodepressant effect in connection with the dose. At high pharmacological doses (> 100mg/kg), melatonin suppresses antibody formation. At these high doses, prolonged melatonin binding to the receptor leads to down - regulation and immune response. causes suppression (Maestroni, 1995). Free radicals are very important in graft rejection. The usefulness of melatonin administration in organ transplantation depends on the course of the inhibitory effect of melatonin on the immune response and the antioxidant effect of the molecule. The safe use of this agent in transplantation requires the absence of toxicity (Reiter and Maestroni, 1999).

Circadian Rhythm and Its Relationship with Diseases

External factors such as light, temperature, melatonin, shift working hours and night shifts, long-distance flight travel, feeding time affect the circadian rhythm. Time incompatibility between clocks, disruption of the harmony of activities with circadian clocks and metabolic rhythms increase the risk of various diseases such as diabetes, insulin resistance, obesity, insulin resistance, cardiovascular diseases, x cancer and neurodegenerative diseases (Aydoğdu and Akbulut, 2020).

Insulin Resistance

Target cells and tissues, as well as pancreatic islet cells, are peripheral. Oscillators exist. Some of them react to variations in blood glucose caused by insulin or by central metabolism regulation on appetite control (Shi et al, 2013). Glucose tolerance tests clearly reveal the circadian effect on glucose tolerance. After a glucose load at noon, blood glucose remains at lower concentrations. This shows the relationship of glucose absorption in the gastrointestinal tract with the circadian rhythm. Loss of Bmal1 as a result of mutation in the core clock gene; It hinders gluconeogenesis, slows down the daily fluctuations in glucose and triglyceride levels, and leads to glucose intolerance. Mice with mutated circadian clocks have been shown to develop hypoinsulinemia and hyperglycemia (Sözlü and Şanlier, 2017).

Obesity

One of the major health issues affecting both industrialized and developing nations is obesity. It is a problem for people of all age groups and reduces the quality of life and life expectancy. In addition, it causes cardiovascular diseases, metabolic diseases such as diabetes and blood pressure, various diseases such as cancer and even death (WHO, Obesity and overweight, 2020). Circadian rhythm; It is effective in the release of insulin, glucagon, leptin, adiponectin, and

ghrelin hormones, which are effective in the development of obesity and in the regulation of appetite. Incorrect timing of the circadian clock; leads to discord between nutrition, behavior, and physiology and adverse health outcomes (Weyer et al, 2001). In a study, it was observed that leptin and ghrelin levels decreased in individuals who spent few hours asleep, and with this decrease, appetite increased. In conclusion, it is said that short sleep time is associated with an increase in BMI (Akbay, 2020). Another study of 143 participants found that night shifts and working late hours were associated with an increased risk of obesity and diabetes. This is because shift work forces people to eat and be active at bedtime, resulting in increased body stress (Shan et al, 2018). Obesity habits of eating late in the evening and skipping breakfast A study was conducted to examine the relationship with the prevalence of the heights and weights of a total of 19,687 Japanese women, whose ages ranged from 40 to 74, were determined; The respondents were asked whether they had habits such as eating late at night, snacking before going to bed, and skipping breakfast. The results of the study found that people who ate a late dinner or snack before bed tended to skip breakfast, which was associated with weight gain (Okada et al, 2019).

Cardiovascular Diseases

The heartbeat adapts physiologically to changes in environmental factors throughout the day. The circadian clock is important in cardiac homeostasis, which is associated with daily activities. When there is a mismatch between environmental stimuli and circadian clocks, it is inevitable for cardiovascular diseases to occur and progression of the disease course (Takeda and Maemura, 2011). As evidence that disruptions in the circadian system affect cardiovascular diseases, shift workers are at greater risk for cardiac problems. In a study on this subject, Japanese male workers were selected as participants. The effect of shift and non-shift work on the occurrence and progression of cardiac problems was investigated. As a result, it has been determined that shift workers may experience higher cardiac problems than non-shift workers, and therefore their mortality risk is as high as 2.32 times (Fujino et al, 2006).

Neurodegenerative Diseases

It has been demonstrated that the connection between the sleep-wake cycle and the internal processes that produce circadian rhythms is useful in understanding the neurobiology of psychiatric diseases. There are observations of a relationship between circadian system disruptions and bipolar disorder (Foster et al, 2013). It is known that Parkinson's disease progresses with fluctuations during the day. The circadian system disruptions seen in Parkinson's patients may increase the progression of the disease. Conditions like this have been observed in a similar fashion in other neurodegenerative disorders like Alzheimer's disease.

Cancer

Circadian genes have clock functions that regulate daily protein secretion and affect the secretion of other genes with the circadian rhythm. Negative changes in the circadian rhythm that occur with modern life cause disruptions in the regulation of these genes and protein secretion, which is responsible for cell proliferation. As a result, many pathological conditions such as tumor formation and cancer progression occur (Savvidis and Koutsilieris, 2012).

Apoptosis

Studies in humans have shown that melatonin, a circadian hormone, slows tumor growth and development. It also has intriguing properties for modulating tumor responses to radiotherapy and chemotherapy. Pinealectomy accelerates tumor formation in animals, but melatonin administration reverses this development. The direct oncostatic and anticancer effects of melatonin in tumor cells are attributed to its immune modulatory and antioxidant effects (Carrillo-Vico et al, 2006). Anti-apoptotic effects of melatonin Many experimental studies have examined the effects of melatonin on apoptosis and the results are summarized under 3 headings;

1. Inhibition of apoptosis in immune cells (antiapoptotic effect);

2. Prevention of cell death in neuronal cells (antiapoptotic effect);

3. Acceleration of apoptosis in cancer cells (proapoptotic effect).

As a result, all studies conducted under these headings revealed the mechanism of the effects of melatonin in apoptosis, the importance of oxidative stress in apoptosis and the role of antioxidant effects of melatonin in apoptosis. Today, the demonstration of the roles of free radicals in various pathologies and aging, especially in cancer, cardiovascular and neurodegenerative diseases, has brought the use of antioxidants to the agenda and studies on this subject have gained momentum. Compared to many other antioxidants, melatonin maintains its currency with its strong radical scavenging effect and its ability to increase antioxidant enzyme activities. However, studies are still ongoing for melatonin to be used in clinical practice.

It is a strong immunomodulatory and antioxidant drug that can stop cell death in oxidative stress situations (Nabavi et al, 2018). Melatonin can stop redox activity, inflammation, and cell death processes, making cancer cells more susceptible to chemotherapy and radiation (Nabavi et al, 2018). Due to its direct role as a free radical scavenger, melatonin is regarded as a radioprotector and chemoprotectant, minimizing DNA damage and cell death. Melatonin has a stronger ability than other antioxidants like glutathione to combat free radicals created by radiation (Karbownik and Reiter, 2000). Studies have shown that melatonin supports cellular antioxidant defense in the aftermath

of radiation exposure (El-Missiry et al, 2007). Melatonin's direct and indirect antioxidant effects may be crucial in reducing DNA damage, genotoxicity, and apoptosis after radiation exposure.

Following radiotherapy and chemotherapy, apoptosis is one of the most significant mechanisms of tumor cell death and is crucial for the management of tumors. Melatonin is known to promote apoptosis through DNA damage and alterations in mitochondria and to boost endogenous ROS generation (Li et al, 2017). It is also known that melatonin increases the antioxidant defense system of the majority of cancer cells and upregulates apoptotic receptors on the surface of tumor cells (Reiter et al, 2017). Melatonin may stimulate apoptosis, but its exact mechanism is still unknown, as is how it can affect tumor cells by radio- or chemo-sensitizing them.

Factors such as regular sleep habits, dark-light periods, nutrition and relaxing herbal teas can be effective to protect against all negative effects that may develop due to melatonin deficiency. In addition to exogenous melatonin administration, utilizing plants may be necessary protocols to maintain body homeostasis and body defenses or to treat diseases (Yildiz et al, 2021). Vitamin deficiency, which has become a global health problem due to changes in lifestyle and dietary habits, is a common cause of macrocytic anemias (Tahiroglu, 2023). In melatonin deficiency, along with a series of neuropsychiatric disorders, mineral, protein and amino acid reserves may become a natural defense mechanism against all these adversities.

Conclusions

In this study, it is seen that it is getting harder to adapt to day and night cycles in today's conditions, and the circadian rhythm is negatively affected by this new order. Daily activities that differ with modern technology; Environmental factors such as light, nutrition, temperature, physical activity, working hours and modern living conditions cause disruptions in the circadian rhythms of individuals. Disruptions in the circadian system and inconsistency between the central and peripheral clocks increase the risk of various diseases such as insulin resistance, diabetes, obesity, cardiovascular diseases, digestive system diseases, cancer and neurodegenerative diseases, immunodeficiency and cancer. Shift workers are among the people whose circadian system is disrupted. Nutritional recommendations for these people are of vital importance for the protection of their health. Fruits and vegetables, whole grain products, dried legumes, which are among the foods with high fiber content, should be included in the nutrition program. Complex carbohydrates should be given priority, as simple carbohydrate sources can cause irregularities in blood sugar. Due to the circadian rhythm of human biology, it is adjusted to work during the day and rest at night. It is known that disruptions are seen in the circadian system as a result of long travels. In order to adapt the circadian rhythm to the destination

region and to reduce the health problems caused by jet lag on individuals, the balance should be stabilized in accordance with the time zone of the destination region. When arranging treatment programs for neuropeptides, hormones, cytokines or stimuli that act on oscillating physiological systems, it is important to pay attention to the body's biological clock system and to regulate it accordingly. By finding the right time to achieve the targeted effect, treatment methods can be developed and unwanted negative side effects can be minimized. Melatonin is an important hormone with many bioactivities such as strengthening the immune system, antioxidant, anti-inflammatory, circadian regulator, preventing cancer development, and antipsychotic. The relationship between food and health is the most important complement to life. Melatonin and its precursor tryptophan have been identified in many foods. There are many studies showing that consumption of food containing melatonin increases the level of melatonin in human serum. However, functional new products are introduced to consumption day by day and new technologies are applied in production. Studies examining how mechanisms affect melatonin levels and bioavailability, particularly in these products, are necessary. Additionally, a healthy diet should promote and contain functional goods that are melatonin-fortified. This review explains how the circadian system works as well as how internal and environmental influences that affect people might have an impact on it. Nutritional status has been discussed in all aspects and the effects of the consequences of modern life, which leads to the deterioration of the circadian system, on diseases have been revealed by studies in this field. In order to lower the prevalence of many diseases, it is vital to monitor the circadian system, which is directly associated to our health. At the same time, it is crucial to pay attention to significant side effects including apoptosis, inflammation, and oxidative stress.

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Chapter 11

THYROID GLAND TUMORS

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Introduction

Thyroid gland comes from the Greek word thyreos (shield). It is the first endocrine gland to develop embryologically and is located in the anterior part of the neck. It is located anterior to the trachea, below the level of the cricoid cartilage. It consists of two lateral lobes and the isthmus connecting them. The thyroid gland weighs approximately 15-45 grams. Each lateral lobe is 4x2x2 cm and its isthmus is 1.5x0.5 cm (Moore & Persaud 2009; Mescher 2019).

The thyroid gland consists of follicle structures lined with single-layered epithelial cells, with colloid material in the center. Thyroid follicles are considered the structural and functional unit of the thyroid gland. The shape of follicle epithelial cells varies depending on synthesis activity. The cytoplasm of these cells expands with the increase in cell activity. Follicle cells with very large granular cytoplasm due to accumulation of mitochondria are called Hurthle cells (oncocytes). Another type of cells with endocrine function in the thyroid gland are parafollicular cells or C cells. They are found singly or in small groups between follicle epithelial cells or in the interstitial connective tissue between follicles. They are larger cells than follicular epithelial cells, and have an eccentrically located nucleus and neurosecretory granules containing calcitonin in their cytoplasm (Mescher 2019; O'Toole et al.1985).

Thyroid cancers are the most common neoplasm of the endocrine system. It constitutes approximately 3% of all malignant tumors. It is 3 times more common in women than in men. The incidence of thyroid cancer increases with age and plateaus after the age of 50 (Arrangoiz et al. 2019; Chen et al. 1988). The incidence of thyroid cancer has increased 2-3 times in the last 3 decades. The mortality rate is generally low, approximately 8 per 1 million people (Adaş et al. 2012). Although five-year survival rates are close to 100% in differentiated thyroid cancer, local recurrence may occur in approximately 20% of patients and distant metastasis may occur in approximately 10% (Eustatia-Rutten et al. 2006; Arrangoiz et al. 2019; Nagataki & Nystorm 2002). Although most thyroid cancers are idiopathic, radiation exposure is considered the main environmental factor. In addition, personal or family history of thyroid cancer and diseases with a genetic predisposition to the development of thyroid cancer are among other risk factors (Nagataki & Nystorm 2002; Cordis 2006; De Felice & Di Loura 2004).

The World Health Organization (WHO) tumor classification organizes tumors according to their anatomical regions and is updated regularly. The fifth edition of the WHO Classification of Endocrine and Neuroendocrine Tumors was published in 2022. In this classification, thyroid tumors are listed according to the tumor cell they originate from, their pathological and molecular features, and their biological behavior (Jung et al. 2022; Juhlin et al. 2022) (Table 1).

Table 1. *Tumors of the Thyroid Gland in the 2022 WHO Clasification of Endocrine and Neuroendocrine Tumors*

1. Developmental abnormalites
- Thyroglossal duct cyst
- Other congenital thyroid abnormalities

2. Follicular cell-derived neoplasm
- Benign tumors
  - Thyroid follicular nodüler disease
  - Follicular thyroid adenoma
  - Follicular thyroid adenoma with papillary architecture
  - Oncocytic adenoma of thyroid
- Low risk neoplasms
  - Non-invasive follicular throid neoplasm with papillary-like nuclear features
  - Thyroid tumors of uncertain malignant potential
    - Follicular tumor of uncertain malignant potential
    - Well-differentiated tumor of uncertain malignant potential
  - Hyalinizing trabecular tumor
- Malignant Neoplasms
  - Follicular thyroid carcinoma
  - Invasive encapsulated follicular variant papillary thyroid carcinoma
  - Papillary thyroid carcinoma
  - Oncocytic carcinoma of thyroid
  - Follicular-derived carcinomas, high-grade
    - Poorly diferrentiated thyroid carcinoma
    - Diferrentiated high-grade thyroid carcinoma
  - Anaplastic follicular cell-derived thyroid carcinoma

3. Thyroid C-cell-derived carcinoma
- Medullary thyroid carcinoma

4. Mixed medullary and follicular cell-derived carcinomas
- Mixed medullary and follicular cell-derived thyroid carcinoma
- Mixed medullary-papillary carcinoma
- Mixed medullary-follicular carcinoma

5. Salivary gland-type carcinomas of thyroid
- Mucoepidermoid carcinoma of thyroid
- Secrotary carcinoma of salivary gland type

6. Thyroid tumors of uncertain histogenesis
- Sclerosing mucoepidermoid carcinoma with eosinophilia
- Cribriform morular thyroid carcinoma

7. Thymic tumors within the thyroid
- Thymoma family
- Spindle epithelial tumor with thymus-like elements
- Thymic carcinoma family
  - Intrathyroidal thymic carcinoma

8. Embryonel thyroid neoplasms
- Thyroblastome

WHO: World Health Organisation

Follicular Cell Derived Neoplasms

Follicular cell-derived neoplasms; It is examined under 3 headings: benign tumors, low-risk neoplasms, and malignant neoplasms.

Benign tumors

The formation of multiple benign nodules of different sizes in the thyroid gland is defined as multinodular goiter. These nodules are generally hyperplastic and non-clonal lesions. Molecular analysis revealed that some of these nodules were monoclonal and represented neoplastic proliferations. It is stated that it is not always possible to distinguish neoplastic and hyperplastic lesions by histopathological examination. For these reasons, the term **"follicular nodular disease (FND)"** began to be used in the latest WHO classification. However, the terms adenomatous nodule, adenomatous hyperplasia, nodular hyperplasia, and multinodular goiter continue to be used (Jung et al. 2022; Juhlin et al. 2022; Mete & Asa 2012; Baloch et al. 2022).

Follicular adenoma (FA) is an encapsulated, benign thyroid tumor showing follicular cell differentiation. The actual incidence is not clear. It is more common in women than in men. Its etiology includes iodine deficiency and radiation exposure. FA is mostly solitary and surrounded by a thin capsule. The capsule is macroscopically and microscopically complete. The cut face is gray-white and brown. It is approximately 1-3 cm in size and areas of bleeding and cystic degeneration can be observed. FA is separated from the surrounding thyroid tissue by a fibrous capsule. It has a different structural and cytological appearance than the surrounding thyroid tissue and may show signs of compression. Normofollicular, macrofollicular, microfollicular, trabecular/solid patterns can be seen. Tumor cells are cubic, columnar, and polygonal in shape, and the nuclei are generally uniform. Mitosis is rare. Secondary changes such as bleeding, edema, fibrosis, calcification, and cystic degeneration may be observed. The differential diagnosis of FA includes follicular thyroid carcinoma (FTC), adenomatoid nodules, and encapsulated follicular variant papillary thyroid carcinoma (EFVPTC). The capsule structure is generally thicker in FTC than in FA. There is no capsule and/or blood vessel invasion in FA. To differentiate FA and FTC, the entire capsule of the nodule must be sampled. In adenomatoid nodules, there are many nodules without a clear capsule structure. They have a similar appearance to the surrounding thyroid tissue and do not cause pressure symptoms. In EFVPTC, the cells in the nodule have nuclear features of papillary thyroid carcinoma (PTC). The larger the follicles in a thyroid nodule, the less likely it is to be neoplastic (Baloch & LiVolsi 2007; LiVolsi & Baloch 2011; Rosai et al. 2006; Serra & Asa 2008) (Figure 1A).

Follicular adenoma with a papillary structure is a well-circumscribed, encapsulated tumor with intrafollicular papillary growth. Papillary structures are

short, unbranched, and have a large edematous structure. PTC nuclear features are not seen. It is a tumor-associated with autonomic hyperfunction. PTC is included in the differential diagnosis (Juhlin et al. 2022; Mete & Asa 2012).

Oncocytic follicular adenoma accounts for approximately 10-15% of FAs. For the diagnosis of this tumor, more than 75% of the tumor cells must consist of oncocytic cells. Oncocytic cells have granular eosinophilic extensive cytoplasm associated with damaged mitochondria. Oncocytic tumors may undergo necrosis following fine needle aspiration or spontaneously. Hurthle cell is called oncocytic cell in the 2022 WHO classification. The diagnoses of Hurthle cell adenoma and Hurthle cell carcinoma are no longer used (Jung 2022; Maximo & Sobrinho-Simoes 2000; Mete & Asa 2010) (Figure 1B).

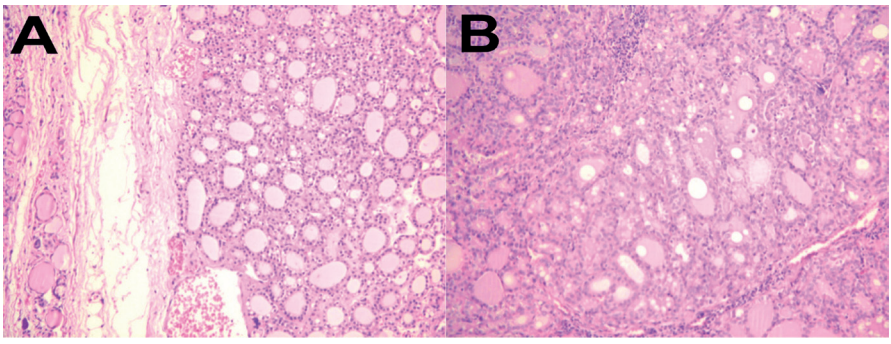

Figure 1A. *Follicular adenoma in a microfollicular pattern separated from the surrounding thyroid tissue by a fibrous capsule (HE x 100).* **B.** *Oncocytic follicular adenoma consists of oncocytic cells with large granular eosinophilic cytoplasm (HE x 100).*

Low risk neoplasms

Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is a term that has begun to be used instead of the noninvasive subtype of EFVPTC. Due to the benign behavior of noninvasive EFVPTC, it was aimed to avoid the use of the expression carcinoma and to prevent unnecessary surgeries and treatments. For the first time in 2016, Nikiforov et al. recommended by. NIFTP diagnostic criteria are:

-There is a capsule around the tumor or it is separated from the surrounding thyroid tissue by a good margin,

-Follicular growth pattern (macro and microfollicles, there should be no papillary structure containing fibrovascular core, solid/insular/trabecular patterns should be below 30%, there should be no psammoma body).

-PTC nuclear score should be 2-3 (size and shape, membrane irregularities, chromatin features).

-There should be no columnar, long cells, or cribriform patterns.

-Absence of tumor necrosis and 3 or more mitoses in an area of 2 mm^2.

-There should be no vascular and/or capsule invasion (Geramizadeh & Malekiz 2019; Seethala et al. 2018; Nikiforov et al 2016).

Thyroid tumors are tumors of uncertain malignant potential, have a follicular growth pattern, and are suspicious of vascular or capsule invasion. PTC has been classified according to its nuclear characteristics into follicular tumors of undetermined malignant potential (FT-UMP) and well-differentiated tumors of uncertain malignant potential (WDT-UMP). There are areas suspicious for vascular and capsule invasion in FT-UMP, but PTC nuclear features are not seen. For correct diagnosis, the entire nodule and capsule must be sampled and examined. In WDT-UMP, there are areas suspicious for vascular and capsule invasion, as well as PTC nuclear features (LiVolsi & Baloch 2011).

Hyalinizing trabecular tumor (HTT) is a tumor originating from follicle cells, showing a trabecular structure, and containing intratrabecular hyalinized matrix. It is a rare tumor and is most often seen between the ages of 40-50 and in women. It is usually smaller than 3 cm, single, encapsulated/well-circumscribed. The cut face is homogeneous yellow in color and white lines and spots can be seen. It is characterized by polygonal or spindle-shaped cells with large eosinophilic cytoplasm forming trabeculae and nests. Tumor cells are located perpendicular to the axis of the trabeculae. There is an accumulation of basement membrane-like hyalinized material that stains PAS positive within the trabeculae and nests. In some cases, round pale yellow bodies may be seen in the paranuclear area of the tumor cell cytoplasm. The differential diagnosis includes PTC, medullary thyroid carcinoma, and paraganglioma (Carney et al. 2008; Galgano et al. 2006; Nose et al. 2008).

Malignant Neoplasms

Follicular thyroid carcinoma (FTC) accounts for approximately 5% of thyroid cancers. It is most common between the ages of 40-50 and in women. Its incidence increases in areas with iodine deficiency. The tumor usually appears as single, encapsulated, large solid nodules. The tumor consists of a follicular, solid, and trabecular pattern of round, well-circumscribed nucleated cells with a fibrous capsule. For a malignant diagnosis in FTC, signs of capsule and/or vascular invasion must be seen. Full-thickness invasion of the capsule is required. Invasion within vessels located within or outside the tumor capsule is considered vascular invasion. FTC is classified into 3 groups about prognosis: minimally invasive, encapsulated angioinvasive, and extensively invasive. Minimally invasive PTC, only full-thickness capsule invasion is seen. Encapsulated angioinvasive PTC is considered limited if there is less than

4 vessel invasion, and widespread if there is 4 or more vessel invasion. RAS mutations are observed at a rate of 40-50% in FTC (Jung et al. 2022; Juhlin et al. 2022) (Figure 2A).

Invasive encapsulated follicular variant papillary thyroid carcinoma (IEFVPTC) was no longer a subtype of PTC in the latest WHO classification and was accepted as a separate entity. IEFVPTC is well circumscribed or encapsulated and also invades the surrounding thyroid parenchyma. Vascular invasion and distant metastases may occur. RAS mutations seen in FA and FTC are also seen in this tumor (Jung et al. 2022; Juhlin et al. 2022).

Papillary thyroid carcinoma (PTC) is the most common tumor of the thyroid, accounting for approximately 85% of all thyroid cancers. Although it is most common between the ages of 20-50, it can be seen at any age. It is 3 times more common in women than in men. The most important risk factor is radiation exposure. Children are more sensitive to the effects of radiation than adults. Because the enlargement of the thyroid occurs in childhood. The average diameter of the tumor is 1-3 cm and it may be multifocal. Macroscopically, it can exhibit very different properties. There may be encapsulated, well-circumscribed, non-encapsulated, irregularly circumscribed, cystic degeneration, and calcification areas. Papillary structures can be observed in PTC, but the presence of papillary structures is not required for diagnosis. The tumor may display a papillary, follicular, solid, insular, or trabecular pattern. Lamellar concentric calcifications called psammoma bodies can be seen in PTC. The main diagnostic features in PTC are nuclear features. PTC nuclear features include:

-Tumor cell nuclei are 2-3 times larger than surrounding follicle cell nuclei.

-Nuclei appear crowded, and overlapping.

-Nuclei appear empty or frosted glass.

-Nuclei have lost their round shape and are oval and long.

-Nuclei are irregularly bordered.

-There is a nuclear notch (groove) that gives the nucleus the appearance of a coffee bean.

-Intranuclear pseudoinclusion is seen.

Subtypes of PTC are classical, infiltrative follicular, tall cell, columnar cell, hobnail, solid/trabecular, diffuse sclerosing, Warthin-like, and oncocytic types. The most aggressive of these subtypes are tall cell, columnar cell and hobnail PTC. Papillary thyroid microcarcinoma (tumor 1 cm or less) was removed from being a separate subtype in the latest WHO classification. Immunohistochemical markers are sometimes used to distinguish PTC from other benign nodules. Staining with CK19, HBME-1 and Galectin-3 is accepted in favor of PTC. The most common mutation in PTC is BRAF mutation, with a

rate of 50-60%. RET/PTK and NTRK gene rearrangements, and mutations in the RAS and TERT genes can also be seen (Lim et al. 2013; Fischer & Asa 2008; Ghossein et al. 2007; Beesly et al. 2002) (Figure 3A, B).

Oncocytic carcinoma of thyroid are tumors consisting of oncocytic cells (over 75%) and showing capsule and/or vascular invasion. It has been considered a separate entity from FTC due to its molecular properties and prognosis (Jung et al. 2022; Maximo & Sobrinho-Simoes 2000; Mete & Asa 2010) (Figure 2B).

Poorly differentiated thyroid carcinoma (PDTC) is located between differentiated follicle cell-derived tumors such as PTC and FTC and anaplastic thyroid carcinoma (ATC) in terms of its morphological features and prognosis. Sometimes differentiated thyroid tumors can be seen in the background, sometimes not. The tumor is approximately 5 cm in size. The cut surface is gray-white in color, solid in structure, and areas of necrosis can be seen. PDTC is diagnosed with the Turin criteria:

-Solid/trabecular/insular growth pattern.

-Lack of PTC nuclear properties.

-Presence of at least one of these; tumor necrosis, 3 or more mitoses in 10 high power fields, tortuous nuclei.

In PDTC, positive staining with thyroglobulin, TTF-1, cytokeratins and PAX8 is observed in immunohistochemical staining (Asioli et al. 2010; Bongiovanni et al. 2014; Burman et al. 2014).

Differentiated high-grade thyroid carcinoma (DHGTC) is used in cases of tumor necrosis and/or 5 or more mitoses in 10 high-power fields in differentiated follicular cell-derived tumors. Apart from RAS and BRAF mutations seen in differentiated thyroid tumors, p53 mutations are also seen in PDTC and DHGTC (Jung et al. 2022; Juhlin et al. 2022).

Anaplastic follicular cell-derived thyroid carcinoma (ATC) is a high-grade tumor consisting of undifferentiated cells. It constitutes 1% of all malignant thyroid tumors. It is often seen in people over the age of 60. Its prognosis is quite poor. Macroscopically, it invades widely inside and outside the thyroid tissue. The tumor is often larger than 5 cm. Large areas of bleeding and necrosis are seen. Spindle cell, giant cell, and squamous cell patterns can be observed in the tumor. Mitosis is frequent. In the latest WHO classification, primary squamous cell carcinoma of the thyroid was accepted as a subtype of ATC. In ATC, immunohistochemical markers of the thyroid are generally negative but are stained positively with cytokeratins. The most common p53 mutation is observed in ATC (Begum et al. 2004; Eloy et al. 2015; Juhlin et al. 2022).

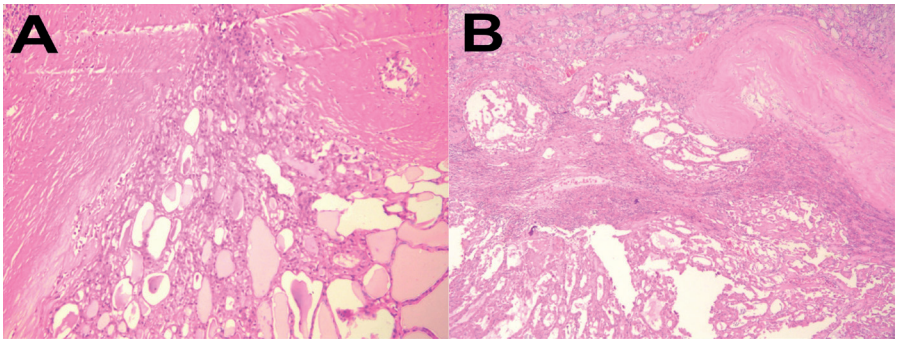

Figure 2A. *Follicular thyroid carcinoma with capsule invasion (HE x 100).* **B.** *Oncocytic carcinoma consists of oncocytic cells spreading outside the capsule (HE x 100).*

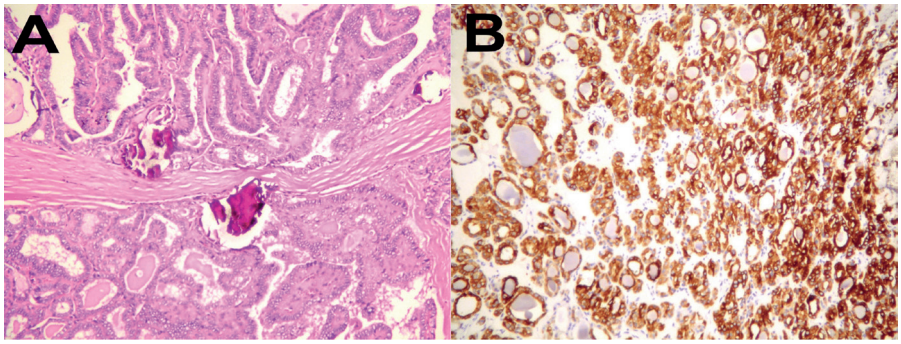

Figure 3. *Papillary thyroid carcinoma.* **A.** *Ground glass nucleus, tumor cells showing nuclear crowding, overlapping, papillary structure and psammoma bodies (HE x 100).* **B.** *Positive staining in tumor cells with CK19 (CK19 x 100).*

Thyroid C-Cell-Derived Carcinoma

Medullary thyroid carcinoma (MTC) originates from the C (parafollicular) cells of the thyroid. It constitutes 2-10% of thyroid tumors. It is often seen between the ages of 40-50. 25% of MTCs are hereditary. It is associated with multiple endocrine neoplasia (MEN) type 2a, MEN type 2b and familial medullary thyroid carcinoma syndrome. The tumor is well-circumscribed, unencapsulated, gray-white in color. Their sizes can vary widely. The tumor consists of polygonal, round, spindle-shaped cells with salt-and-pepper chromatin that form layers, nests, and cords in a fibrovascular stroma. Amyloid accumulation is observed at a rate of 80% in the tumor. MTC is considered high-grade MTC based on the presence of one of the following: tumor necrosis, 5 or more mitoses per 2 mm^2, and Ki67 proliferation index of 5% and more. MTC should be kept in mind in the differential diagnosis of all thyroid tumors. Because it can display very different appearances. Immunohistochemical stains are helpful in differential diagnosis. While positive staining is observed with calcitonin and CEA in

MTC, no staining with thyroglobulin is observed. RET mutations are the most common molecular change in MTC (Abraham et al. 2011; Fernandez et al. 2004; Massoll et al. 2004; Juhlin et al. 2022) (Figure 4A, B).

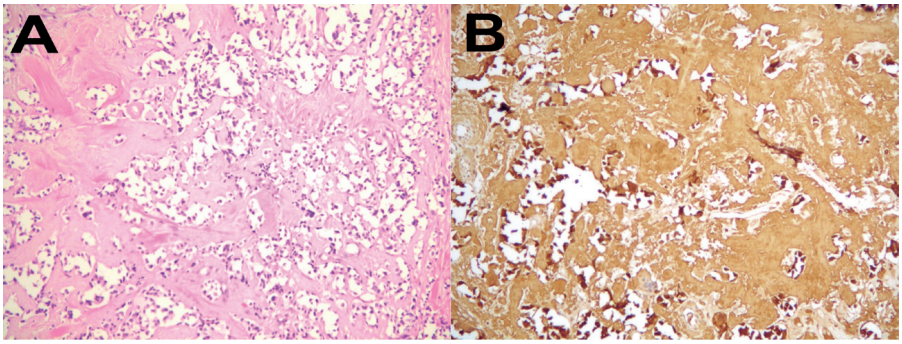

Figure 4. *Medullary thyroid carcinoma.* **A.** *Polygonal shaped tumor cells in the fibrovascular stroma (HE x 100).* **B.** *Positive staining in tumor cells with calcitonin (Calsitonin x 100).*

Salivary gland-type carcinomas of thyroid

Salivary gland-type tumors in the thyroid gland are very rare. These tumors are thought to arise from ectopic salivary gland cells located in the thyroid. This group includes mucoepidermoid carcinoma and secrotary carcinoma. Morphologically, they look similar to their counterparts in the salivary gland (Jung et al. 2022; Juhlin et al. 2022).

Thyroid tumors of uncertain histogenesis

This group was added to the 2022 WHO classification. This group includes sclerosing mucoepidermoid carcinoma with eosinophilia and cribriform morular thyroid carcinoma. Sclerosing mucoepidermoid carcinoma with eosinophilia was among the salivary gland-type carcinomas of the thyroid category in the previous classification. Cribriform morular thyroid carcinoma was the subtype of PTC in the previous classification. Unlike PTC, it shows nuclear and cytoplasmic β-catenin expression (Jung et al.2022; Juhlin et al. 2022).

Thymic tumors within the thyroid

Thymoma, spindle epithelial tumor with thymus-like elements and intrathyroidal thymic carcinoma are included under the title of thyroid gland thymic tumors (Jung et al. 2022).

Embryonal thyroid neoplasms

Thyroblastome is an extremely rare high-grade thyroid tumor. It consists of primitive follicle cells and small cells in a mesenchymal stroma. DİCER-1 mutations are the most common genetic change (Jung et al. 2022).

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Chapter 12

THE PHYSIOLOGY OF TRP CHANNELS AND ITS ROLE IN EPILEPSY

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Definition and History of TRP Channels

In 1989, Transient Receptor Potential (TRP) channels were serendipitously discovered in the retinal cells of Drosophila vinegar flies. Additionally, TRP-like (TRPL) channels were identified within the eyes of Drosophila by Montell and Rubin in the same year (Montell and Rubin, 1989). Subsequent research has established that both TRP and TRPL channels play pivotal roles in the process of phototransduction. Phototransduction is the intricate mechanism by which absorbed light quanta are converted into an electrical signal within photoreceptor cells, as extensively elucidated in their work published (Katz and Minke, 2009).

Furthermore, as research progressed, it became evident that TRP channels are ubiquitously expressed across a diverse array of cell types and tissues in vertebrates. Notably, photoreceptors exhibiting *Trp* gene mutations have been designated as such due to their characteristic transient (aphasic) voltage changes when continuously exposed to light. Moreover, the terminology employed in this context pertains to cation channels rather than the designation of calcium (Ca^{2+}) channels. This choice arises from their permeability to Ca^{2+} and sodium (Na^{+}) ions, which are predominantly found in higher concentrations outside the cellular milieu than within.

TRP channels serve as 'cellular sensors' that exhibit responsiveness to alterations in various cellular environments. These encompass changes in temperature, stress or pressure, chemical composition, oxidation or reduction states, osmolarity, and pH levels, spanning both acidic and alkaline conditions (Clapham, 2003; Moran et al., 2011). However, the mechanisms governing the regulation of TRP channels in vivo remain a subject of ongoing research, with aspects of this regulation yet to be fully elucidated.

TRP channel proteins are nonselective cation channels ubiquitously expressed in the cellular membranes, excluding the nuclear and mitochondrial membranes (Gees et al. 2010). These TRP channels, primarily located in the plasma membrane, are essential for regulating ion fluxes such as potassium (K^{+}), magnesium (Mg^{2+}), Na^{+} and Ca^{2+} within specific organelles (Nilius and Owsianik, 2011). Moreover, they exert a significant influence on the functioning of organelles such as lysosomes and impact various cellular activities (Moran, 2018). TRP channels are linked to diverse physiological functions in both excitable and non-excitable tissues. Notably, they are implicated in sensory signaling processes, encompassing nociception, taste perception, pressure sensation, temperature sensing, vision, and pheromone signaling. Additionally, TRP channels have a fundamental role in preserving homeostasis, influencing functions such as muscle contraction, vascular relaxation, and cell proliferation (Gees et al., 2010). In the central nervous system (CNS), both neurons and glial cells express multiple TRP channels,

playing a significant role in neurogenesis, structural/functional plasticity, as well as cellular homeostasis (Katz et al., 2017; Nilius, 2012; Vennekens et al., 2012).

TRP channels, along with other ion channels expressed in brain cells, have been implicated in the progression of neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Additionally, specific TRP subfamilies are primarily expressed in neurons and microglia, both of which play essential roles in the transmission of neuropathic pain, as noted (Haraguchi et al., 2012). TRP channels play a multifaceted role in the generation of various inflammatory mediators, which are closely associated with neuroprotection and neurotoxicity. This contribution is facilitated through their involvement in intracellular calcium regulation, signaling pathways, and the transmission of pain (Ji and Suter, 2007; Lee and Kim, 2017; Miyake et al., 2014). Given these significant implications, TRP channels are presently the subject of research as potential therapeutic targets for addressing both neurodegenerative disorders and pain management.

Subfamilies/Types of TRP Channels

TRPV (vanilloid) subfamily: The TRPV subfamily comprises six members, categorized into two groups based on sequence homology: TRPV1-4 and TRPV5/6. The former, known as thermo-TRPV channels, notably contribute to heat and pain perception (Patapoutian et al., 2003). In contrast, TRPV5 and TRPV6 are characterized as Ca^{2+} selective channels under physiological conditions. (Zhao et al., 2021). Distinctive to the TRPV subfamily is the presence of the N-terminal Ankyrin Repeat Domain (ARD), consisting of six repetitions. This domain serves a unique role in TRPV channels as it establishes connections with ARDs from other subunits, forming a characteristic marginal domain that encloses a cytoplasmic gap. Subsequent to the ARDs, a helix-loop-helix (HLH) domain serves as a binding domain, while a pre-S1 helix binds to the Transmembrane Domain (TMD). Notably, a conserved TRP helix extends from the C-terminus of the S6 helix, running in parallel to the membrane. This TRP helix is situated between the S4-S5 linker and the HLH domain (Zhao et al., 2021).

TRPM (melastatin) subfamily: The TRPM (Transient Receptor Potential Melastatin) subfamily stands as the largest and most diverse subgroup within the broader TRP channel superfamily, comprising eight distinct members denoted as TRPM1-8. Furthermore, these members are grouped into four categories determined by their sequence resemblance: TRPM1/3, TRPM4/5, TRPM6/7, and TRPM2/8. Remarkably, the majority of TRPM subfamily members serve as non-specific cation channels that allow the passage of Ca^{2+} ions, while TRPM4/5 exclusively permits monovalent cations such as Na^{+} or K^{+}. TRPM6/7, conversely, facilitates the passage of Mg^{2+} ions, playing a pivotal

role in maintaining intracellular magnesium homeostasis (Zhao et al., 2021).

A distinguishing feature of TRPM4/5/8, unlike other TRP channels, is their possession of voltage-dependent gating mechanisms (Huang, 2020). TRPM channels have been recognized for their involvement in diverse physiological processes, including temperature and oxidative stress sensing, regulation of cellular apoptosis, and transmission of taste signals (McNulty and Fonfria, 2005; Simon et al., 2013). Furthermore, the malfunction or dysregulation of TRPM channels has been associated with various pathological conditions, encompassing neurodegenerative diseases, cardiovascular dysfunction, metabolic disorders, and inflammatory diseases (Abriel et al., 2012; Sun et al., 2015).

TRPC (canonical) subfamily: The TRPC (Transient Receptor Potential Canonical) channels are considered foundational members of the TRP superfamily, owing to their close homology with the initially discovered TRP channel in Drosophila melanogaster. Within the TRPC subfamily, there exist five members: TRPC3/6/7, TRPC1, TRPC4/5, and TRPC2, the latter being a human pseudogene. These TRPC subfamily members are further categorized into four distinct subgroups based on their primary amino acid sequences and modes of activation. Diacylglycerol (DAG), a signaling molecule generated by the breakdown of phosphatidylinositol 3,5-bisphosphate (PIP2) through the action of phospholipase C (PLC), has the direct capability to activate TRPC3/6/7 channels. This activation can occur in response to stimulation by G-protein-coupled receptors (GPCRs) or receptor tyrosine kinases (Trebak et al., 2003). While TRPC1 and TRPC4/5 were traditionally considered insensitive to DAG, recent research has shown that, when the associated sodium/hydrogen (H^+) modifier regulatory element is deleted, TRPC4 and TRPC5 can become responsive to DAG (Hofmann et al., 1999; Storch et al., 2017). Moreover, TRPC channels are subject to modulation by various chemicals within the PLC signaling pathway, including inositol triphosphate (IP3), phosphatidylinositol 3,5-bisphosphate (PIP2), calcium-calmodulin (CaM), and intracellular Ca^{2+} ions (Wang et al., 2020).

TRPA (ankyrin) subfamily: TRPA1, commonly referred to as the wasabi receptor, stands as the sole member of the TRPA (Transient Receptor Potential Ankyrin 1) subfamily. It exhibits predominant expression in nociceptive neurons and is also found in non-neuronal cells, notably epithelial cells. TRPA1 is known for its ability to detect a wide range of harmful stimuli, including severe cold, aromatic compounds, and environmental irritants. The implication of TRPA1 in processes related to inflammation and pain perception has rendered it an attractive target in pharmaceutical research, with potential applications in disorders such as familial episodic pain syndrome, asthma, and cough (Moran, 2018). Structurally, TRPA1 features an extensive N-terminal tail housing a 17-ankyrin repeat domain (ARD). The regulatory

mechanisms governing the N-terminal domain (NTD) are akin to those observed in TRPV channels, where large N-terminal ARDs are connected to the Transmembrane Domain (TMD) via a binding domain and the pre-S1 domain. In the C-terminal domain (CTD), the binding domain consists of a short α-helix and two β-strands that collectively form a β-stranded β-sheet. In the typical TMD architecture of TRPA1, two additional regions known as PH1 and PH2 (Permeable Helices 1 and 2) are present. A β-stripe connects the TRP-like helix within the CTD, a short helix, and a vertically oriented helix-helix domain. This configuration encompasses a coupling domain, encompassing the binding domain, pre-S1 domain, and TRP-like helix. Importantly, this coupling domain features an electrophilic agonist binding pocket (Suo et al., 2020).

TRPML (mucolipin) subfamily: The members of the TRPML (Transient Receptor Potential Mucolipin) subfamily, formally designated as MCOLN (Mucolipin Transient Receptor Potential) channels, encompass TRPML1-3. In contrast to many TRP channels, which are predominantly located in the plasma membrane, TRPML channels are predominantly localized within late endosomes and lysosomes, where they play a significant role in the endocytotic pathway (Venkatachalam et al., 2015). Significantly, mutations in TRPML1 that impact its function are responsible for Lysosomal Storage Disease Mucolipidosis Type IV, a condition marked by compromised lysosomal function (Bassi et al., 2000; Sun et al., 2000). Structurally, TRPML channels share similarities with other TRP channels regarding their Transmembrane Domain (TMD) architecture. However, they lack substantial intracellular N-terminal (NTD) or C-terminal (CTD) domains, as well as recognized structural features such as Ankyrin Repeats (ARs) and the canonical TRP domain. Instead, a prominent feature that distinguishes the TRPML subfamily is the Extracytosolic/Lumen Domain (ELD). Situated between the S1 and S2 segments, this domain protrudes from the membrane, detecting ambient concentrations of Ca^{2+} ions and protons H^{+} (Viet et al., 2019). Furthermore, TRPML channels possess two Permeable Helices (PHs), PH1 and PH2, akin to TRPA1. The intracellular side of the S1-S3 helices extends across the membrane to form mucolipin domains, playing a pivotal role as a recognition site for phosphoinositides, mainly PI(3,5)P2 and PI(4,5)P2 (Fine et al., 2018).

TRPP (polycystine) subfamily: TRPP (Transient Receptor Potential Polycystin) channels were first discovered in association with a common genetic disorder that can be life-threatening, involving the formation of cysts in vital organs such as the kidneys, liver, and pancreas, ultimately resulting in impaired kidney function. This condition is primarily attributed to mutations in the TRPP1 channel, encoded by the Pkd2 gene, and is recognized as autosomal dominant polycystic kidney disease (Igarashi and Somlo, 2002). In accordance with sequence homology, TRPP channels are categorized

into two groups, namely PKD1 and PKD2. Members of the PKD1 protein family, including PKD1, PKDREJ, PKD1LD, PKD1L2, and PKD1L3, share a common structural architecture comprising 11 Transmembrane (TM) helices, a substantial extracellular domain, and an intracellular C-terminal domain (CTD). Notably, PKD1 proteins do not function independently as ion channels; instead, they form hetero-oligomeric complexes with PKD2 channels in a 3:1 ratio, a configuration predominantly localized on primary cilia within renal epithelial cells (Su et al., 2018). Members of the PKD2 (TRPP) channel group encompass TRPP1 (also known as TRPP2, PKD2, APKD2), TRPP2 (also known as TRPP3, PKDL2, PKDL, PKD2L), and TRPP3 (also known as TRPP3, PKDL2, PKDL, PKD2L), with a shared Transmembrane Domain (TMD) structure akin to other TRP channels (Shen et al., 2016). Specifically, TRPP1 is typically located within the endoplasmic reticulum (ER) and the plasma membrane, TRPP2 primarily within the ER, and TRPP3 on the plasma membrane and/or ER (Katsianou et al., 2018).

Molecular Structure of TRP Channels

Channel proteins have a fundamental impact on the vitality and functionality of nearly every cell, and channels allowing the passage of Ca^{2+} ions are especially crucial since Ca^{2+} acts as both a charge carrier and one of the primary secondary messengers. Prior to the discovery of TRP (Transient Receptor Potential) channels, the two primary categories of Ca^{2+}-permeable channels were voltage-gated and ligand-gated channels (Hille, 1992). The TRP channel superfamily represents a novel class of Ca^{2+}-permeable channels. Despite possessing the canonical six transmembrane segments (S1-S6) and the pore-forming region situated between transmembrane segments S5 and S6, these channels differ in that the positively charged residues in the S4 segment have been substituted with uncharged amino acids (Phillips et al., 1992).

The TRP channel superfamily comprises seven related subfamilies, denoted as TRPC, TRPM, TRPV, TRPA, TRPP, TRPML, and TRPN. Notably, all these subfamilies, except TRPN, are present in mammals (Montell et al., 2002).

In mammals, the diverse TRP channel types have been systematically classified into six subfamilies, encompassing a total of 28 distinct TRP channel types. These subfamilies include TRPC with seven subtypes, TRPV with six subtypes, TRPM with eight subtypes TRPP with three subtypes, TRPML with three subtypes, and TRPA alongside a no-mechano-potential (NOMCP, TRPN) subfamily (Clapham, 2003; Clapham, 2007).

Several members within the TRP channel superfamily have been shown to have essential functions in sensory transmission pathways, including thermosensation, mechanosensation, taste perception, recognition of strong odors, pheromone sensing, and osmolarity control (Clapham, 2003; Julius,

2005; Nishida et al., 2006). Consequently, there has been a notable upsurge in interest and research focused on elucidating the functions of the TRP channel subfamily. It is important to recognize that TRP channels extend their influence far beyond sensory perception. They actively participate in various other physiological processes, including the regulation of salivary fluid production, modulation of inflammatory responses, control of cardiovascular function, regulation of smooth muscle tone, maintenance of pressure homeostasis, and the balance of intracellular Ca^{2+} ions and Mg^{2+} ions. Additionally, TRP channels are integral to the proper functioning of lysosomes (Minke and Cook, 2002; Montell, 2001; Nilius and Voets, 2005). Moreover, TRP channels are involved in a wide range of cellular processes, including the regulation of cell adhesion, control of cellular growth and differentiation, modulation of cell proliferation, coordination of cell death mechanisms, and the establishment of cell polarity (Abramowitz and Birnbaumer, 2009). Given the critical roles played by TRP channels in a multitude of physiological activities, it is not surprising that several subfamilies within this channel superfamily have been linked to human disorders (Nilius et al., 2007).

TRP CHANNELS' PHYSIOLOGY AND ROLE IN EPILEPSY

TRP Channels' Physiology

The activation of TRP channels facilitates the passage of cations across the cellular membrane, leading to cell depolarization and triggering a diverse array of cellular responses. These channels, which respond to a broad spectrum of stimuli and are ubiquitously distributed in virtually all cell types within the body, are postulated to fulfill numerous physiological roles (Nilius, 2013 Furthermore, extensive research in this field has revealed that TRP channels play crucial roles in a variety of disorders that impact both the peripheral and central nervous systems (Morelli et al., 2013). They also exert influence over the respiratory system and contribute to conditions affecting the genitourinary system, gastrointestinal system, cardiovascular system, and immunological system, as well as metabolic disorders such as obesity and diabetes (Preti et al., 2012, Skryma et al., 2011, Holzer, 2011, Watanabe et al., 2013, Smith and Nilius, 2013, Zhu et al., 2009).

Additionally, TRP channels are activated by the elevation of intracellular Ca^{2+} ions resulting from the activation of G-protein-coupled receptors (GPCRs), and they play a pivotal role in mediating downstream signaling events. TRP channels' activity is additionally regulated by several intracellular molecules, such as phosphatidylinositol 4,5-bisphosphate (PIP2), diacylglycerol (DAG), adenosine triphosphate (ATP), and calmodulin (Wu et al., 2010).

Intracellular Ca^{2+} ions play a multifaceted role in brain signaling, exerting a regulatory influence on brain physiology to maintain the health of neurons (Kawamoto et al., 2012). The flow of Ca^{2+} across the plasma

membrane stands as a fundamental requirement for essential brain activities, primarily mediated through glutamate receptor channels, voltage-gated calcium channels, sodium-calcium exchangers, and TRP (Transient Receptor Potential) channels (Bezprozvanny, 2010; Hwang et al., 2021; Kumar et al., 2016). The impact of Ca^{2+} signaling extends to various neuronal functions across diverse physiological roles, necessitating precise regulation to avert uncontrolled responses that may culminate in pathological conditions (Kumar et al., 2016 Nonetheless, the continuous influx of Ca^{2+} can induce endoplasmic reticulum stress, mitochondrial dysfunction, and neuronal cell death, mediated by multiple proteases (Bezprozvanny, 2010). Indeed, the decline in cellular activity resulting from the actions of reactive nitrogen (oxygen) species and disturbances in pH homeostasis underpin the pathogenesis of neurodegenerative diseases (Harguindey et al., 2019; Popugaeva et al., 2017). Notably, heightened Ca^{2+} currents and disturbances in pH levels are particularly pronounced in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, and age-related disorders (Hong et al., 2020; Thapak et al., 2020).

In the developing nervous system, TRP channels play pivotal roles in processes such as neurite outgrowth and growth cone direction, which are fundamental to the establishment of neural connectivity. Guiding cues, exemplified by Netrins and Brain-Derived Neurotrophic Factor (BDNF), exert attractive or repulsive effects on growth cones by inducing Ca^{2+} ion influx. Notably, TRPC3 has emerged as a candidate implicated in BDNF-mediated modulation of growth cone guidance, as it becomes activated by BDNF within the developing mammalian brain (Li et al., 1999). Substantiating this possibility, the knockdown of TRPC3 expression via small interfering RNAs (siRNAs) results in the inhibition of BDNF-induced reorientation of growth cones within cultured cerebellar granule cells (Li et al., 2005).

TRPC channels also operate postsynaptically in reaction to the activation of metabotropic receptors. For instance, the activation of metabotropic serotonin receptors within the dendrites of thalamic interneurons triggers the release of the neurotransmitter γ-aminobutyric acid (GABA) from dendrites, mediating local GABAergic inhibition. Notably, the dendritic release of GABA is significantly attenuated in thalamic interneurons from TRPC4 knockout mice (TRPC4 -/-) (Munsch et al., 2003). Conversely, TRPC1 seems to generate an excitatory postsynaptic current following the activation of metabotropic glutamate receptors in Purkinje cells. Perturbations that interfere with the activity of TRPC1 have been observed to lead to a reduction in excitatory postsynaptic currents (Kim et al., 2003).

TRP Channels' Role in Epilepsy

Epilepsy stands as a relatively prevalent neurological disorder, affecting approximately 70 million individuals worldwide (Trinka et al., 2019). This condition encompasses a spectrum of potential clinical syndromes that may manifest at various stages of life, often stemming from genetic mutations and developmental malformations in the cortical region (Duncan et al., 2006; Sisodiya, 2004). The hallmark of epilepsy lies in its recurrent and spontaneous epileptic seizures, originating from the synchronized bursting of neurons in disparate regions of the brain. Despite significant research efforts aimed at unraveling the etiology of epilepsy, the precise molecular and cellular pathways remain incompletely understood. Presently, the predominant approach to epilepsy treatment involves pharmacological agents that target ion channels, such as Ca^{2+}, glutamate, and γ-aminobutyric acid (GABA) channels (Curcic et al., 2019). Notably, epilepsy can indeed result from mutations affecting ion channels (Thakran et al., 2020). In broad terms, epileptogenesis may stem from either the loss of function of K^+ channels or augmentation in the function of Na^+ channels or non-selective cation channels, culminating in heightened neuronal excitability, which is a contributory factor to the manifestation of epilepsy (Muona et al., 2015). Furthermore, mutations resulting in increased currents and sustained elevated activity in different voltage-gated sodium channels have also been linked to the development of epilepsy (Wengert and Patel, 2021).

While the onset of epilepsy is influenced by a multitude of factors, ion channels occupy a pivotal role in the regulation of various neuronal properties, including membrane excitability. Epilepsy manifests as generalized cortical spike-and-wave seizures, often stemming from the dysregulation of these ion channels. Recent studies have revealed the potential role of TRP channels in the pathophysiology of epilepsy (Sawamura et al., 2017). Specifically, diminished concentrations of Ca^{2+} ions and Mg^{2+} ions result in increased depolarization of pyramidal neurons, rendering them more susceptible to epileptiform activity, particularly in immature and dysplastic cortices compared to mature and healthy counterparts.

Developmental and epileptic encephalopathies (DEE) represent a class of disorders characterized by epilepsy accompanied by intellectual disability. Ion channel mutations have emerged as causal factors in DEE (D'Adamo et al., 2020). Notably, among the earliest identified ion channel mutations implicated in DEE were intermediate function mutations affecting the KCNQ2 (Potassium Voltage-Gated Channel Subfamily Q Member 2) channel (Schroeder et al., 1998).

Epileptic seizures arise from abnormal synchronized electrical discharges, either focal or generalized, within the central nervous system. The

complex communication between neurons is finely regulated by a system of stimulating and inhibiting circuits. Disruptions in this balance, stemming from both an augmentation in excitatory mechanisms and a deterioration in inhibitory mechanisms, may precipitate epileptic discharges. Two fundamental processes underlie neuronal electrophysiological excitability and communication: the generation of action potentials mediating axonal conduction and the transmission of signals between cells at synapses. Given that ion channels constitute the foundational elements of both processes, any malfunction induced by mutations has the potential to directly influence brain excitability, thereby triggering epileptic seizures (Lerche, 2001).

Recent investigations have uncovered a significant role for TRP channels, known for their non-selective Ca^{2+} ion permeability, in the context of seizure activity associated with epilepsy. A noteworthy revelation in this regard associates the TRPV1 channel with the development of epilepsy (Nazıroğlu, 2015). These findings are buttressed by prior research that reported an upregulated expression of TRPV1 in critical brain regions such as the hippocampus in rat models, the cortex in individuals with mesial temporal lobe epilepsy, and the dentate gyrus in mice afflicted with temporal lobe epilepsy (Bhaskaran and Smith; 2010; Saffarzadeh et al., 2025; Sun et al., 2013).

To further substantiate these experimental and clinical observations, the blockade of the TRPV1 channel using capsazepine (CPZ) and 5'-iodoresiferatoxin (IRTX) has been shown to effectively prevent epileptic seizures (Yilmaz et al., 2011). Moreover, in an acute rat model of temporal lobe epilepsy, the simultaneous of capsaicin with WIN 55, 212-2, a cannabinoid agonist, was observed to result in a reduction in epileptic seizures (Carletti et al., 2016).

TRP channels hold a pivotal role in mediating sensory signals, exerting profound effects on various cellular functions and signaling pathways. Mutations in the genes encoding TRP channels have been linked to a range of inherited diseases affecting multiple systems in the human body, including the cardiovascular, renal, skeletal, and nervous systems (Clare, 2010).

Epilepsy susceptibility frequently emerges in idiopathic epilepsies, and genetic anomalies, including mutations affecting genes responsible for Ca^{2+}-dependent ion channels, contribute to the genesis of this neurological disorder. Notably, dysregulation of calcium homeostasis and calcium signaling stands as crucial variables in the process of epileptogenesis. Potassium channels, on the other hand, govern the resting membrane potential and therefore wield a pivotal role in regulating neuronal excitability. Based on their structural characteristics, biophysical properties, pharmacological sensitivities, and physiological functions, potassium channels can be classified into various subtypes, including voltage-gated (Kv), inward rectifier, Ca^{2+}-activated,

and tandem pore K^+ channels. The involvement of potassium channels in the pathogenesis of numerous neurological disorders has been extensively explored, as underscored by N'Gouemo (N'Gouemo, 2011).

A study examining PTZ-induced epilepsy in mice observed that the activation of TRPV1 channels reduced apoptosis and ameliorated epileptic seizures (Gonzalez-Reyes et al., 2013). Another experimental animal model involving penicillin-G-induced seizures found that the activation of TRPM8 had the capacity to suppress seizures (Moriyama et al., 2019). Additionally, a study demonstrated that the knockout of TRPM2 resulted in a reduction in epilepsy-induced neurodegeneration and autophagy in the mouse hippocampus, with the TRPM2 channel contributing to the mitigation of epilepsy-induced cognitive impairment (Hu et al., 2020). Significantly, investigation in humans unveiled that *de novo* TRPM3 mutations were causal factors behind cognitive disability and epilepsy (Dyment et al., 2019).

The TRP superfamily of channels is implicated in a plethora of functions within both excitable and non-excitable cells. While TRP channels are widely recognized for their pivotal roles in sensory physiology, they extend their influence to broader sensory functions. These channels not only facilitate an organism's perception of its environment but also enable individual cells to sense their surroundings (Venkatachalam and Montell, 2007).

Various TRP channel family members have been associated with epilepsy, including TRPC1, TRPC3-7, TRPM2, TRPM7, TRPV1, TRPV4, and TRPA1. TRPC channels, in particular, are renowned for their significance in neuronal development and survival during brain maturation. Furthermore, they are believed to play pivotal roles in various epileptogenic processes. For instance, enhanced TRPC1 expression has been observed in cortical lesions of epileptic patients, regulated by astrocyte-induced epilepsy (Zang et al., 2015). In an experimental model employing the pilocarpine-induced Status Epilepticus (PISE) paradigm, researchers discovered that genetic deletion of TRPC3 resulted in reduced seizure sensitivity to pilocarpine, accompanied by an increase in TRPC3 expression. Curiously, this deletion paradoxically induced hyperexcitability in the brain, elevating susceptibility to epileptiform activity (Phelan et al., 2017; Zhou and Roper, 2014). Conversely, downregulation of TRPC6 expression was observed in chronic epileptic rats, with genetic deletion of siTRPC6 increasing seizure susceptibility and seizure-induced neuronal damage specifically in the dentate gyrus, sparing CA1 and CA3 neurons in the hippocampus (Kim and Kang, 2015). Furthermore, genetic deletion of TRPC1/4 lowered seizure-induced neuronal cell death, whereas TRPC5 mutant mice displayed a reduced seizure frequency and decreased seizure-induced neuronal cell death in the CA1 and CA3 regions of the hippocampus (Phelan et al., 2013). Interestingly, the TRPC7 channel emerged as a key participant in spontaneous epileptiform bursts in CA3 regions, with

its reduction likely associated with a decrease in PISE susceptibility in TRPC7 knock-out mice (Phelan et al., 2014).

The TRPM family also has a crucial role in the development of epilepsy. Mutations in EFHC1 might underlie the potentiation of TRPM2-mediated neuronal apoptosis activity in epilepsy (Katano et al., 2012). Additionally, it has been documented that TRPM7 becomes activated during epileptic events (Aarts and Tymianski, 2005). Notably, genetic ablation of TRPM7 has been shown to attenuate ROS-mediated activation and suppress the induction of a cation current by oxygen-glucose deprivation (OGD) (Aarts et al., 2003).

While primarily linked to the development of neurogenic pain and inflammation in sensory neurons, the TRPV1 channel is also present in diverse brain regions, such as the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus, striatum, midbrain, and amygdala (Caterina and Julius, 2001; Julius and Basbaum, 2001; Cristino et al., 2006). Elevated expression of TRPV1 has been observed in the hippocampus of rats, the dentate gyrus of mice with temporal lobe epilepsy, and the cortex of patients with temporal lobe epilepsy (Bhaskaran and Smith, 2010; Saffarzadeh et al., 2015; Sun et al., 2013). The activation of the TRPV1 channel has been linked to the onset of epilepsy (Nazıroğlu and Övey, 2015). Researchers have reported that inhibiting the TRPV1 channel exerts protective effects on hippocampal and DRG neurons against epilepsy and epilepsy-induced calcium entry, using compounds such as capsazepine, 5'-iodoresiferatoxin, and resolving (Nazıroğlu and Övey, 2015).

Furthermore, the activation of TRPV3 has been demonstrated to mitigate epileptiform field potentials and reduce the amplitude of field postsynaptic potentials evoked in CA1 neurons of the hippocampus and the third layer of the neocortex (Müller et al., 2006). In contrast, TRPV4 activation by its specific agonist, GSK1016790A, has been found to upregulate pro-inflammatory cytokines (TNF-, IL-1, and IL-6). Interestingly, in a different study employing the pilocarpine-induced status epilepticus (PISE) model of epilepsy, TRPV4 exhibited a selective impact. It was demonstrated that its antagonist, HC-067047, effectively blocked these effects. Consequently, TRPV4 antagonists have been shown to enhance cell survival following status epilepticus (Wang et al., 2019).

In the kainic acid-induced epilepsy model, the expression of the TRPV4 protein remained constant, whereas there was an increase in TRPA1 protein expression, contradicting previous research on TRPV4 (Hunt et al., 2012; Wang et al., 2019). This inconsistency could be due to the varied distribution of TRP channels in different brain regions. Additionally, the same study revealed elevated levels of p-PKCa and pERK1/2 expression, along with reduced PKC expression in the hippocampus of kainic acid-induced epilepsy.

These findings suggest that PKC isoforms and ERK1/2 may be part of the downstream pathway involved in the cellular and molecular response (Lin and Hsieh, 2014).

TRPC channels also play a significant role in epileptogenesis, similar to their involvement in other disorders. These channels are crucial for neuronal proliferation, brain development, and survival. Genetic ablation (gene silencing) of TRPC3 has been associated with a reduced susceptibility to seizures in the pilocarpine-induced Status Epilepticus (SE) model. Consequently, elevated TRPC3 expression may increase the risk of recurrent seizures in the cortex (Phelan et al., 2017; Zhou and Roper, 2014). However, in contrast to TRPC3, TRPC6 expression was downregulated in chronic epileptic rats, and genetic ablation (gene silencing) of TRPC6 increased seizure susceptibility and neuronal vulnerability in the dentate gyrus but not in CA1 and CA3 neurons of the hippocampus (Kim and Kang, 2015).

Conclusion

TRP channels represent promising targets for drug development. Inhibitors of TRP channels hold potential for the treatment of conditions such as pain, asthma, and neurodegenerative disorders associated with heightened TRP channel activity.

In both experimental and clinical studies, TRP channels have emerged as intriguing therapeutic targets, and a multitude of compounds designed to modulate these channels are currently undergoing clinical development (Brederson et al., 2013; Moran et al., 2011).

The significance of TRP channels extends to their wide range of physiological functions within both the CNS and Peripheral Nervous System (PNS). Furthermore, they play a central role in the onset of numerous diseases and disorders by disrupting calcium homeostasis (Lee et al., 2021). These channels are ubiquitously present in multiple brain regions, including the spinal cord, and serve as vital signaling proteins involved in lipid metabolism and glucose homeostasis (Zhu et al., 2011).

Given their involvement in both physiological and pathological processes within the CNS and PNS, TRP channels are considered prospective therapeutic targets for addressing conditions such as neuropathic pain in the PNS and neurological disorders like epilepsy in the CNS.

The physiological functions and regulatory mechanisms of TRP channels have a significant impact on their involvement in various diseases. This includes genetic and acquired channelopathies, as well as a range of disorders where blocking one or more TRP channels could potentially alleviate symptoms or offer therapeutic benefits (Nilius and Szallasi, 2014).

One noteworthy example is TRPM3, which is expressed in both glial cells and neurons within the CNS. The discovery that primidone, a well-established drug for treating epilepsy and essential tremor, effectively blocks TRPM3 in the brain at therapeutic doses underscores the potential of TRPM3 as a target for neurological pharmacology (Krügel et al., 2017). It can be speculated that excessive activation of TRPM3 may contribute to white matter damage, and a centrally acting TRPM3 antagonist could potentially modify the course of the disease by preserving white matter integrity. This highlights the ongoing significance of the TRP family as a promising and valuable therapeutic target for a wide range of illnesses (Koivisto et al., 2021).

Further research into the structures of TRP channels is expected to greatly benefit drug development efforts. The increasingly diverse roles of TRP channels in physiology are crucial for advancing therapeutic interventions. While developing modulator drugs that specifically target TRPs has proven challenging, TRPs hold immense promise in the fields of chronic pain management, neurology, oncology, dermatology, pulmonology, cardiology, urology, and rare diseases.

Exploring the role of TRP channels, as one type of non-selective membrane channel, in epilepsy and identifying effective blockers for these channels may lead to additional treatment options for controlling this complex neurological disorder.

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Chapter 13

APPROACH TO KNEE OSTEOARTHRITIS IN PRIMARY CARE

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INTRODUCTION

Osteoarthritis (OA) is the most common joint disease in the world and develops as a result of disruption of the normal balance between the formation and destruction of articular cartilage and subchondral bone (1). According to clinicians, arthritis refers to inflammation of the joints and includes more than 100 rheumatoid diseases or conditions that affect the joints, the tissues surrounding the joints, and other connective tissues (2). According to the American College of Rheumatology, osteoarthritis is considered a disease with heterogeneous features at clinical, physiological, anatomical and molecular levels (3).

Due to the excessive degeneration of weight-bearing joints, the joint most frequently affected symptomatically by osteoarthritis is the knee joint (2). In addition to the knee joint, the hip, spine, hands, feet and fingers are the other joints with the most common involvement (4). Progressive cartilage destruction, especially in the knee, brings with it limitation of joint range of motion, severe chronic pain and serious muscle atrophy (2).

Although osteoarthritis is defined as a degenerative joint disease, genetic, biological, biochemical, mechanical and nutritional causes play a role in the formation of the disease. Therefore, these reasons should also be taken into consideration for successful treatment (5).

EPIDEMIOLOGY

Although there are many types of arthritis that affect the joints, the most common type is osteoarthritis. Like many chronic diseases, the incidence of osteoarthritis increases with age and affects women over the age of 65 the most (4,6). It is known that the incidence rate in women is 42.1%, while it is 31.2% in men (7). According to another study, 9.6% of men and 18% of women over the age of 60 worldwide are reported to experience symptomatic osteoarthritis (8).

When evaluated radiographically and symptomatically, the prevalence of hip osteoarthritis was 19.6% and 4.2%, respectively; the prevalence of knee osteoarthritis was 25.4% and 15.4%; the prevalence of radiographic foot osteoarthritis has been reported to be 0.1%-61% (9, 10, 11)

It is stated that 14 million people in the United States experience symptomatic knee osteoarthritis, more than 2 million people who experience symptomatic osteoarthritis are individuals under the age of 45, and more than 6 million people are between the ages of 45-65 (12).

According to the 2019 data of the Turkish Statistical Institute, the incidence of osteoarthritis in the Turkish population was determined to be 11.2% (13).

PATHOLOGY

Osteoarthritis, which affects all components of the joint such as cartilage, ligaments, joint capsule, subchondral bone and synovial tissue that make up

the synovial joint, is a disease in which the balance between bone destruction and repair is disrupted with the effects of many genetic, metabolic, mechanical and biochemical factors. Especially in the early stages of the disease, cartilage fibrillation begins, and while the fibrillation is more superficial, degeneration may progress to the deeper layers over time. Therefore, the primary changes of the disease appear as loss of articular cartilage, subchondral bone formation and osteophyte formation. The most important mediators that cause cartilage damage are metalloproteinases secreted from synovial cells and chondrocytes. In addition, tumor necrosis factor alpha and interleukin-17 release are other mediators that cause the cartilage destruction process (4, 14,15,16).

Change begins in the subchondral bone with the deterioration of bone collagen production, increased osteoclastic activity, alkaline phosphatase and non-collagen protein production. As the degeneration of the cartilage increases, the end parts of the cartilage tear, pieces of the joint begin to break off, and the thickness of the cartilage decreases. This situation causes osteophyte formation (4,14,15,16).

ETIOLOGY

In current sources, osteoarthritis is thought to originate from biochemical and mechanical reasons and is a dynamic event in which construction and destruction occur together (17). Although the molecular pathogenesis of OA is unknown, various genetic, biochemical, mechanical and environmental factors are thought to play a role. In fact; Cartilage damage, repair efforts that begin with the damage, and sclerosis in the subchondral bone are observed (18).

The most common cause of osteoarthritis is idiopathic and is called primary osteoarthritis. OA that occurs due to trauma, infection, developmental, genetic, metabolic disease, anatomical and neurological reasons is called secondary osteoarthritis (19).

RISK FACTORS

Considering the etiology of osteoarthritis, it appears to be a multifactorial disease. Many factors such as age, obesity, gender, lifestyle, change in eating habits, physical inactivity, joint morphology, occupational strain, proprioception disorder, previous traumas causing serious damage to the joint, metabolic dysfunction, circadian rhythm, presence of other comorbid diseases and genetic disposition. Many factors are seen as risk factors for osteoarthritis (4,20,21).

In the literature, osteoarthritis risk factors are grouped in various ways. Accordingly, when the evaluation is made in terms of primary and secondary osteoarthritis, no known cause can be found in primary osteoarthritis, while it is known that there are risk factors in the etiology of secondary osteoarthritis

resulting from joint destruction such as trauma, infection, and congenital deformity.It is stated that joint destruction increases due to factors such as age, gender, obesity, occupational strains and sports activities (22).

On the other hand, risk factors include personal risk factors (sociodemographic characteristics and family history, obesity and metabolic syndrome, nutrition and vitamin factors, smoking, bone mass and bone density, socioeconomic level) and joint related risk factors (bone/joint structure, injury). There are two main groups of approaches: muscle mass and muscle strength, joint load and joint alignment, occupation and physical activity status (8,23,24).

Another type of classification of risk factors is to divide them into two main categories: systemic and local factors. Under the heading of systemic factors; There are unchangeable risk factors such as age, gender, genetics and modifiable risk factors such as obesity, bone mineral density and nutrition quality. Under the heading of local factors, there are external factors such as trauma, physical activity, occupational activities and internal factors such as joint malalignment, ligament deficiencies, muscle strength deficiency, loss of proprioception (25).

More than 25% of osteoarthritis cases have comorbid diseases such as obesity, diabetes, pulmonary and cardiovascular disease, hypertension, metabolic diseases, musculoskeletal disorders and depression (20).

One of the strongest and major risk factors for osteoarthritis is age. With advancing age, the incidence of osteoarthritis and the number of joints affected by osteoarthritis increases. The incidence of osteoarthritis is quite high, especially in individuals over the age of 65. The reason why the incidence of osteoarthritis increases with age is explained by the decrease in the rate of chondroitin sulfate in the structure of proteoglycans with aging, the increase in the amount of keratin, mitochondrial DNA damage and inflammatory cytokines being added to the process, resulting in chondrocyte destruction. As a result of chondrocyte and cartilage destruction or damage, reactive oxygen products increase, causing thinning and weakness in the cartilage structure. In addition, tissue damage that develops with age, deterioration in physical activity, and muscle atrophy also increase the risk of osteoarthritis (26).

Since the protective effect of estrogen, especially on the knee and hip bones, is known, and therefore the decrease in estrogen levels after menopause increases the susceptibility to the disease, the probability of osteoarthritis in women is 2.6 times higher than in men. Hormonal changes that occur with menopause cause increased calcium absorption from the bone into the blood, and therefore bone loss also increases (8,26).

One of the most important risk factors for osteoarthritis is obesity. The risk of osteoarthritis increases 4-5 times in women with obesity. Damage to the

knee joint is especially severe due to obesity. It is known that mechanical stress on articular cartilage increases with increasing body weight. Apart from the mechanical load on the knee joint, obesity increases the risk of osteoarthritis as it is also associated with deterioration of posture, decreased walking and physical activity, and deterioration of the biomechanical structure of the knee joint (8,25,26).

Another common cause of knee osteoarthritis is trauma. Repetitive major or minor traumas within the joint, damage to the ligament and meniscus, tear or history of previous surgery accelerate the degeneration process of the joints and increase the incidence of osteoarthritis (27).

Factors such as insufficient dietary intake of vitamins C and D and dairy products, and low serum vitamin D and vitamin K levels increase the incidence of osteoarthritis. It is known that vitamin E, in particular, is effective in reducing the need for analgesics due to its pain relieving feature that positively affects chondrocytes (10,23).

Genetics are important determinants of knee osteoarthritis and are responsible for 50% of the susceptibility to the disease.Especially since the probability of Heberden nodule being seen in the daughter of a mother with distal interphalangeal joint involvement is 2 times higher, the susceptibility to osteoarthritis increases. Heberden's nodule and Bouchard's nodule are clearly seen in primary osteoarthritis with knee and hip involvement, and are carried by the dominant gene in women (26).

Repetitive movements performed in certain professions cause damage to the joints, causing osteoarthritis. Exercise, which is effective in increasing bone mineral density, can cause increased damage to the joints with increasing age, thus predisposing to osteoarthritis. In addition to sports activities, the risk of osteoarthritis increases in farmers, workers in carpet weaving factories and boxers, as the load on the joints increases and the joints are damaged (8).

CLINICAL FEATURES

When osteoarthritis is symptomatic, the most important complaint is pain (28). The pain usually has an insidious onset, is intermittent, deep and aching, and increases with activities such as walking, going up and down stairs and squatting. The pain usually has an insidious onset, is intermittent, deep and aching, and increases with activities such as walking, going up and down stairs and squatting. As the disease progresses, rest pain and night pain are added to the picture (29). Pain intensity may not always be compatible with radiological findings (30). Since there is no nerve tissue in the cartilage tissue, pain originates from intra-articular and periarticular structures. Factors such as osteophytes irritating the periosteum, trabecular microfractures, intraosseous pressure in the subchondral bone, distension in the capsule, synovitis, bursitis,

and spasm in the muscles around the joint may cause pain (29).

One of the most important clinical symptoms in knee osteoarthritis is joint stiffness. Joint stiffness usually occurs after inactivity. The stiffness can be relieved with movement in less than 30 minutes.

Restriction of movement in the joint; It develops due to inappropriateness of joint surfaces, muscle spasm, capsular contracture or mechanical obstruction by osteophytes and free bodies (28).

Crepitation is one of the most important physical examination findings of osteoarthritis. Crunching sounds made when irregular surfaces slide over each other (30).

There may be soft tissue swelling in the joint due to effusion and synovitis. Synovitis and effusion are more common in the knee joint than in other joints. Swellings caused by surrounding soft tissue inflammations such as osteophytes and fatpad may also be observed (30). In advanced cases of osteoarthritis, deterioration of the joint surfaces, instability in the surrounding ligaments and loss ofmuscle strength may occur. These changes cause deformity in the knee joint. Varus deformity is more common than valgus deformity (31).

EVALUATION OF PATIENTS

Radiological Evaluation

Although the diagnosis of knee osteoarthritis can be made by clinical findings and physical examination, it is necessary to determine the degree of joint involvement as well as joint damage for diagnostic confirmation. Conventional plain radiographs are generally the preferred initial diagnostic method to determine the structure-pain relationship in knee osteoarthritis. While radiographic examination has several limitations, magnetic resonance imaging (MRI) has the ability to show all structures in the knee joint (32).

Conventional Radiography

Due to the low radiographic sensitivity, it may not be possible to detect bone changes in knee osteoarthritis by radiography in the early stages. However, when joint changes are seen on conventional radiography, it may not be necessary to resort to advanced imaging techniques. Changes that can be detected in radiography related to osteoarthritis are joint space narrowing, subchondral sclerosis, osteophytes, subchondral cysts and joint mice (33).

Subchondral sclerosis occurs with increased subchondral bone activity in the early stages of osteoarthritis. Osteophytes are protrusions that form in the capsule recess at the outer edge of the joint, where mechanical load is least (31). Osteophytitis is the radiographic feature best associated with knee pain among men and women in the community (34).

Narrowing of the joint space indicates the compression of the joint cartilage in the area exposed to load. In later stages, cysts may be seen as increased radiolucencies in the subcortical region. In more advanced stages, joint remodeling, deformities and loss of joint space may occur (31). Chondrocalcinosis may increase especially with aging and can be seen in 4.4% of patients over the age of 50 (35).

While extension radiographs of the knee in weight-bearing and non weight bearing states are limited in evaluating the disease status, joint space width and bone changes in the tibiofemoral joint are visualized in all standing semi flexion knee radiographs. It has been reported that the axial view of the patellofemoral joint, rather than the lateral view, is more effective in detecting joint changes in osteoarthritis (36). The presence of osteophytes in the patellofemoral joint is more sensitive but less specific than in the tibiofemoral joint. Radiographic evaluation of the tibiofemoral and patellofemoral regions should be included in all studies (34).

Magnetic Resonance Imaging

MRI is not necessary in most patients with symptoms of osteoarthritis or conventional radiographic features. However, knee MRI can be performed in patients with symptoms such as locking or instability and joint pain; It has an important diagnostic role in cases indicating meniscal or ligament damage. Various types of lesions can be expected to be observed with MRI in knee osteoarthritis. These include cartilage anomalies, osteophytes, bone edema, subarticular cysts, bone erosion, meniscus tears, ligament abnormalities, synovial thickening, joint effusion, intraarticular foreign bodies and periarticular cysts (32, 33, 34). MRI is sensitive in detecting changes in cartilage in the absence of radiographic findings of osteoarthritis and can provide ultra-early detection of osteoarthritis (37, 38).

Ultrasonography

Although this imaging technique differs from other imaging techniques because it does not involve radiation, it has disadvantages such as not passing through bone tissue easily, being operator dependent, and lacking standardized diagnostic criteria. Synovial effusion, synovial hypertrophy, Baker cysts and surrounding soft tissues can be observed with the ultrasonography technique (31).

Laboratory Evaluation

Mild synovitis may occur in patients with knee osteoarthritis, but markers of inflammation such as erythrocyte sedimentation rate and C-reactive protein levels are usually normal. Synovial fluid in knee osteoarthritis is of the non inflammatory type (39).

DIFFERENTIAL DIAGNOSIS

Axial involvement of osteoarthritis is as common as peripheral involvement. Cervical and lumbar involvement is most common. Osteoarthritis is one of the diseases that most commonly affects the cervical spine.

In the differential diagnosis of osteoarthritis, pathologies such as trauma, rheumatoid arthritis and seronegative spondylarthropathies, bone tumors, and thoracic outlet syndrome may cause a similar clinical picture with pain and nerve root compression. Neurological examination in cervical spondylosis is normal except for radiculosis or myelopathy (40, 41).

In the differential diagnosis of osteoarthritis in the lumbosacral spine, spondylolisthesis, congenital spine fusions, scoliosis, metabolic, neoplastic and inflammatory diseases, Maigne syndrome, sacroiliac joint dysfunctions and other problems related to this region (sacroiliitis) should be considered (42).

Distal interphalangeal joint involvement is common in patients with psoriasis, Reiter's syndrome, ulcerative colitis, and gout (42).

Primary inflammatory polyarthritis often involves proximal interphalangeal joints (42).

In involvement of the hip joint, rheumatoid arthritis, ankylosing spondylitis with peripheral joint involvement, osteonecrosis, infections and tumors should be taken into consideration (42).

In knee joint involvement, osteonecrosis, pigmented villonodular synovitis, infections, tumors, inflammatory arthritis, and crystal deposit diseases should be considered (42).

In cases of chronic gout, thickening and enlargement of the metatarsalphalangeal joint is observed (42).

TREATMENT

The aim of treatment is to reduce pain, protect and improve joint functions and increase the quality of life (43). International guidelines present nonpharmacological treatments as the first line of treatment for knee osteoarthritis. Exercise, patient education, and weight loss are the first recommended treatment methods (44).

There are publications showing functional improvement in patients with knee osteoarthritis with education and exercise. 9825 patients with knee and hip osteoarthritis were given 3 sessions of training for two weeks, neuromuscular exercise was performed twice a week for 6 weeks, and as a result, the symptoms and medical drug intake of these patients decreased and their physical functions increased (45).

As with all chronic diseases, one of the basic elements in the treatment of osteoarthritis is educating patients about their diseases (46, 47). There are studies showing that it is beneficial to use a multidisciplinary approach in the education of patients, individual or small group counseling of 6-10 people, written and printed materials, video images, phone calls and social training (47,48).

Although nonpharmacological treatment is very important, less than 40% of patients with knee osteoarthritis receive these treatments. Clinical practice and rehabilitation are still inadequate. Although serious side effects can occur with long-term use of pharmacological treatments, they are used as the dominant treatments (49).

EULAR(European League Against Rheumatism) 2003 Knee OA Treatment Recommendations

1)Pharmacological and non pharmacological methods should be used together in the primary treatment of knee osteoarthritis.

2)In the treatment of knee osteoarthritis, attention should be paid to obesity, physical activity, age, accompanying comorbid diseases, pain severity, inflammation findings, extent and location of damage.

3)Non pharmacological treatment; It should include education, exercise, use of assistive devices (cane, insoles, etc.) and weight loss.

4)Paracetamol should be the first choice in pharmacological treatment and should also be preferred in long term treatment if beneficial.

5)If there is no response to paracetamol, nonsteroidal anti inflammatory drugs (NSAIDs) should be considered and given in combination with gastroprotective drugs.

6)In cases where NSAIDs are contraindicated, ineffective or have side effects, opioids should be given or combined with paracetamol.

7)Topical drug applications are effective and reliable.

8)Drugs that have a symptomatic slow effect, such as glucosamine sulfate, chondroitin sulfate, diacerein and hyaluronic acid, have both symptomatic and structural effects in osteoarthritis.

9)Intraarticular corticosteroids are recommended for acute exacerbations of knee osteoarthritis.

10)Surgical treatment should be considered in severe and persistent pain

Nonpharmacological Treatment Methods

1)Weight Loss

More than a third of the world's population is classified as overweight or obese.According to studies, more than 55% of the society will be classified as overweight or obese in 2030 (50).

In women with a body mass index (BMI) of 25 kg/m2, losing 5.1 kg of weight reduces the risk of developing knee OA by more than 50%. In a study, in patients with mild to moderate knee osteoarthritis and an average BMI between 33.6 and 36.4 kg/m2, pain complaints decreased significantly as a result of losing weight by 5% to 10%. Patients classified as overweight should aim to lose 7.7% of their minimum body weight to clinically improve their physical function (51).

2)Exercise

There are studies showing that exercise therapy has similar benefits to pharmacological treatment for pain, which is one of the most important complaints of osteoarthritis patients. Patients with knee osteoarthritis are recommended to strengthen the quadriceps muscle with regular exercise programs and to continue exercising regularly (52). For this purpose, strengthening, stretching and aerobic exercises can be applied in the treatment of patients to increase joint range of motion (53). In addition, aerobic exercises such as walking (3-5 days/week, 30-60 minutes) and swimming may be recommended to patients (54). It has been shown in various studies that the effect of exercises on limitation of movement becomes evident, especially from the 6th month (55).

Since exercise and lifestyle changes have such an important place in the treatment of osteoarthritis, increasing patients' compliance with exercise programs is an important element (56). Some suggestions are offered to healthcare professionals to increase patients exercise compliance (56, 57). These suggestions:

1)Patient Education

2)Follow up of patients at close intervals

3)Patients should follow up on their own and for this purpose keeping a diary

4)Follow up by a healthcare professional via phone or e-mail

5)Providing social support for family and relatives

6)Increasing exercise programs from mild to moderate levels

7)Providing psychological support

Treatment parameters used in the rehabilitation program, such as duration, frequency, type and intensity of the exercise program to be applied to the patient, should be adjusted according to the patient (58).

Fundamentally, the socioeconomic level of the patient, fear of movement, lack of confidence, time constraints and pain that may occur in the early stages of treatment are important reasons why exercise programs are not carried out by patients (59).

In order to increase the success of patients, physiotherapists should supervise exercise performance, provide their patients with self management confidence, implement group exercises, and be in constant contact by phone if necessary. To increase interest in exercise in patients with osteoarthritis; In follow up sessions, pain and functional activity should be constantly questioned, and the patient's exercise frequency and duration and exercise techniques should be reviewed and adjusted (60).

Patient Education

Patient education plays an important role in self-management and compliance with medication in patients with osteoarthritis. Appropriate patient education is needed to better manage the disease and improve the fit between patients' expectations and treatment outcomes. However, it is stated in the literature that patients have insufficient information about the disease. (61,62).

As a result of the study conducted in 13 countries, the messages that should be given to patients were determined (63). These messages:

1-The aim of regular physical activity and exercise programs is to reduce pain, prevent the progression of the disease and improve daily functions.

2)Overweight or obese patients losing weight

3)Without surgery, osteoarthritis symptoms are significantly reduced.

Other messages that should be given about the disease: It is to emphasize that symptoms vary from person to person and that osteoarthritis is not an inevitable part of aging and that long-term drug use should be avoided (58).

Pharmacological Treatment

Acetaminophen (up to 4 g/day) is the first oral treatment option in patients with mild to moderate pain, but when used in doses higher than 4 g/day, hepatotoxicity may occur as an undesirable effect. Patients who do not respond to acetaminophen treatment should be given non steroidal anti-inflammatory drug (NSAID) treatment (64, 65, 66). Capsaicin or lidocaine treatments can be used as topical treatment in patients who cannot tolerate systemic treatments (66).

Another treatment option for patients with moderate or severe pain, knee osteoarthritis with effusion, and who have not responded to oral analgesic or NSAID treatment is intraarticular (IA) corticosteroid injection (64,67).

In addition, IA hyaluronic acid injection can be applied to these patients, and this treatment shows later but longer effectiveness than IA corticosteroid treatment (64).

Recent studies have found that glucosamine treatment is effective on pain. For this reason, it is recommended to give patients glucosamine and chondroitin for treatment purposes; However, if no response is obtained within the first 6 months of treatment, this treatment method should be terminated (68,69). The recommended dose of glucosamine in treatment is 1500 mg/day and the dose of chondroitin is 1200 mg/day (69,70). In order to observe the effectiveness of glucosamine and chondroitin treatments, they must be used for at least a month. Gastrointestinal side effects of both glucosamine and chondroitin treatments are much less compared to NSAIDs. Recently, combined glucosamine and chondroitin treatments have become very common (69,71).Weak opioids can also be used to treat refractory pain (47,70).

As a result, it is thought that osteoarthritis will become an important health problem due to the increasing elderly population (67,68). Effective treatment of this disease, which is so common in the society and causes significant loss of function, in primary care will reduce disease related loss of function and referrals to second and third care. In this context, primary care physicians' providing exercise training, which has been proven to be important in the treatment of osteoarthritis, and encouraging their patients during the time allocated to the patient-physician encounter may increase patients' compliance with exercise treatment.

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Chapter 14

EVALUATION OF RISK FACTORS AND ORTHODONTIC TOOTH MOVEMENT ACCELERATION METHODS FOR ORTHODONTICALLY INDUCED INFLAMMATORY ROOT RESORPTION (OIIRR) - A LITERATURE REVIEW

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1. Orthodontic Tooth Movement

The process of orthodontic tooth movement is contingent upon the remodeling capacity of the alveolar bone and the soft tissues encompassing the tooth. The alveolar bone is a tissue that exhibits dynamic properties in response to mechanical forces. Osteocytes, which are the predominant cellular component of bone, serve as mechanosensory cells within the skeletal system. Osteoblasts, which are the cellular entities accountable for the process of bone formation, persistently reside on the surface of bones throughout their lifespan. Osteoclasts are the cellular entities accountable for bone resorption and play a pivotal role in regulating the rate at which orthodontic tooth movement occurs. Tooth movement is accompanied by the physiological occurrence of pressure and tension regions within the periodontal ligament, which arise from various biological processes. The immediate occurrence of pressure and tension zones leads to the deformation and constriction of blood vessels, resulting in cellular damage within the periodontal tissues.[1]

The periodontal ligament (PDL) serves as the target tissue for orthodontic forces. Initially, vasoconstriction takes place on the side experiencing pressure. The decrease in oxygen levels is attributed to alterations in blood circulation (Hypoxia). Apoptosis can be triggered in certain cells, leading to the formation of necrotic regions, depending on the magnitude of force applied and the extent of blood flow alteration (Hyalinized areas).[2–4]

An aseptic, acute inflammatory response ensues, whereby chemokines and cytokines are released by the cells in the local area, thereby initiating the immune response. Chemokines and cytokines, the majority of which exhibit proinflammatory properties, play a crucial role in the regulation and sustenance of the inflammatory response. This step is achieved through the process of the recruitment of inflammatory cells and osteoclast precursor cells within the extravascular space of the PDL. The process of differentiating osteoclast precursor cells into multinucleated giant cells (osteoclasts) is facilitated by maintaining elevated levels of chemokines and cytokines.[2–4]

Osteoclastogenesis refers to the process by which osteoclast precursor cells undergo differentiation, specifically on the pressure side of the alveolar bone. This differentiation leads to the formation of osteoclasts, which subsequently facilitate bone resorption and contribute to tooth movement. Cytokines are known to have a significant impact on this process: The binding of these molecules to the receptors for tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) induces the differentiation of osteoclasts from their progenitor cells. Furthermore, the cytokines IL-1 and IL-6 play a role in stimulating the release of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) from osteoblasts and inflammatory cells. Osteoclasts, in turn, interact with

the receptor activator of nuclear factor kappa-B (RANK) and macrophage colony-stimulating factor (M-CSF) present on their cell surface.[2–4] The molecules exhibit affinity for their respective receptors. Osteoprotegrin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) serve as paracrine regulators in the processes of osteoclastogenesis and cementoclastogenesis. The release of osteoprotegerin (OPG) from cells within the region serves as a pseudo-receptor for receptor activator of nuclear factor kappa-B ligand (RANKL). This interaction blocks the binding between the receptor activator of nuclear factor kappa-B (RANK) and RANKL, thereby exerting an antagonistic influence on the formation of mature osteoclasts. A high level of osteoprotegerin (OPG) on the compressive side of the tooth results in a reduction in the rate of osteoclastogenesis, thereby slowing down the process of tooth movement.[5]

2. Orthodontic Treatment Induced Inflammatory Root Resorption (OIIRR)

There are two main types of root resorption:

2.1. Internal Root Resorption: Internal root resorption occurs within the root canal space of the tooth. It typically starts on the inner surface of the tooth, near the pulp chamber, and progresses inward. Internal root resorption is often asymptomatic and may be detected during routine dental examinations or through dental X-rays. The causes of internal root resorption can vary, including trauma, chronic inflammation, or pulpal pathology. Treatment usually involves removing the affected tissue and filling the resorbed area.[6]

2.2. External Root Resorption: External root resorption, also known as external inflammatory root resorption, occurs on the outer surface of the tooth root.[7] It can be further classified into two subtypes:

a. Surface Resorption: Surface resorption involves the destruction of the outer layer of the root surface, known as cementum. It may be caused by factors such as trauma, prolonged orthodontic force, or pressure from impacted teeth. Surface resorption is typically slow-progressing and may be detected as a pink or yellow spot on the root surface.[6]

b. Inflammatory Resorption: Inflammatory resorption occurs in response to inflammation or infection in the surrounding periodontal tissues. It can be caused by factors such as periodontitis, tooth decay, dental trauma, or orthodontic treatment. Inflammatory resorption can affect both the cementum and dentin of the root. If left untreated, it can lead to a significant loss of tooth structure.[6]

Orthodontically induced inflammatory root resorption (OIIRR), also known as orthodontically induced apical root resorption (OIARR), refers to a specific type of root resorption that occurs as a result of orthodontic treatment.

It is characterized by the inflammatory destruction of the root structure near the apex of the tooth.[8–11]

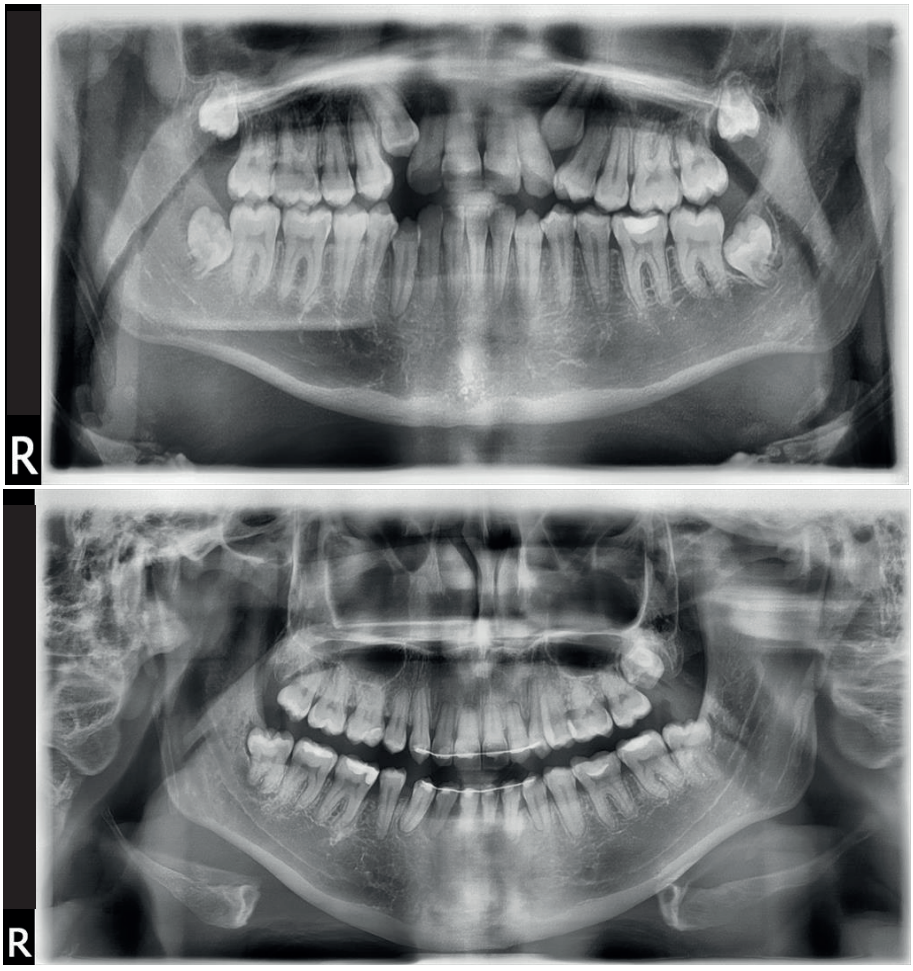

Figure 1. *Panoramic radiograph of the patient with orthodontically induced inflammatory root resorption (OIIRR) before and after treatment*

OIIRR is considered a pathological process that can affect the roots of teeth during orthodontic treatment. It is different from the physiological root resorption that naturally occurs when primary teeth are replaced by permanent teeth.[8]

The exact mechanisms underlying OIIRR are not fully understood, but it is thought that excessive or prolonged orthodontic forces can lead to an inflammatory response in the periodontal ligament and connective tissue.

This inflammation can trigger the activation of osteoclasts. The severity of OIIRR can vary among individuals and may depend on factors such as the magnitude and duration of orthodontic forces, individual susceptibility, root morphology, and treatment protocols.[9–11]

The prevalence of orthodontic root resorption varies across studies. Kjaer's study revealed a significant correlation between dental morphological characteristics and a tendency for root resorption in context of orthodontic treatment.[12] Mirabella and Årtun observed a high prevalence of apical root resorption in adult orthodontic patients.[13] Motokawa et al. reported root resorption in 86.4% of orthodontic patients.[14] Bayir and Bolat documented an incidence of severe root resorption following orthodontic treatment of 14.8%.[15] Alsagr et al. found higher resorption rates in upper anterior teeth compared to lower anterior teeth.[16] Giovanni and Eliezer documented a notable incidence of significant root resorption in patients who underwent treatment utilizing the edgewise technique.[17]

To minimize the risk of OIIRR, orthodontists employ various strategies, including careful treatment planning, appropriate force magnitude, periodic monitoring, and regular adjustments to the treatment plan. Regular dental check-ups and X-rays are important for detecting any signs of root resorption early on, allowing for timely intervention if necessary.[8]

Tooth resorption during orthodontic treatment can occur for several reasons[15]:

a. Mechanical Factors: The application of forces to the teeth during orthodontic treatment can sometimes result in excessive pressure on the roots. Prolonged or excessive force can lead to root resorption. This type of resorption is known as external root resorption and usually occurs on the side of the root facing the direction of force.[18]

b. Genetic Factors: Some individuals may have a genetic predisposition to root resorption. They may be more susceptible to this condition even with minimal orthodontic force applied.[19–21]

c. Duration of Treatment: Longer orthodontic treatment durations may increase the risk of root resorption. Prolonged exposure to orthodontic forces can potentially contribute to root damage.[18,22,23]

d. Root Proximity: The susceptibility to root resorption during orthodontic treatment is higher in teeth with shorter roots or roots that are in close proximity to neighboring teeth. This phenomenon occurs due to a reduced availability of root surface area to sustain the exerted forces. The teeth that are primarily impacted by inflammatory root resorption resulting from orthodontic treatment include the maxillary incisors, mandibular incisors, and first molars.

To minimize the risk of tooth resorption during orthodontic treatment, it is essential to have regular check-ups with the orthodontist. X-rays and other imaging techniques may be used to detect any signs of root resorption early on.[11,12,24]

If significant root resorption occurs during orthodontic treatment, the orthodontist may modify the treatment plan, adjust the forces applied, or even discontinue the treatment if necessary. The treatment approach to external root resorption is dependent on the severity and extent of the resorptive process. Mild cases may necessitate observation and preventative interventions, such as the maintenance of optimal oral hygiene practices. In cases of more advanced complexity, the course of treatment may encompass the extraction of the tooth, the application of a filling material to the resorbed region, and the potential consideration of restorative or endodontic interventions aimed at maintaining the integrity of the tooth.[6,8,17,25,26]

OIIRR can occur when orthodontic forces are applied to endodontically treated teeth, increasing the risk of resorption. Furthermore, orthodontic treatment with extraction has been found to cause more apical root resorption in vital teeth than in endodontically treated teeth.[25]

3. Orthodontic Tooth Movement Acceleration Methods

The duration of orthodontic treatment is affected by many factors, such as the severity of the case or the treatment plan, and takes approximately 24-36 months.

Root resorption, decalcification, gingival inflammation, white spot lesions, and a decrease in patient satisfaction may occur depending on the prolongation of the orthodontic treatment period. For this reason, accelerated orthodontic treatment has been an interesting topic for both orthodontists and patients.

Non-surgical and surgical methods for accelerated tooth movement have been described in the literature.

3.1. Non-surgical Methods

- Local or systemic application of chemicals: Epidermal Growth Factor
  Parathyroid Hormone
  L-Thyroxine
  1.25 Dihydroxyvitamin D3
  Prostaglandins
  Osteocalcin
- Gene Transfer Treatments

- Relaxin
- Vibration
- Electrical current
- Magnetic field
- Photobiomodulation
- Low Intensity Pulsed Ultrasound (LIPUS) [27–33]

3.2. Surgical Methods

- Osteotomy
- Corticotomy
- Wilcko's Method: The topic of interest is Accelerated Osteogenic Orthodontics (AOO) or Periodontally Accelerated Osteogenic Orthodontics (PAOO)
- Corticotomy with Piezosurgery
- Piezocision and Corticision
- Microosteoperforation[34–36]

When the related publications, systematic reviews, and meta-analyses are examined, the following information is obtained:

The study conducted by Chan et al. aimed to assess the impact of microosteoperforation on inflammatory root resorption resulting from orthodontic treatment by applying buccal tipping force to the first premolar teeth, which have an extraction indication. The extracted teeth were analyzed using Micro-CT imaging. According to the available report, a higher incidence of root resorption was observed in the microosteoperforated group undergoing buccal tipping force on maxillary 1st premolar teeth as compared to the control group. The investigation of repair mechanisms in resorption areas subsequent to micro-osteoperforation was suggested as a topic for future research.[37]

Patterson et al. reported that in the maxillary first premolar teeth with buccal tipping force, more root resorption occurred in the piezocision group compared to the control group. They argued that the effect of the regional accelerator phenomenon emerged with the application of piezocision, and an increase in OIIRR resorption occurred.[38]

In a controlled clinical study by Ng et al. in which they examined the effects of low-level laser therapy on OIIRR, less root resorption was observed in the laser-treated group than in the control group. The mean resorption volume was 0.381 mm^3 in the laser applied group and 0.495 mm^3 in the

control group, and the difference between the two groups was statistically significant.[39]

The animal study conducted by Murphy et al. examined the impact of various orthodontic forces and corticision applications on root resorption. The insignificance of the difference in root resorption levels between light and heavy forces was observed through the utilization of histomorphometry and microCT examinations.[40]

The animal study conducted by Yadav et al. aimed to examine the impact of mechanical vibration on orthodontic root resorption. The impact of a 15-minute daily application of mechanical vibration at frequencies of 5, 10, and 20 Hz on root volume does not yield statistically significant results.[41]

Donald et al. evaluated the amount of root resorption of the maxillary central incisor in nonextraction cases with and without corticotomy. In the measurements made with periapical radiography, it was stated that 1.1 mm more root resorption occurred in the control group than in the corticotomy group.[22] However, Patterson et al. stated that the application of corticotomy did not increase the amount of root resorption.[42]

4. Evaluation of Orthodontic Treatment Induced Inflammatory Root Resorption (OIIRR) in Patients Treated with Clear Aligners

Clear Aligners are a type of orthodontic appliance that applies controlled forces to the teeth to achieve tooth movement and alignment.[43]

While aligners are generally considered a more conservative orthodontic treatment option compared to traditional braces, they still involve the application of forces to the teeth. The potential risk of root resorption exists with any orthodontic treatment, including clear aligner treatment, although the incidence and severity may vary.[43–45]

The forces exerted by aligners are typically more gentle and continuous compared to the forces applied by braces. This may theoretically reduce the risk of root resorption. However, it's important to note that the risk of root resorption depends on various factors.[46]

The current researche indicates that the incidence of root resorption with clear aligner treatment is generally low. However, more long-term studies are needed to better understand the relationship between clear aligners and root resorption and to identify potential risk factors associated with clear aligner treatment. The majority of research on root resorption has focused on traditional fixed braces rather than aligners.[44,45,47] Additionally, the available studies have relatively small sample sizes. New research and advancements in clear aligner technology may impact our understanding of the relationship between aligners and root resorption.[43–48]

In order to minimize the potential risk of root resorption during clear aligner treatment, the protocol employed is similar to that used in braces treatment. Orthodontists regularly perform a comprehensive assessment of the patient's dental and medical history, conduct a thorough examination, and acquire relevant imaging, such as X-rays, to evaluate the current condition and health of the teeth and roots.[43,45,46]

5. The Effect of Hormones and Medication Administration on Orthodontic Treatment Induced Inflammatory Root Resorption (OIIRR)

Estrogen is a hormone that plays a role in various physiological processes, including bone remodeling. Studies have indicated that estrogen may have a potential influence on orthodontic tooth movement and the occurrence of root resorption. Estrogen may enhance tooth movement by promoting bone remodeling and increasing osteoclast activity, which is involved in the resorption of bone during tooth movement.[49] However, other studies have reported conflicting findings, suggesting no significant effect of estrogen on tooth movement. Several studies indicate that estrogen could have a protecting influence on root resorption by modulating the equilibrium between bone formation and resorption. It has been proposed that estrogen may help maintain the integrity of the root surface and protect against excessive resorption. However, additional research is needed to establish a clear association between estrogen and root resorption.[49]

The effect of medication administration on the rate of root resorption development and tooth movement in orthodontics in humans has been investigated in various studies. Bisphosphonates, for example, have been found to potentially inhibit root resorption during orthodontic tooth movement, but they may also interrupt tooth movement and alter treatment outcomes.[50] Thyroxine administration's influence on root resorption and tooth movement stays ambiguous.[51] Strontium ranelate has been shown to reduce osteoclasts and suppress root resorption and tooth movement in rats.[52] Prostaglandin E2 and Ca++ administration did not significantly affect root resorption after tooth movement.[53] NSAIDs like paracetamol, meloxicam, aspirin, and acetaminophen were found not to affect tooth movement.[54] Inhibition of odontoclastogenesis and root resorption has been observed with the use of anti-c-Fms antibody.[55] The increased incidence of root resorption caused by senescent cells induced by mechanical stress can be reduced through the administration of senolytic agents.[56] There is evidence to suggest that alveolar corticotomies are linked to a reduction in root resorption during orthodontic treatment.[57] In general, the influence of medication administration on the process of orthodontic tooth movement and the development of root resorption is a multifaceted and complicated phenomenon, wherein various medications may have distinct effects on the outcomes.

6. The Diagnostic Methods To Determine Orthodontic Treatment Induced Inflammatory Root Resorption (OIIRR)

After orthodontic treatment, various diagnostic techniques can be used to evaluate and identify root resorption.[6,8,9,26] These methods include:

6.1. Clinical Examination: The initial stage in diagnosing root resorption involves a comprehensive clinical examination conducted by a dental professional, specifically an orthodontist or dentist. The dental professionals will conduct a visual examination of the teeth, assess the occlusion, and observe for any signs or symptoms of root resorption, such as atypical tooth movement, alterations in tooth coloration, or increased sensitivity.[6,8,9,26]

6.2. Radiographic Imaging: Radiographic imaging plays a crucial role in the evaluation of root resorption, with dental X-rays being the most common technique employed for this purpose. Various types of X-rays may be employed for diagnostic purposes:

a. Periapical X-rays: Periapical radiographs offer a comprehensive visualization of individual teeth and their close anatomical features. Root resorption can be effectively assessed and identified through the utilization of dental radiographs, which serve as a valuable tool for evaluating tooth roots.[6,8,9]

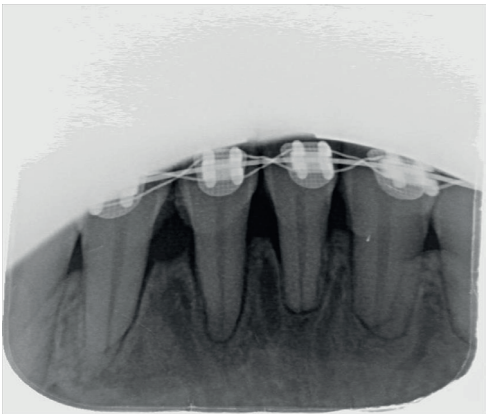

Figure 2. *Detailed examination of orthodontically induced inflammatory root resorption (OIIRR) with periapical radiography*

b. Panoramic X-rays: Panoramic X-rays are a diagnostic imaging technique that provides a comprehensive visual representation of the oral cavity, encompassing the maxilla, mandible, dentition, and associated anatomical structures. They can provide an overview of root resorption in multiple teeth.[6,8,9,26]

c. Cone Beam Computed Tomography (CBCT): Cone-beam computed tomography (CBCT) scans are capable of providing three-dimensional representations of dental structures, including the teeth and jaws. They can provide a more detailed assessment of root resorption and aid in treatment planning if significant resorption is detected.[7,58]

d. Digital Imaging: Digital imaging techniques, such as digital radiography, offer advanced capabilities that enable improved visualization of root resorption. Digital images have the capability of receiving magnification, contrast adjustment, and effortless sharing, thereby facilitating precise diagnosis and monitoring of root resorption.[6,8]

6.3. Transillumination: Transillumination is a diagnostic technique that entails the use of light to identify alterations in tooth structure, such as regions of resorption. It can be a helpful adjunctive diagnostic tool.[26]

6.4. Palpation: Gentle palpation of the teeth and surrounding tissues may reveal any abnormalities, including areas of root resorption. Nevertheless, this approach is characterized by a higher degree of subjectivity and is less frequently employed in comparison to alternative diagnostic methodologies.[6]

OIIRR can be a gradual process and may not be immediately apparent after orthodontic treatment. Therefore, regular follow-up visits with an orthodontist or dentist are crucial for monitoring the teeth and detecting any signs of root resorption. Early detection allows for timely intervention and appropriate management to prevent the progression of resorption and potential complications.

7. The Treatment Options of Orthodontic Treatment Induced Inflammatory Root Resorption (OIIRR)

The treatment options for OIIRR depend on the severity and extent of the resorption, as well as the overall health and stability of the affected teeth.

7.1. Monitoring: In cases of mild root resorption where the extent of resorption is minimal and not progressing, the orthodontist may choose to monitor the condition closely. Regular check-ups and dental imaging (X-rays) will be scheduled to assess the progression and stability of the resorption. Preventive measures, such as maintaining good oral hygiene and avoiding excessive forces on the affected teeth, may be recommended to minimize further damage.[8–11,24]

7.2. Adjusting The Orthodontic Treatment Plan: If ongoing orthodontic treatment is contributing to root resorption, the orthodontist may modify the treatment plan. This may encompass modifying the magnitude of forces applied to the teeth, changing the orthodontic appliance utilized, or potentially suspending the treatment temporarily until the resorption stabilizes. The

optimal course of action will be determined by the orthodontist, taking into account the specific needs of the individual.[11,14,24]

7.3. Root Canal Treatment: In cases where root resorption has progressed significantly and affected the pulp, root canal treatment may be necessary. Root canal treatment helps to preserve the tooth structure, alleviate any symptoms, and prevent further resorption.[17,25]

7.4. Extraction and Replacement: Extraction may be deemed necessary in instances of severe root resorption, where the affected tooth is considered unsalvageable or presents a potential risk to the nearby teeth. Following the extraction procedure, the absence of the tooth can be remedied through the utilization of a dental implant or alternative prosthetic restorations, which serve to reinstate both the functional and aesthetic components of the dental arch. Effective treatment planning for patients requires collaboration among the orthodontist, general dentist, and, if necessary, the endodontist.[9,11,14,17,25]

7.5. Photobiomodulation Therapy (PBMT): PBMT, also known as low-level laser therapy (LLLT), is a non-invasive treatment modality that uses low-intensity lasers or light-emitting diodes (LEDs) to stimulate cellular processes and promote tissue healing and regeneration. PBMT has been studied for its potential effects on various dental and oral conditions, including orthodontically-induced root resorption. Current literature has suggested that PBMT may have a beneficial effect on reducing root resorption associated with orthodontic forces.[39] It is proposed that PBMT can help modulate the cellular response in the periodontal ligament, reducing inflammation, accelerating bone formation, modulating the activity of cells involved in bone remodeling, and promoting healing processes, which may potentially minimize the extent of root resorption.[39,59–61] However, the mechanisms through which PBMT exerts these effects are not fully understood. The optimal parameters for PBMT, such as the specific wavelength, energy density, treatment duration, and timing, have not been firmly established. These parameters can vary depending on the specific PBMT device used and the targeted tissues. While PBMT shows promise as a potential adjunctive therapy for reducing root resorption associated with orthodontic forces, further research is needed to determine its effectiveness, optimal protocols, and long-term outcomes.[59–61]

8. Conclusions

- The complex and incompletely understood relationship between biological factors, auxiliary treatments, and root resorption necessitates that orthodontists have knowledge regarding the medications, hormones, and vitamins administered to their patients.

- The patient should be given information about root resorption, a detrimental side effect.

- Patients who are at heightened risk for root resorption, such as those diagnosed with asthma, should be identified and subjected to careful assessment in light of the potential for resorption.

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Figure Legends:

Figure 1: Panoramic radiograph of the patient with orthodontically induced inflammatory root resorption (OIIRR) before and after treatment

Figure 2: Detailed examination of orthodontically induced inflammatory root resorption (OIIRR) on periapical radiography

Chapter 15

TOXICOLOGICAL INSIGHTS INTO COVID-19 TREATMENT: A REVIEW OF ANTIBIOTICS, NITAZOXANIDE, IVERMECTIN, REMDESIVIR AND MOLNUPIRAVIR

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In late 2019, a new coronavirus known as SARS-CoV-2 emerged, causing the global pandemic of Coronavirus Disease 2019 (COVID-19). This virus, which shares genetic similarities with other coronaviruses, has proven highly pathogenic and contagious (1).

SARS-CoV-2 primarily uses a receptor called ACE2, found in various organs but highly expressed in the lungs and small intestine, to infect host cells. Research has shown that SARS-CoV-2 binds even more tightly to ACE2 than its relative, SARS-CoV (1, 2).

While SARS-CoV and MERS-CoV have intermediate hosts like civets and camels, SARS-CoV-2 might have one too. Evidence suggests pangolins could serve as such hosts, with a close genetic match to the virus (2, 3).

SARS-CoV-2 can recognize ACE2 in various animals, indicating a wide range of potential hosts. Differences in ACE2 activity across species may influence susceptibility to the virus (3, 4).

SARS-CoV-2 infects specific lung cells, leading to rapid replication and potential immune responses that can result in severe conditions like cytokine storms, acute respiratory distress, and respiratory failure, which contribute to COVID-19-related deaths (4).

COVID-19, also referred to as Novel Coronavirus Disease (SARS-CoV-2), was initially identified on January 13, 2020, following research conducted in December 2019 among a group of patients in Wuhan, the capital of Hubei province, China, who presented with respiratory symptoms, including fever, cough, and shortness of breath (5).

Symptoms typically encompass fever, dry cough, and shortness of breath. However, asymptomatic cases have also been reported. In severe instances, complications may include pneumonia, profound respiratory failure, sepsis, septic shock, renal failure, and fatality. The rate at which symptoms manifest remains uncertain. Notably, all patients subjected to chest computed tomography (CT) scans have exhibited pneumonia characterized by abnormal findings (5).

Pharmaceutical Measures Employed for COVID-19 Preceding Current Antiviral Therapies

1. Antibiotics

At the onset of the disease, neither the FDA nor any other regulatory body had approved a specific drug treatment. However, considering the viral nature of the disease, antiviral medications have gained prominence as potential treatment options. In situations where any drugs prove inadequate, the utilization of off-label drugs has been employed as one of the therapeutic approaches during the ongoing pandemic (6).

In the context of toxicological interactions and the significance of side effects, it is crucial to understand the limitations of using antibiotics and certain drugs, such as chloroquine and hydroxychloroquine, in the treatment of COVID-19.

The causative agent responsible for COVID-19 is the SARS-CoV-2 virus, which falls under the category of viruses. It is important to emphasize that antibiotics are not effective against viruses. Antibiotics are specifically designed to treat bacterial infections, not viral ones. Consequently, the use of antibiotics is not recommended for the treatment or prevention of COVID-19. In cases where bacterial coinfection occurs alongside COVID-19, which is relatively rare (approximately 3.5% of cases in studies), empirical antimicrobial agents have not been shown to provide any significant clinical benefits (7).

In monitoring COVID-19 patients, especially during the early stages of infection, healthcare providers may consider the use of antibiotics if there is an increase in inflammatory biomarkers such as serum C reactive protein (CRP) and procalcitonin (PCT). Azithromycin, an antibiotic with antiviral and immunomodulatory properties, has been used in the treatment of COVID-19. It is believed that azithromycin's properties may help mitigate the hyperinflammatory stage of the disease and prevent complications like lung fibrosis and excessive cytokine production (6, 7).

Chloroquine and hydroxychloroquine, on the other hand, are drugs primarily used for malaria treatment and rheumatoid arthritis. These drugs have been explored for their potential antiviral effects against SARS-CoV-2. In vitro studies have shown that these drugs may be effective against the virus, particularly in kidney-derived Vero E6 cells (8, 9). However, their clinical efficacy in treating COVID-19 has been a subject of debate, and their use should be approached with caution due to potential drug interactions and side effects.

In terms of metabolism and potential drug interactions, both chloroquine and hydroxychloroquine are metabolized by various cytochrome P450 enzymes, including CYP2C8, CYP3A4, and CYP2D6. They are also known to inhibit CYP2D6, which can affect the metabolism of other drugs. Therefore, caution is necessary when co-administering these drugs with inhibitors of CYP2D6 and P-glycoprotein, as well as drugs that have narrow therapeutic indices and are metabolized or transported by these routes. Additionally, these drugs can prolong the QTc interval and may lead to "torsades de pointes," a potentially life-threatening arrhythmia. Combining hydroxychloroquine with certain antituberculosis drugs or azithromycin has been associated with increased risks, particularly in older or diabetic patients. The bioavailability of hydroxychloroquine can also be affected when taken with antacids (10).

Regarding side effects, chloroquine and hydroxychloroquine have been associated with a range of adverse effects, including hematological issues like thrombocytopenia, aplastic anemia, neutropenia, and hemolysis (especially in individuals with glucose-6-phosphate dehydrogenase deficiency). Mild gastrointestinal side effects such as nausea, vomiting, abdominal pain, and diarrhea have also been reported. Importantly, these drugs carry a higher risk of ventricular arrhythmias and torsades de pointes in patients with cardiovascular comorbidities (9, 10).

Clinical trials investigating the use of chloroquine and hydroxychloroquine for COVID-19 have yielded mixed results. Early clinical data showed some promise in terms of improving clinical outcomes and viral clearance. However, larger clinical trials, such as the RECOVERY trial and multicenter studies in Brazil, did not demonstrate significant benefits in terms of mortality or clinical status when compared to standard care (8, 11).

In summary, while chloroquine and hydroxychloroquine initially garnered attention for their potential use in COVID-19 treatment, their effectiveness remains uncertain, and their use has been associated with various risks, including drug interactions and side effects. As a result, organizations like the World Health Organization (WHO) and national health agencies have advised against their routine use in the treatment of COVID-19, and clinical trials have been paused or modified due to safety concerns (11).

2. Nitazoxanidine

Nitazoxanide (NTZ) is a pharmacological compound derived from nitrothiazole benzamide (2-acetyloxy-N-5-nitro-2-thiazoyl). Initially, it was employed by Jean François Rossignol as an anthelmintic agent for addressing liver and intestinal parasitic infections; beyond its primary use, nitazoxanide, categorized as an anti-parasitic drug, has demonstrated effectiveness in treating various viral infections and protozoan infections (12). Its broad-spectrum antiviral activity extends to viruses such as influenza, parainfluenza, respiratory syncytial virus, rotavirus, and norovirus (12).

Furthermore, nitazoxanide and its active metabolite, tizoxanide, have exhibited significant efficacy against SARS-CoV-2 and MERS CoV in laboratory experiments conducted on Vero E6 cells (13). This potent antiviral activity across a range of viruses is attributed to its interaction with cytoplasmic RNA and the type 1 interferon pathways. Nitazoxanide is known to activate interferon (IFN), restore innate immunity, and inhibit the release of proinflammatory cytokines, thereby mitigating the inflammatory reactions triggered by SARS-CoV-2. It's worth noting, however, that while the in vitro activity of nitazoxanide against SARS-CoV-2 is promising, additional randomized clinical trials are imperative to assess its efficacy and safety for treating COVID-19 (14).

Numerous in vitro studies have indicated that nitazoxanide and its active metabolite, tizoxanide, do not exert inhibitory effects on cytochrome P450 enzymes. Consequently, it is expected that concurrent administration of nitazoxanide with other drugs will not lead to significant drug interactions (15). The active metabolite, tizoxanide, binds to plasma proteins at a rate exceeding 99.9%. Therefore, caution is warranted when administering nitazoxanide alongside drugs that exhibit high plasma protein binding but possess a narrow therapeutic index, such as warfarin (10, 14). Common side effects associated with nitazoxanide use include headache, abdominal pain, vomiting, and urine discoloration. Rarely observed side effects encompass dizziness, increased diarrhea, urticaria, skin rash, dyspnea, and gastroesophageal reflux (10, 14).

In a double-blind placebo-controlled study, nitazoxanide treatment was administered to 257 children hospitalized with acute influenza-like respiratory diseases. However, no significant differences were found in terms of treatment duration and hospital stay between the treatment group and the placebo group. Further analysis revealed that nitazoxanide treatment did not confer any substantial benefits. Thus, more clinical trial results are required to ascertain whether this agent, which has demonstrated in vitro effectiveness against SARS-CoV-2, offers clinical benefits in the context of COVID-19 (16). In a randomized controlled trial involving 392 COVID-19 patients with mild symptoms, participants were allocated to either the nitazoxinide or placebo group. Among these patients, 198 received placebo treatment, while 194 were treated with 500 mg/day nitazoxanide for 5 days. After 5 days, the viral loads of patients in the nitazoxinide-treated group were significantly reduced. However, secondary outcomes did not exhibit significant differences (17). In this context, nitazoxanide has demonstrated efficacy against COVID-19 but does not expedite recovery.

Nitazoxanide does not directly exhibit a beneficial effect against SARS-CoV-2. Nevertheless, a recent study has suggested that nitazoxanide may interfere with the N-glycosylation of the spike protein of SARS-CoV-2. This implies that it might be an effective agent for prophylaxis and treatment of mild to severe COVID-19. Nonetheless, further in silico and in vitro studies, as well as additional clinical trials and prospective investigations, are essential to validate the potential beneficial impact of nitazoxanide on the pathogenesis of SARS-CoV-2 infection (14).

Nitazoxanide may cause side effects, well known ones are stomach pain, headache, nausea and discolored urine, and hair lose (18). The systematic examination revealed a lack of substantiated clinical advantages associated with the administration of nitazoxanide for individuals with mild or moderate cases of COVID-19. Furthermore, the meta analyse investigation identified a decline in white blood cell count (WBC), lactate dehydrogenase (LDH), and D-dimer levels in patients subjected to nitazoxanide treatment; however, it

is important to note that the magnitude of this effect was deemed to be of modest to moderate significance; among the 211 adverse events documented in patients who received nitazoxanide, a substantial majority, accounting for 98.6%, were of mild to moderate intensity and in the majority of studies, gastrointestinal symptoms emerged as the prevailing adverse events (19).

3. Ivermectin

Ivermectin, belonging to the avermectin group, is primarily recognized as an antiparasitic agent used extensively in veterinary medicine. It is efficacious against both endoparasites (helminths) and ectoparasites (mites). The World Health Organization (WHO) has included ivermectin in its List of Essential Medicines due to its notable effectiveness against various parasites (20). Beyond its antiparasitic properties, clinical studies have revealed that ivermectin also exhibits antiviral, antibacterial, and anticancer activities. The mechanism behind its antiviral activity involves the inhibition of virus replication by disrupting the interaction between viral proteins and a human cargo protein complex known as importin (IMPa/β1). An Australian research team conducted an in vitro study demonstrating ivermectin's ability to hinder the replication of SARS-CoV-2, suggesting its potential as a promising therapeutic agent against the virus. However, before considering its use in COVID-19 treatment, further in vitro, in vivo, and randomized clinical trials are essential (21).

Ivermectin is metabolized by liver enzymes while it should not be co-administered with drugs from the monoamine oxidase inhibitor group, such as amitraz, as it can lead to sedation and side effects when used concurrently (10, 21). When used in accordance with recommended indications and doses, ivermectin is generally well-tolerated. Nevertheless, deviations from proper usage may result in side effects. Common side effects associated with ivermectin use include weakness, drowsiness, fever, rash, diarrhea, nausea, and vomiting. Rare but serious side effects encompass neurotoxicity and liver damage (21).

Results of controlled clinical trials investigating the effect of ivermectin in COVID-19 treatment:

In a randomized controlled trial involving 478 COVID-19 patients with a mild course, participants were allocated to either the standard care plus placebo group or the standard care plus ivermectin group. The study compared the two groups in terms of symptom relief at 14 days. The results indicated that the addition of ivermectin to standard care had little to no effect on improvement compared to the standard care plus placebo group (22).

A study combining data from six randomized controlled trials assigned 2860 COVID-19 patients with mild symptoms to various groups, including

the ivermectin plus standard care group and the standard care plus placebo group (21). The comparison focused on mortality rates on day 28. The study revealed 28 deaths in the ivermectin group and 38 deaths in the comparison group. Ivermectin treatment's impact on all-cause mortality at day 28 was reported to have little to no effect compared to the comparison group (21).

Ivermectin has demonstrated in vitro efficacy against SARS-CoV-2; nonetheless, it is crucial to emphasize that this observed in vitro activity transpired at significantly elevated concentrations, far surpassing those typically reached within human plasma and pulmonary tissue following standard therapeutic dosages (23). The utilization of Ivermectin as an intervention in COVID-19 treatment beared the potential for adverse health outcomes, predominantly associated with neurological dysfunction. This risk was particularly pronounced in instances where patients engage in self-medication and employ formulations originally intended for veterinary purposes (24).

Despite in vitro trials demonstrating ivermectin's efficacy against SARS-CoV-2, there wass insufficient clinical evidence to support its use as a treatment for COVID-19. In light of the lack of conclusive evidence regarding ivermectin's efficacy against COVID-19, the World Health Organization (WHO) had recommended its use only in the context of clinical trials (21, 25).

4. Remdesivir

Remdesivir is an antiviral drug developed by Gilead Sciences. It has previously been used in the treatment of Ebola and Marburg virus infections. Remdesivir is a prodrug that gets metabolized to an analog of adenosine triphosphate. Its antiviral effect is attributed to its inhibition of RNA-dependent RNA polymerase, effectively interfering with viral RNA synthesis. Importantly, Remdesivir exhibits a broad spectrum of antiviral activity, being effective against various virus families (26).

In numerous non-clinical studies involving coronaviruses, Remdesivir has demonstrated both prophylactic and therapeutic efficacy. In vitro testing on Vero E6 cells has indicated its activity against SARS-CoV-2. Notably, the first intravenous use of Remdesivir in a COVID-19 case in the United States reportedly resulted in significant clinical improvement (27, 28).

Regarding its pharmacokinetics and potential interactions (10, 28):

- Remdesivir serves as a substrate for various cytochrome P450 enzymes, P-glycoprotein (P-gp), and organic anion-transporting polypeptide OATP1B1. However, its metabolism primarily occurs via hydrolysis, with its active metabolite being GS-441524.

- Clinically significant interactions are unlikely due to rapid distribution, metabolism, and clearance following intravenous administration.

- Co-administration with rifampicin, carbamazepine, or phenytoin may lead to reduced Remdesivir exposure, making their concurrent use inadvisable.
- Remdesivir does not have a notable effect on QTc intervals.
- When administered alongside chloroquine or hydroxychloroquine, it has been observed that these antimalarial drugs can antagonize Remdesivir's intracellular metabolic activation and antiviral activity. Hence, their combined use is not recommended.

It's essential to emphasize that Remdesivir's pharmacological interactions and potential antagonism with certain medications should be carefully considered in clinical practice, and any adverse effects should be closely monitored due to the significance of these interactions.

In hospitalized COVID-19 patients with severe symptoms, the administration of Remdesivir resulted in clinical improvement in 36 out of 53 cases, corresponding to a 68% success rate. However, it's important to note that this study lacked a placebo or active comparator group, which makes it challenging to make a definitive assessment regarding the clinical effectiveness of Remdesivir (27). In clinical trials, the most frequently observed adverse effects associated with Remdesivir encompass nausea, vomiting, and elevated transaminase levels. Furthermore, hypersensitivity reactions, including anaphylaxis, have been reported. Less commonly, side effects such as headache, extremity pain, respiratory failure, organ insufficiencies, reduced albumin and potassium levels, diminished red blood cell counts, and decreased platelet counts responsible for coagulation have been documented. It is crucial to emphasize the significance of these adverse effects and their implications, as they underscore the importance of monitoring and managing potential toxicity concerns. These toxicological interactions and side effects of Remdesivir warrant vigilant attention in clinical practice, considering their potential impact on patient well-being and treatment outcomes (28).

Remdesivir operates as an antiviral ribonucleoside analog, specifically mimicking adenosine. Its activation transpires intracellularly, followed by penetration into the host cell, where it effectively halts viral RNA transcription. Historically, certain nucleoside analogs have been associated with inadvertent interference in mitochondrial RNA or DNA polymerases, potentially leading to mutational modifications in mitochondrial DNA (mtDNA). Given this precedent concerning the mitochondrial toxicity of ribonucleoside analogs, researchers embarked on an investigation to ascertain the impact of Remdesivir on mitochondrial functionality. Employing in vitro methodologies and in vivo rodent models subjected to Remdesivir treatment, the study revealed noticeable amplifications in mtDNA copy numbers within Mv1Lu cells (reflecting a 35.26% increase ± 11.33%) and the liver (indicating

a 100.27% increase ± 32.73%). Nonetheless, these enhancements yielded only marginal alterations in mitochondrial performance (29).

5. Molnupiravir

Molnupiravir, an antiviral agent classified as a nucleoside analog, has been granted authorization for the treatment of COVID-19. Its mechanism of action involves conversion to the active metabolite N4-hydroxycytidine upon ingestion. This active metabolite is subsequently integrated into the viral genome, inducing a form of lethal mutagenesis. It is worth noting that the use of Molnupiravir was not recommended during pregnancy due to findings from preclinical animal studies indicating potential risks to embryonic development (30). The RNA-dependent RNA polymerase (RdRp) serves as a crucial enzyme in the replication of COVID-19 and appears to have a pivotal role in the pathophysiology of the disease. Molnupiravir specifically targets the RdRp, positioning itself as a potential candidate for the treatment of COVID-19. While findings from animal studies suggest the effectiveness of molnupiravir against COVID-19, it was imperative that well-designed randomized clinical trials that would be conducted in the future to substantiate its therapeutic benefits in individuals afflicted with COVID-19 (31, 32).

In clinical studies, frequently observed adverse effects encompass instances of headaches and diarrhea, with the molnupiravir group (12.5%) experiencing a lower incidence in comparison to the placebo group (18.8%). A noteworthy 93.3 percent of these adverse effects were categorized as mild in severity. Study's outcomes underscore the well-tolerated nature of molnupiravir. However, it is crucial to note that one participant had to discontinue early due to the development of a skin rash. Participants were allocated in a 1:1 ratio to investigate the influence of dietary intake on the pharmacokinetics of molnupiravir. They were administered either 200 mg of molnupiravir in a fed state or 200 mg of molnupiravir under fasting conditions. While there was an observed reduction in the rate of absorption under fed conditions, the overall exposure to the drug remained unaltered (33).

These findings emphasize the importance of assessing both pharmacokinetic interactions and the significance of potential side effects in clinical trials involving molnupiravir.

Conclusion

In conclusion, the emergence of SARS-CoV-2 in late 2019 led to the global COVID-19 pandemic, characterized by its high pathogenicity and contagiousness. As for pharmaceutical interventions, the use of antibiotics is not recommended for COVID-19 treatment, as it is a viral infection, and antibiotics are effective against bacterial infections. Chloroquine and

hydroxychloroquine, initially explored for their antiviral potential, showed mixed results in clinical trials, with concerns about potential side effects and drug interactions. Nitazoxanide has demonstrated in vitro activity against SARS-CoV-2, but its clinical benefits remain inconclusive. It does, however, exhibit potential in mitigating inflammatory responses. Ivermectin has shown in vitro efficacy against the virus but at concentrations not typically achieved in human plasma, and its use for COVID-19 treatment poses potential adverse health effects, especially when using formulations intended for animals. Although promising in vitro, lacks substantial clinical evidence to support its use in COVID-19 treatment. Additionally, there is a risk of adverse health outcomes, especially when used without medical supervision or with veterinary formulations. In contrast, nitazoxanide has demonstrated some potential in clinical trials, but further research is necessary.

The antiviral drugs Remdesivir and Molnupiravir had shown promise in the treatment of COVID-19. The potential pharmacological interactions and side effects of both drugs underscore their importance in clinical practice, emphasizing the need for continuous monitoring and management of potential toxicity concerns. These findings further highlight the significance of thorough assessment in clinical trials involving these antiviral agents.

In summary, while various drugs have been explored as potential treatments for COVID-19, it is essential to acknowledge that many of these treatments also exhibit their own set of toxicological side effects, albeit generally at tolerable levels. However, the evolving landscape of the pandemic has necessitated a shift towards the development and evaluation of novel pharmaceutical interventions. Rigorous clinical trials are imperative to not only establish the efficacy and safety of these pharmaceutical interventions but also to usher in a new era of evidence-based treatments for the effective management of COVID-19.

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Chapter 16

EVALUATION OF CARDIOVASCULAR DISEASE RISK KNOWLEDGE LEVEL AND HEALTH IMPROVEMENT BEHAVIOURS OF PATIENTS WITH HYPERTENSION

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According to the World Health Organization (WHO), 17.9 million deaths are attributable to cardiovascular diseases (CVD), which are among the leading causes of non-communicable mortality worldwide (31% of all deaths and 44% of deaths due to non-communicable diseases) (Chigom, 2018). According to the cause of death statistics in our country, circulatory disorders are leading at 38.5%, and it is stated that the majority of deaths due to circulatory system diseases are caused by CVD (Turkish Statistical Institute, 2020). The good news about CVD is that it is largely "preventable" (Republic of Turkey Ministry of Health, 2021) because with most community-oriented practices, CVD can be prevented by controlling behavioral risk factors such as tobacco use, unhealthy diet, inactivity and obesity (Liu, 2014). The most significant cardiac risk factors worldwide, according to the INTERHEART study which was conducted in 52 countries, include dyslipidaemia, smoking, hypertension (HT), diabetes, abdominal obesity, psychosocial factors, inadequate consumption of vegetables and fruits, and irregular exercise (Yusuf et al., 2004). Similar findings were obtained in the TEKHARF study conducted to determine cardiac risk factors in our country (Onat et al., 2017).

Due to its great prevalence in the population, rising mortality, and status as the primary cause of disability worldwide, hypertension is one of the major risk factors for CVD (Lamprea-Montealegre, 2018). In addition, the presence of risk factors in patients in addition to the diagnosis of HT has an additive effect on each other in terms of the development of CVD. It is also known that keeping blood pressure under control in hypertension will reduce the development of CVD. This reduction in blood pressure can be achieved by a range of highly effective and well tolerated lifestyle and drug therapies. Despite this, it is seen that the control of blood pressure is poor worldwide, and therefore hypertension continues to be the leading "preventable cause" of CVD and all-cause deaths globally (Williams, 2018).

According to social behaviour models, awareness of the detrimental consequences of behaviour on health is a prerequisite for behavioural changes. CVD prevention efforts are built on public education programmes since insufficient knowledge may result in a lack of motivation for behavioural change (Nissinen et al., 2001; Parker, 2005). According to the studies, understanding behavioural risks is the key to lifestyle change and those who perceive themselves to be at a higher CVD risk are more likely to adopt a healthier lifestyle (Imes and Lewis, 2014; Sheeran, 2014). Therefore, measuring knowledge, risk perception, and intention towards a healthy lifestyle is essential for the development and implementation of targeted public health interventions (Albarqouni et al., 2016; Liu et al., 2020).

In this study, we investigated the level of CVD risk factors knowledge and healthy lifestyle behaviours of patients diagnosed with HT.

Materials and Methods

Study Population

The study was designed as a descriptive and cross-sectional type. The study was conducted on patients who consulted the cardiology outpatient clinic of a university hospital in the Eastern Anatolia Region of Turkey and fulfilled the inclusion criteria (aged 18 and over, followed up with a diagnosis of HT for at least the last year and taking medication, not diagnosed with cancer, voluntarily accepted to participate in the study, and did not have a physical problem that would prevent the completion of the forms) between June 2019 and August 2019. At an 80% power and a 95% confidence level, it was determined by the power analysis that the total should consist of at least 80 patients. Considering the possibility of missing data, 100 patients were included in the study. The research complies that the ethical principles of the Declaration of Helsinki and it was approved by the local ethics committee. Participants voluntarily participated in the research and provided with the informed written consent.

Data Collection

The research data were obtained by using the Healthy Lifestyle Behaviours Scale II (HLBS II), Cardiovascular Diseases Risk Factors Knowledge Level Scale (CVDRF-KLS), and a personal information form prepared by the researchers, containing the demographic data, family types, smoking status, alcohol intake status, and some data related to the diseased of the patients. The data were collected by the researcher using the face-to-face interview technique. The interviews with the patients were carried out after the medical examination, and the interview with each patient lasted an average of 15-20 minutes.

HLBS II; The Turkish validity and reliability tests were conducted by Bahar et al (2008) and adapted to the Turkish population. The scale is a four-point Likert type. The items were designed as 4 (regularly), 3 (frequently), 2 (sometimes), and 1 (never). It consists of 52 items. All of the items were prepared as positive propositions. The score that can be obtained from the scale varies between 52 and 208. The result obtained from the scale is directly correlated with how often each person applies the designated health behaviours. The scale has 6 subheadings consisting of physical activity, spiritual development, interpersonal relationships, nutrition, health responsibility, and stress management. Cronbach Alpha coefficient of the scale is 0.94 (Bahar et al., 2008) was calculated as 0.90 in this study.

CVDRF-KLS; The items in the scale are presented to the participants in the form of a complete sentence that can be true or false. Participants were asked to answer these statements as “Yes”, “No” or “I don’t know”. The

participant receives 1 point for each correct answer. The scale consists of 28 items in total and the items numbered 12, 13, 16, 17, 24, and 26 contain reverse propositions. These items are coded and evaluated inversely to the others. The score range that can be obtained is 0-28. There is a direct correlation between the score obtained from the scale and the level of knowledge of CVD risk factors. Turkish validity and reliability study was conducted by Arıkan et al (2009) and the Cronbach's alpha value was found to be 0.76 (Arıkan et al., 2009). In this study, Cronbach's alpha was calculated as 0.81.

Statistical Analysis

The analyses were performed with IBM SPSS (SPSS Inc., Chicago, Ill, USA) statistical analysis programme. The data were presented as mean (mean), standard deviation (sd), percentage (%), and number (n). The normal distribution of continuous variables was analysed by the Shapiro Wilk test. In the comparisons between two independent groups, the Independent Samples t-test was used when the normal distribution condition was met, and the Mann Whitney u test was used when the normal distribution condition was met. In the comparison of continuous variables with more than two independent groups, the ANOVA test was used when normal distribution condition was met and the Kruskal Wallis test was used when it was not met. In 2x2 comparisons between categorical variables, the Pearson chi-squared test was used when the expected value was >5, Yates' chi-squared test was used when the expected value was between 3-5, and the Fisher's Exact test was used when the expected value was <3. For comparisons greater than 2x2 between categorical variables, the Pearson Chi-squared test was used when the expected value was >5, and Fisher-Freeman-Halton test was used when the expected value was <5. In the comparison of two continuous variables, the Pearson correlation test was used if the condition of normal distribution was fulfilled, otherwise, the Spearman correlation test was used if not met. The statistical significance level was taken as $p<0.05$.

Results

In total, 100 patients were enrolled in the study. The mean age and body mass index of the participants were 64.40±11.3 and 29.54±6.1 respectively, and they had hypertension for 12.33±8.3 years (Table 1).

Table 1. Relationship Between CVDRF-KLS, HLBS II and Age, BMI, and Disease Duration (n=100)

	Age	**BMI**	**Disease Duration**
Mean±SD	64.40±11.3	29.54±6.1	12.33±8.3
Scale	**p**	**p**	**p**
CVDRF-KLS	-.140	-.031	.149
HLBS II	-.101	.123	.019

Health responsibility	.048	.044	.112
Nutrition	.129	-.033	.055
Interpersonal relationships	-.137	.063	.026
Physical activity	-.096	.074	-.055
Spiritual Development	-.139	.129	.046
Stress management	-.007	.180	-.056

HLBS II, Healthy Lifestyle Behaviours Scale II; CVDRF-KLS, Cardiovascular Diseases Risk Factors Knowledge Level Scale; BMI, Body Mass Index.

The majority of patients were female (61% vs. 395), 53% had a family history of CVD, 76% were not informed about CVD, 57% did not attend regular medical check-ups. A significant proportion of participants (72%) never exercised. Other baseline characteristics are presented in Table 2.

Table 2. Relationship Between Baseline Characteristics and CVDRF-KLS and HLBS II Scores

Characteristics (n, %)		CVDRF-KLS Mean ±SD	HLBS II Mean ±SD	Health Responsibility Mean ±SD	Nutrition Mean ±SD	Interpersonal Relationship-sMean ±SD	Physical Activity Mean ±SD	Spiritual Development Mean ±SD	Stress Management Mean ±SD
Sex	Female(61, 61.0)	17.77±4.9	118.72±16.7	20.87±3.8	22.25±3.3	23.00±4.0	12.41±3.9	22.92±3.9	17.51±3.2
	Male (39, 39.0)	18.21±4.6	117.69±19,8	21.21±4.0	21.31±3.9	22.41±5.4	13.49±3.6	22.56±4.7	16.62±3.3
	p	.761	.799	.532	.654	.455	**.050**	.809	.254
Education level	Not literate (33, 33.0)	17.03±5.6	115.48±13.9	20.21±4.1	22.15±3.2	22.33±3.4	12.58±3.5	21.67±3.4	16.82±2.6
	Prim.-sec. (56, 56.0)	17.88±4.1	120.00±20.2	21.32±3.8	21.93±3.7	22.98±5.3	12.84±3.8	23.39±4.8	17.55±3.7
	Hig. and above (11, 11.0)	21.00±4.3	118.27±16.3	21.73±3.8	20.82±3.8	23.00±4.2	13.55±5.0	23.00±2.6	16.18±2.4
	p	.053	.262	.532	.516	.819	.938	.079	.272
Marital status	Married (80, 80.0)	18.01±5.1	117.68±17.8	20.83±4.0	21.55±3.7	22.56±4.8	12.93±3.6	22.91±4.2	17.03±3.1
	Single (20, 20.0)	17.65±3.1	120.90±18.5	21.70±3.5	23.20±2.7	23.60±3.7	12.45±4.7	22.25±4.6	17.70±3.7
	p	.390	.657	.332	**.046**	.327	.271	.339	.354
Tobacco use	Non-smoker (55, 55.0)	17.85±5.1	119.55±16.1	20.87±3.9	22.36±3.3	23.18±4.3	12.58±3.6	23.24±3.8	17.56±3.1
	Smoked (36, 36.0)	18.33±4.3	116.36±21.2	21.03±3.9	21.56±3.7	22.00±5.1	13.39±4.2	21.67±5.0	16.61±3.5
	Smoking (9, 9.0)	16.89±4.8	118.67±14.9	21.67±4.1	20.22±4.3	23.33±4.2	12.11±2.8	24.44±2.7	16.89±3.3
	p	.651	.617	.840	.320	.446	.567	.085	.304
CVD	Yes (66, 66.0)	18.02±5.0	116.7±17.9	20.39±3.9	21.85±3.4	22.59±4.6	12.50±3.4	22.55±4.5	17.06±3.4
	No (34, 34.0)	17.79±4.4	121.4±17.7	22.18±3.5	21.94±3.9	23.12±4.5	13.47±4.5	23.24±3.7	17.35±2.9
	p	.699	.184	**.012**	.642	.667	.240	.499	.767
Number of comorbidities	0 (21, 21.0)	18.76±4.8	124.05±11.2	22.00±2.5	21.86±2.0	24.71±4.4	13.52±3.7	24.38±3.7	17.57±2.0
	1 (45, 45.0)	17.84±4.8	119.53±21.2	21.02±4.4	21.89±4.1	22.69±4.7	13.11±4.2	23.29±4.3	17.73±3.6
	2 (19, 19.0)	18.16±4.5	115.84±15.2	20.63±3.5	22.05±3.7	22.16±4.2	11.68±2.6	22.21±3.6	17.16±3.1
	3 (15, 15.0)	16.80±5.3	109.80±15.3	20.00±4.2	21.67±3.7	21.07±4.3	12.47±3.9	19.73±4.3	14.87±3.0
	p	.688	.069	.496	.978	.114	.385	**.018**	**.016**
Number of medications	1 (56, 56.0)	18.41±4.7	120.07±21.2	20.91±4.4	21.75±3.6	23.20±5.1	13.77±4.4	23.05±4.8	17.39±3.7
	2 (44, 44.0)	16.88±4.8	116.13±12.8	21.28±3.0	21.93±3.6	21.93±3.8	11.80±2.5	22.48±3.3	16.98±2.7
	3 (4, 4.0)	22.00±3.7	115.75±8.8	19.50±4.6	23.25±1.8	25.25±2.8	10.00±1.4	22.00±5.0	15.75±2.8
	p	**.044**	.559	.829	.594	.135	**.020**	.965	.564
Family history of HT	Yes (67, 67.0)	18.24±4.7	118.39±20.0	20.97±3.8	21.58±3.8	22.81±4.8	12.97±4.2	22.78±4.6	17.43±3.6
	No (33, 33.0)	17.33±4.9	118.18±12.9	21.06±4.1	22.48±2.9	22.70±4.0	12.55±2.7	22.79±3.6	16.61±2.4
	p	.388	.982	.606	.225	.823	.785	.837	.188

Family history of CVD	Yes (53, 53.0) No (47, 47.0) **p**	18.25±4.7 17.60±4.9 .580	119.40±19.9 117.11±15.5 .981	21.26±3.8 20.70±4.0 .876	22.09±3.7 21.64±3.4 .723	23.00±4.5 22.51±4.7 .625	13.08±4.3 12.55±3.1 .970	22.89±4.5 22.66±4.0 .726	17.34±3.7 16.96±2.8 .590
Informed about CVD by the physician	Yes (24, 24.0) No (76, 76.0) **p**	20.08±3.0 17.26±5.0 .019	120.17±16.2 117.74±18.4 .392	21.04±2.9 20.99±4.2 .964	21.92±3.3 21.87±3.7 .742	23.08±4.2 22.67±4.7 .692	13.38±4.5 12.66±3.6 .697	23.25±3.6 22.63±4.4 .355	17.67±3.4 17.00±3.2 .125
Medical check-ups	Regular (43, 43.0) Irregular (57, 57.0) **p**	19.09±3.9 17.07±5.2 .061	119.67±19.4 117.30±16.7 .234	21.49±3.5 20.63±4.1 .197	21.95±3.9 21.82±3.3 .357	22.95±4.8 22.63±4.4 .588	12.07±4.2 12.52±2.6 .762	23.12±4.4 22.53±4.1 .360	17.16±3.4 17.16±3.2 .839
Diet adaptation	Yes (60, 60.0) No (40, 40.0) **p**	17.93±4.7 17.95±4.9 .941	119.35 ±18.6 116.78 ±16.9 .271	21.13 ±4.4 20.80 ±3.0 .292	22.07 ±4.0 21.60 ±2.7 .400	23.08 ±4.5 22.30 ±4.7 .188	12.85±3.5 12.80±4.2 .557	22.98 ±4.2 22.48 ±4.4 .297	17.25±3.5 17.03 ±3.0 .449
Exercise	Regular (0, 0.0) Sometimes (28, 28.0) Never (72, 72.0) **p**	18.50±4.1 17.72±5.0 .639	122.9±17.8 116.5±17.7 .109	21.46±3.5 20.82±4.0 .394	22.14±3.6 21.78±3.6 .440	23.21±4.2 22.60±4.7 .538	14.68±4.6 12.11±3.2 **.003**	23.64±3.5 22.44±4.5 .183	17.82±3.0 16.90±3.3 .137

CVD, cardiovascular disease; HT, hypertension; HLBS II, Healthy Lifestyle Behaviours Scale II; CVDRF-KLS, Cardiovascular Diseases Risk Factors Knowledge Level Scale; Prim.-sec., Primary-secondary school; Hig. and above, High school and above.

Table 3 shows the correlation between the patients' CVDRF-KLS and HLBS II scale scores. The participants' CVDRF-KLS score was 17.94±4.8, and the HLBS II scale score was 118.32±17.9. Spiritual development and interpersonal relationships, of the HLBS II subscales, had the highest scores (22.78±4.2, 22.77±4.6, respectively), and physical activity had the lowest (12.83±3.8) score. The CVDRF-KLS and HLBS II scores as well as the subscale scores for interpersonal connections and spiritual development had a weak positive correlation. This relationship was found to be statistically highly significant ($p<0.01$).

Table 3. Correlation Between the Patients' CVDRF-KLS And HLBS II Scale Scores

Scale (Mean±SD)	1	2	3	4	5	6	7	8
CVDRF-KLS (17.94±4.8)	1							
HLBS II (118.32±17.9)	.246*	1						
Health responsibility (21.00±3.9)	.097	.747*	1					
Nutrition (21.88±3.6)	.091	.648*	.548*	1				
Interpersonal relationships (22.77±4.6)	.297*	.803*	.475*	.476*	1			
Physical activity (12.83±3.8)	-.043	.399*	.257*	.156	.091	1		
Spiritual Development (22.78±4.2)	.256*	.847*	.576*	.435*	.751*	.164	1	
Stress management (17.16±3.3)	.171	.773*	.460*	.438*	.547*	.322*	.637*	1

* The correlation shows high significance at the 0.01 level.
HLBS II, Healthy Lifestyle Behaviours Scale II; CVDRF-KLS, Cardiovascular Diseases Risk Factors Knowledge Level Scale

The relationship between CVDRF-KLS and HLBS II and characteristics of sociodemographic, disease, and healthy life behaviours were examined (Table 1 and Table 2). No difference was observed in CVDRF-KLS and HLBS II scores according to age, BMI, and disease duration ($p>0.05$) (Table 1). There was a significant correlation between marital status and the HLBS II nutrition subscale, between the presence of CVD and the HLBS II health responsibility subscale, and between the number of comorbidities and the HLBS II spiritual development and stress management subscales ($p<0.05$). In addition, a significant correlation was found between gender, the number of medications used for HT and exercise suitability, and physical activity on the HLBS II. A statistical relationship was found between the number of medications used for HT and being informed about CVD by the physician, and CVDRF-KLS ($p<0.05$) (Table 2). Other findings are reported in Table 2.

Discussion

The cornerstone of CVD prevention is a behavioural change towards a healthy lifestyle in individuals. To create this, it is vital to determine the individuals' awareness and risk perception of CVD. Therefore, we investigated the CVD risk knowledge and healthy lifestyle behaviours of patients with HT, which is an important risk factor for CVD. This study demonstrated that HT patients have a moderate level of knowledge about CVD risk factors and perform health promotion behaviours at a moderate level. As the level of knowledge of patients about CVD risk factors increases, their health

promotion behaviours also increase. Most patients were not informed about CVD and used spiritual development and interpersonal relationships the most and physical activity the least among health promotion behaviours.

CVD risk factors that contribute to the development of CVD may be variable and invariable. CVD morbidity and mortality can be reduced by managing variable risk factors such as high blood pressure, increased body weight, and insufficient physical activity (Francula-Zaninovic and Nola, 2018). When our study findings were evaluated, it was observed that the majority of the patients were female, overweight, had family members with CVD, were not informed about CVD, did not attend regular physician controls, and did not perform regular physical activity. Clinical trials: Framingham, Multiple Risk Factor Intervention Trial (MRFI) (Neaton and Wentworth, 1992), Asia Pacific Cohort Studies Collaboration (APCSC) (Rodgers, 2005), INTERHEART (Yusuf et al., 2004) showed that multiple risk factors increase the risk of CVD and that the risk of myocardial infarction is gradually increasing. Hypertension is one of the most important preventable risk factors for CVD (Yusuf et al., 2020). The Turkish Hypertension Consensus Report (Aydoğdu et al., 2019) and the Joint National Committee (JNC-8) emphasise taking a detailed medical history and questioning CVD risk factors in patients with HT. In addition, interventions for lifestyle modification are also recommended as a first step (James et al., 2014). In this regard, it is clear that various biological and behavioural risk factors should be paid attention to and necessary precautions should be taken for CVD awareness.

Researches have shown that behavioral risk information has an important and fundamental place in the change of lifestyle. In the same researches, the people who perceive higher CVD risk are more likely to adopt healthy behaviors to their lifestyle (Sheeran, 2014; Imes and Lewis, 2014; Ko and Boo, 2016). In our study, it was observed that HT patients had a moderate level of CVD risk knowledge and a moderate level of a healthy lifestyle, and their health improvement behaviours increased as the level of risk factor knowledge increased. This result supports the literature. Studies have also found that knowledge of certain risk factors is associated with healthy behaviour, but it is also noted that knowledge alone does not motivate behaviour change (Alzaman et al., 2013; Burger et al., 2016; Maruf et al., 2018). Information, which is a powerful modifying and improving factor for healthy living behaviors, should be combined with many conditions such as correct perception, positive health attitudes and socioeconomic factors for sustainability (Glanz et al., 2008). Therefore, evidence on the level of knowledge of CVD risk factors, especially in at-risk individuals, should be considered essential to the development and implementation of evidence-based health policies and targeted public health interventions (Imes and Lewis, 2014; Albarqouni, 2016).

In the literature, it is generally stated that incorporating several healthy lifestyle changes into everyday living at once is the most efficient and long-lasting way to achieve optimal blood pressure (Frisoli et al., 2011; Firmo et al., 2019). In addition, it is important to note that actions must be taken to encourage hypertensive people to adopt healthy behaviours in order to lower blood pressure, improve the efficiency of antihypertensive medications, and lower the risk of CVD (Firmo et al., 2019). In our study, it was observed that, among healthy lifestyle behaviours, the patients performed spiritual development and interpersonal relationships the most, and stress management and physical activity the least. Ineffective stress management and inadequate physical activity are known to be behavioural risk factors that are strongly associated with HT and CVD (Yusuf et al., 2020). In our study, the interpersonal communication and spiritual development behaviours of the patients increased as their CVD risk knowledge levels also increased. In ensuring hypertension control and preventing CVD risks, patients' compliance with treatment, adequate patient participation in disease management, adequate resources and time to carry out patient education and lifestyle change interventions are of great importance. Studies conducted in this context constitute evidence.

The importance of patient education for healthy lifestyle behaviours (Maruf et al., 2018), physician communication skill development (Tavakoly, 2020), patient empowerment through self-efficacy, improvement of patient-physician interactions, and increasing the effectiveness of patient self-management by collaborative care are also noteworthy (Xie, 2020). As there is an increase in patients' healthy living practices and healthy living behaviours, and they indirectly receive positive feedback about knowing HT management and CVD risks and preventing these risks, it can be said that the use of mobile technologies such as smartphones, tablets, patient monitoring devices, and personal digital assistants, has an important position in providing disease management (m-health) and supporting lifestyle changes (Piette et al., 2015; Li et al., 2020).

In our study, CVD risk knowledge levels and healthy life behaviours of the patients were not affected by age, BMI, and disease duration. While no study on the same subject was found in the literature, it was observed that there was no consensus in similar studies (Taşkın et al., 2018; Nyberg et al., 2020; Karatay et al., 2021; Sarıhan and Kul, 2022). These differences between the studies are thought to be due to the differences in the sample group of patients and the lack of comparison due to the lack of similar studies in the literature. In a prospective cohort study in a large group, a linear relationship was found between the number of healthy living behaviours and years without chronic disease. A BMI below 25 was also associated with a longer duration of health (Nyberg et al., 2020). Considering that the patients in our study had a mean

BMI of 29 and a mean disease duration of 12 years, it is again noteworthy that risk factors are modifiable. Therefore, these findings may support healthy choices plus strengthen evidence for interventions on the change of behaviors in daily life, and may also be useful in preventing negative behaviors.

In our study, it was observed that, among the healthy lifestyle behaviours, only physical activity differed according to gender and female hypertensive patients performed less physical activity than male patients. Studies also support this conclusion (Gavin et al., 2011; Hassen et al., 2022) and suggest that men are more likely to comply with and maintain exercise recommendations than women (Gavin et al., 2011). Although there was no statistical significance, the fact that there were more female patients in our study and that their CVDRF-KLS scores were lower than those of the male patients, helps to explain this result given the significance of inadequate physical activity in the development of HT and CVD (Yusuf et al., 2020). The cause for this gender gap, which may result in inconsistent levels of physical activity, may be due to gender disparities in self-efficacy, social support, and motivation, as well as lack of access to equipment, lack of safe and female-specific facilities, and cultural barriers (The Lancet Public Health, 2019). For this reason, CVD preventive interventions aiming to improve physical activity should take such apparent gender differences into account to ensure service uptake and sustainability. Qualitative studies are needed to obtain in-depth information on this issue.

In our study, while the level of CVD risk factors knowledge did not vary statistically by the marital status of HT patients, it was observed that married patients had more healthy eating habits. In one study, women's intention toward a healthy diet was found to be higher than men's (Hassen et al., 2022). According to our results, healthy eating behaviour was higher in women and HT diet adherence was higher in all patients. Although these results are not statistically significant, they can be explained by the high proportion of women in this study and the role of women in the household in our society. In this direction, the cultural household characteristics of the society should also be taken into account in healthy lifestyle changes for CVD risks.

While the presence of CVD in HT patients and family history of CVD or HT did not affect the level of knowledge of CVD risk factors and healthy lifestyle behaviours scale scores in our study, in a previous study, it was found that most of the patients were not aware that family history was a risk factor for CVD (Hassen et al., 2022). In developing countries and our society, the lack of knowledge about CVD risk factors also affects the awareness and attitudes of individuals. This situation constitutes an important obstacle to the control of the disease (Ahmed et al., 2013). Bayındır et al (2015) revealed that patients' and their families' levels of knowledge were boosted by their high levels of physical activity, health responsibility, spiritual support, interpersonal interactions, nutrition, and stress management. In our study, it was observed

that individuals without CVD were more inclined towards responsible healthy behaviours among the healthy lifestyle behaviours. Establishing a connection between adequate disease control and a possibly reduced risk of CVD may help to explain this. The results show that intervention is needed to increase healthy lifestyles. As emphasised in the studies, increasing awareness through education is an output that will directly increase the level of knowledge of CVD risk factors and has been established by studies (Glanz et al., 2008; Alzaman et al., 2013; Tayakoly et al., 2020). Also in our study, it was observed that HT patients who were informed about CVD had higher CVD risk knowledge levels. All of this information indicates that both situation-specific and general strategies based on risk factors for the individual, society and the environment are needed to reduce CVD and mortality.

In our study, it was noteworthy that while the majority of the patients followed their diets, the number of patients who did not go to regular physician controls and never exercised was also high. The statistical significance between exercising individuals and physical activity was an expected result. This suggests that there is a need for developing for tracking the patient to encourage to healthy lifestyle behaviors. The European Society of Cardiology's recommendations place a strong emphasis on the value of patient involvement and education, which may raise patients' knowledge and motivation levels (Kirchhof et al., 2016). Self-management practises for HT control and CVD risk education have been much emphasised more attention recently. In a randomised controlled trial, a substantial improvement in physician-patient communication skills, blood pressure results, self-efficacy in medication adherence, and health literacy were observed in HT patients treated by trained physicians compared to control group patients (Tavakoly et al., 2020). The efficiency of mHealth-supported self-management in this area has been proven in a systematic review (Li et al., 2020).

Conclusion

Our data, which consisted of direct information obtained from HT patients, revealed important information on awareness of CVD risk factors. Patients' CVD risk knowledge and healthy life behaviours are not at the desired level. Learning more about CVD raises one's awareness of the danger. A direct correlation exists between the level of CVD risk knowledge and change in healthy lifestyle behaviours. Based on these findings, it is essential to offer innovative and personalised interventions aimed at improving patients' knowledge of CVD risk factors and promoting healthy lifestyle behaviours. In this context; The most important issue that to be considered in the design and implementation of monitoring and evaluation systems to improve patient track services is to determine how patients perceive their CVD risk and risk factors. In addition, it is recommended to plan interventional studies to design and implement innovative systems where patients can be trained and empowered to manage their CVD risk factors.

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Chapter 17

HUMERUS SHAFT FRACTURES

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Humerus shaft fractures occurs in 3-5% of total fractures (1, 2). These fractures show a bimodal distribution according to age. It is mostly associated with high-energy traumas in young people, but it occurs as a result of low-energy traumas in elderly patients (1). The most common injury mechanisms are; falls on outstretched hands, motor vehicle accidents, and direct trauma to the arm (3). Fractures can also occur with muscle contractions alone (4). Fractures have also been reported during throwing sports (4). Humerus shaft fractures are seen in the proximal 1/3 region in 30%, in the middle 1/3 region in 60%, and in the distal 1/3 region in 10% (5).

Typical clinical deformities are observed in humerus shaft fractures depending on the location of the fracture and the effect of the surrounding muscles. If the fracture is proximal to the attachment site of the pectoralis major muscle, the proximal fragment is displaced into abduction and external rotation by the effect of the rotator cuff, and the distal fragment is displaced medially by the effect of the pectoralis major. If the fracture is just below the attachment point of the pectoralis muscle, but proximal to the attachment site of the deltoid muscle, the distal part is displaced laterally by the effect of the deltoid, and the proximal part is displaced medially by the effect of the pectoralis major, latissimus dorsi and teres major. If the fracture is distal to the deltoid insertion site, the proximal part is abducted and flexed, and the distal part is displaced proximally (6).

The most common nerve lesion in long bone fractures is radial nerve paralysis accompanying humerus fractures. The average incidence has been reported as 11% (7, 8). Approximately 3/4 of nerve injuries occur primary (during an accident), the rest are secondary (following fracture manipulation). In addition, iatrogenic radial nerve lesions seen after surgical treatment of humerus fractures are as important as other etiologies, although they are rarely reported in the literature.

Humeral shaft fractures are mostly treated conservatively. In studies by Sarmiento et al., satisfactory results were obtained in many patients with conservative treatment (9). In conservative treatment, hanging cast, U splint, abduction splint, skeletal traction, functional brace are used. The most important disadvantages of conservative treatment are; the long duration of treatment is that the entire extremity becomes dysfunctional and movement limitation in the joints may develop during the treatment. In today's industrial society, work loss as well as fracture healing is also taken into consideration. This situation has led to an increasing trend towards surgical treatment. On the other hand, there are also developments in implant technology.

ANATOMY

For the appropriate treatment of humerus shaft fractures, knowing the anatomical features of the arm is important in the treatment. The term

humeral shaft fracture is used for fractures in the proximal humerus, distal to the surgical neck, and distally, proximal to the supracondylar region.

Humeral shaft is a cylindrical bone and takes a triangular shape towards the distal. Intramedullary canal ends 2 to3 cm proximal to the olecranon fossa. The main muscles whose insertion is the humeral shaft are the deltoid muscle, pectoralis major muscle, and corocobrachialis muscle. The main muscles whose origin is the humeral shaft are brachialis, triceps brachii, and brachioradialis muscle.

Especially in proximal shaft fractures, the pectoralis major and deltoid muscles constitute the main deforming force at the fracture line. Interposition of the biceps tendon also causes nonunions in proximal shaft fractures.

The most important neurovascular structure in humeral shaft fractures is the radial nerve. If we briefly talk about radial nerve anatomy; the radial nerve consists of the posterior cord (C5-T1 ventral branch) of the brachial plexus. It is the only nerve that contains fibers from all segments of the brachial plexus. It is located in the sulcus nervi radialis, between the lateral and medial heads of the musculus triceps brachii together with the arteria profunda brachii. It is in the groove between the musculus brachialis and musculus brachioradialis, in the distal part of the arm. It divides into two terminal branches, superficial and deep, in front of Epicondylus lateralis. Musculus triceps brachii, musculus anconeus, musculus subanconeus, musculus brachioradialis and musculus extensor carpi radialis longus are the muscles that are directly stimulated by the radialis nerve. All extensor muscles on the back of the forearm (musculus extensor carpi radialis brevis, musculus supinator, musculus extensor digitorium, musculus extensor digiti minimi, musculus extersor carpi ulnaris, musculus pollicis longus, musculus extensor indicis, musculus abductor pollicis longus and musculus extensor pollicis brevis) , It is stimulated by the ramus profundus of the radial nerve. Nervus radialis is most commonly injured in humeral shaft fractures. A droopy hand is seen, but the extension of the forearm is not affected because the branches that stimulate the long and medial heads of the musculus triceps brachii are separated from the radial nerve in the axilla. The points where it crosses the humeral shaft are approximately 20 cm proximal to the medial epicondyle on the medial side and approximately 14 cm proximal to the lateral epicondyle on the lateral side.

The main structure that provides blood supply to the arm is the arteria brachialis. Nutritional arteries are the structures that arise from the arteria profunda brachii and play an important role in the nutrition of the humerus. Studies have shown that the blood supply is a single feeding artery in most of the humerus (10, 11). The location of this feeding artery is generally in the middle and distal third of the humerus, distal and medial to the attachment

point of the deltoid muscle (10, 11). For this reason, while the possibility of disruption of this circulation is less in proximally located fractures, fractures affecting the nutritional foramen in the middle and distal third region are more risky in terms of nonunion.

CLASSIFICATION

Many factors need to be considered when defining the classification of humeral shaft fractures. These include conditions such as the mechanism of injury (low energy, high energy, accompanying gunshot wound), location of the fracture (proximal, middle, distal) and extension to the joint, accompanying soft tissue injury, accompanying nerve (especially radial nerve) and vascular injury, the quality of the bone (osteopenic, pathological, normal), availability of prosthesis, etc. Although such descriptive statements provide a broad perspective on the picture of humeral shaft fracture, they are not sufficient for research and clinical studies. Therefore, more objective classification arrangements have been tried to be created to meet this need.

The most commonly used classification system for humeral shaft fractures is the AO/OTA (Arbeitsgemeinschaft für Osteosynthesefragen / Orthopaedic Trauma Association) classification system. In this classification, they are divided into three main groups according to the fracture type. Type A simple fractures, there is a single circumferential fracture line in the humeral shaft. There are two important fracture fragments, proximal and distal (fragments smaller than 10% circumferentially are ignored). Type B wedge-shaped fracture line, there is one or two fracture fragments in between, but after reduction, there is contact between the distal and proximal fragments and length is achieved. Type C comminuted fractures, there are one or two fracture fragments in between, but after reduction, contact cannot be achieved between the distal and proximal fragments. There is a fracture segment without a wedge in between. This segment may be intact or fragmented.

All main groups are divided into subgroups. Type A simple fractures are divided into subgroups as spiral, oblique and transverse. Linear fractures that angle more than 30° to the shaft axis are called oblique. Type B wedge fractures are divided into subgroups: intact and fragmented. Type C segmental fractures are divided into subgroups: intact and fragmented.

In addition to the AO classification, the Gustilo Anderson classification is used for open fractures. If we need to remember this classification, Type 1 is open wounds less than 1 cm with minimal tissue damage, Type 2 is open wounds between 1 and 10 cm accompanied by moderate soft tissue damage, and Type 3A is wide open wounds generally more than 10 cm. These are high-energy injuries that have tissue damage but can be closed without requiring a flap. Type 3B is an open fracture with extensive periosteal stripping that requires a graft or flap. Type 3C is a type with severe soft tissue damage

accompanied by vascular damage that requires vascular repair.

INSPECTION AND IMAGING

In multiple trauma, it may not be possible to take a history from the patient, but important information can be obtained from accident witnesses, ambulance personnel or family relatives. These types of patients can be evaluated in detail after they become stable. The frequency of open wounds, ipsilateral fractures, compartment syndrome and vascular nerve injuries is increased in these patients. There is a high risk of forearm compartment syndrome in injuries where the same side forearm fracture and humerus fracture occur together (floating elbow). Injuries such as accompanying arterial injuries, scapulothoracic dislocations, and hemopneumothorax can be life-threatening. In cases of humeral shaft fractures, the extremities are often short and varus upon examination at the emergency department.

Neurovascular examination is very critical in these fractures, especially the radial nerve examination should be checked and recorded before and after the procedure. Radial nerve palsy most commonly occurs with humeral distal shaft spiral fracture, called Holstein-Lewis (12). The frequency of radial nerve palsy is 22% in this type of fracture and 8% in other types of fractures (12).

Routine AP and lateral radiographs are used in imaging. A point that should be noted here is that the joint above and below the fracture line should be evaluated by seeing them on radiographs. Transthoracic lateral radiography is useful in evaluating deformity in humeral shaft fractures, especially in the sagittal plane. Another advantage of transthoracic radiography is that it allows lateral view to be taken without moving the distal humerus. Not moving the fracture line reduces the possibility of injury to the soft tissue and neurovascular structure. Traction radiography is not routinely indicated, but it can be used to see the distal and proximal extension of the fracture line in severely shortened fractures.

TREATMENT

Nonoperative Treatment

Conservative treatment of humeral shaft fractures is a subject with long-term, well-demonstrated successful results, and different authors describe different casts and splints. For the majority of humeral shaft fractures, successful results are observed with conservative treatment and follow-up (9,13,14). With conservative treatment, due to the wide range of motion of the shoulder and elbow joints, angular, axial and rotational unsuitable unions can be tolerated and functional limitations are very low. Fracture reduction is greatly aided by the effect of gravity on the arm. Many deformities can be hidden due to the fact that it does not bear weight and the muscle mass surrounding the humeral body. Acceptable limits for conservative treatment

can be listed as follows; anterior angulation of less than 20 degrees, varus/valgus angulation of less than 30 degrees, and shortness of less than 3 cm can be considered (15). Severe soft tissue damage, bone loss, vascular damage requiring repair, and brachial plexus damage are considered contraindications to conservative treatment (16). Having a radial deficit does not constitute a contraindication for conservative treatment (17).

There are different methods used in conservative treatment. One of these is the hanging casting technique. This technique was first described in 1933. It is used in displaced humeral shaft fractures with shortness, oblique and spiral fractures, distal humerus fractures, and comminuted fractures. In this technique, the forearm and humerus are cast, with the elbow flexed at 90 degrees and the forearm in a neutral position. In the humerus, the cast is extended 1 inch above the fracture line. On the forearm, the cast is brought up to the wrist. The cast is attached to the patient's neck to create traction. The cast should be light to prevent excessive traction. One of the difficulties of this method is that the patient must maintain his position while sleeping; the patient must sleep in a sitting or semi-sitting position. There should be nothing supporting the elbow from below during the day.

Another method in conservative follow-up is the technique called coaptation splint or U-splint. In this method, the cast begins over the shoulder and progresses along the lateral surface and extended from under the elbow to media and axillary. Humerus is wrapped in U -shaped. Then the wrist is attached to the patient's neck with a winding. In this way, a small elbow movement is allowed. Unlike the hanging cast technique, there is no cast weight that attracts down; increasing the diastasis of the fracture line and excessive gap formation is prevented. The most common deformity is varus and extension deformity. It can be done in the cast valgus position to prevent this. Today, this method is mostly applied until swelling descends before the functional bracing.

Another method, the functional bracing today is the main element of conservative treatment. In 1977, Sermiento et al. were described for conservative treatment of shaft fractures (18). Brace, which is made personalized or produced in certain molds, is wrapped around the broken arm. Brace usually administered after 1-2 weeks of immobilization. The first edema is expected to pass after fracture. Brace should reach 2.5 cm distal of the axilla and 2.5 cm proximal of humerus condyles. Sling should not be used to provide fracture reduction with gravity support. The biomechanical goal in functional bracing is to accelerate fracture healing by allowing some movement on the fracture line. Early starting elbow movements also reduces stiff elbow. In addition, having muscle contractions around the fracture accelerates fracture healing (17). Over time, edema decrease and muscle atrophy may cause thinning around the arm. In this case, brace should be tightened with the appeared

intervals. However, care should be careful that the extremely tight brace is the formation of ulcers in the skin. Brace should be worn full -time during the day. It can only be removed during a shower or during control radiographs. In this method, the first 3 week weekly control radiographs and fracture line are evaluated. Then, a fracture is evaluated with control radiographs every 3-4 weeks.

In April 2020, a multicentered retrospective study was conducted on conservative follow -up in Humerus shaft fractures (19). In this study, 29 percent of 1182 patients who were followed with functional bracing was needed and then the need for surgical intervention. The reasons for surgical intervention were seen as nonunion (60%), malalignment beyond acceptable parameters (24%), inability to tolerate functional bracing (12%) and exploration requirement due to radial nerve damage (3.7%) (19).

Operative Treatment

In some cases, surgical treatment is recommended for humeral shaft fractures. These situations include bilateral humerus fractures, pathological fractures, floating elbows (both arm and forearm fractures), multi-trauma patients, brachial plexus damage, vascular injuries, open fractures, and symptomatic nonunion cases. There are different opinions about radial nerve damage. It is not considered a definitive indication for surgery.

In a study conducted on this subject in 2020, radial palsy healing in humerus fractures was analyzed (20). In this study, the prevalence of radial palsy was evaluated as 12.3% in 7262 humerus fracture cases. In cases followed conservatively, the rate of spontaneous recovery of radial palsy was evaluated as 77.2%. In cases where conservative follow-up failed and surgical exploration was performed after the 8th week, the radial palsy recovery rate was evaluated as 68.1%. In cases where early (within the first 3 weeks) surgical exploration was performed and fracture repair was performed, the radial palsy recovery rate was evaluated as 89.8% (20).

One of the surgical methods is open reduction plate osteosynthesis. The aim of this method is to provide anatomical reduction by opening the fracture area and to provide compression and fixation between the fracture ends with interfragmentary lag screws and compression plates. Providing compression between the fracture ends in simple fractures increases the rate of union, but it is not possible to provide compression in comminuted fractures. In comminuted fractures, fixation is provided with bridge plates after the anatomical alignment is achieved. The use of locking or non-locking screws may vary depending on the surgeon's preference and bone quality. In general, it is recommended to use locking screws that pass through both cortices in osteoporotic bones. In a study, two locked screws and three non-locking screws were evaluated to have comparable results (21).

The basic approaches used during surgical intervention are as follows. The anterior approach is used for shaft fractures in the middle third. The skin is opened with a curved incision along the lateral border of the humerus, starting from the coracoid. The brachialis muscle is passed through split. The anterolateral approach is used for proximal and middle third shaft fractures. It is the extension of the deltopectoral approach to the distal. In deep dissection, the radial nerve is dissected between the brachialis and brachioradialis muscles. Posterior approach is used for middle and distal third shaft fractures. In this approach, the radial nerve is dissected between the triceps fibers in deep dissection.

Another option in surgical treatments is the intramedullary nailing (IMN) technique. Nowadays, proximal entry (anterograde) or distal entry (retrograde) locking nails are mostly used. After the nail is sent to the medulla, it is locked with screws both proximally and distally. For proximal entry nails, the skin is passed through a 4 cm skin incision inferior to the anterolateral edge of the acromion. The rotator fibers are reached by passing the deltoid fibers bluntly. Excessive extension of the incision inferiorly should be avoided. The axillary nerve passes approximately 7 cm below the acromion level. For distal entry nails, they are entered through a longitudinal incision between the triceps fibers from the posterior of the elbow. Entry is planned just proximal to the olecranon fossa.

Nail application inherently has better cosmetic results with smaller incisions and scar tissue. Studies have shown that shoulder pain is higher in IMN applications compared to plate and screw applications, but no significant difference was observed in terms of shoulder functions (22). Studies have shown no significant difference between IMN and plate screw applications in terms of the development of radial palsy (23).

In a study conducted in March 2000, the results of humeral IMN treatment and plate screw treatment were compared (24). In this study, after 16 weeks of follow-up, the union rate was evaluated as 87 percent in patients who received IMN, and the union rate in patients who received plate and screws was evaluated as 93 percent. As a result, both methods are successfully used in the surgical treatment of humerus fractures with similar success and complication rates.

Minimally invasive plate osteosynthesis (MIPO) is one of the techniques that has increased in popularity recently. The aim here is to create a method that combines the advantages of plate and screw application with the advantages of the IMN technique, such as less soft tissue damage and smaller incisions. In this method, a plate is placed through two different small incisions without opening the fracture line. A possible problem here is the possibility of secondary radial nerve damage. It can be thought that this risk increases

because the fracture line is fully opened and the radial nerve is not directly seen, as in the open reduction plate screw osteosynthesis method. However, one study compared MIPO and open reduction and plate screw application (ORIF) (25). In this study conducted on a total of 361 cases, the development of radial palsy after MIPO and ORIF applications was found to be low in both methods (25). In addition, in the study, union rates of MIPO application were evaluated as higher.

Another surgical method is external fixation. Nowadays, it is mostly used temporarily in patients with severe soft tissue damage or polytrauma, before plate and screw applications and IMN application. In addition to these situations, external fixation application is also used in the follow-up of infected nonunion cases. Although some studies mention its use in the treatment of acute simple humerus fractures, its use for this purpose in practice is limited.

In the external fixation application technique, the anterolateral surface is preferred for proximal pins. To reduce axillary nerve damage, it is entered through a small incision and a pin is sent to a safe position after reaching the bone. For distal pins, the lateral edge of the distal humerus is preferred. Here too, before sending the pins, it is recommended to enter through a small incision and reach the bone to reduce radial nerve damage. It is recommended that the most distal pin be sent just proximal to the olecranon fossa.

COMPLICATIONS

As with every fracture and treatment, some complications may develop in humeral shaft fractures or in the treatment of these fractures.

Nonunion

Fracture union is defined clinically as the absence of movement and pain in the fracture area, and radiologically as the occurrence of trabecular and cortical bone healing. It is called "delayed union" when there are radiological signs of healing, but union does not occur within the expected time depending on the location of the fracture (usually between 4 and 6 months in long bones). "Nonunion" is defined clinically as the presence of pathological movement and pain, and radiologically as the absence of union findings. The absence of union findings in long bones within 9-12 months is considered as nonunion.

In humeral shaft fractures, nonunion cases may develop as a result of fracture healing interruption during both operative and nonoperative treatments. Nonunions in the humerus, like all long bones, appear atrophic or hypertrophic. While biological problems generally come to the fore in atrophic nonunions, the main problem in hypertrophic nonunions is instability.

The reason for nonunion occurring after conservative follow-up is the opening in the fracture line due to muscle forces or gravity, especially

in simple fractures. But the most basic problem clinical and radiological examinations are not frequently performed in these patients who are treated with a conservative approach. If the gap in the fracture line is too wide, atrophic nonunion is usually encountered. In comminuted fractures or fractures that develop deformity after muscle forces, hypertrophic nonunion due to instability can be seen.

Diagnosis can be made by plain radiographic examination, but CT imaging is also useful in terms of treatment planning. It is usually treated with ORIF with or without bone grafting.

In the literature, primary nonunion rates are seen between 2% and 33% in nonoperative treatments and between 5% and 10% in operative treatments. When evaluated according to fracture locations, nonunion rates are higher in fractures in the proximal third region. Some factors that increase the likelihood of developing nonunion can be listed as follows. Metabolic endocrine disorders (osteoporosis, vitamin D deficiency), infection, smoking, obesity, treatment non-compliance, malnutrition, open fractures, insufficient stability in the fracture line, and limited mobility in the elbow and shoulder joints.

Especially mistakes made during surgical treatment can cause fractures to fail to union.Excessive space in the fracture line, application of short plates, preoperative planning errors, selection of inappropriate implants, indication errors in treatment selection, treating comminuted fractures as simple fractures, failure to ensure proper alignment and instability are the most common causes of nonunion after surgical treatment. But most importantly, the main reason for nonunion is damage to the soft tissue cover and disruption of tissue biology during surgical treatment. For all these reasons, atrophic or hypertrophic type humeral nonunions are encountered. In these cases, treatment should be directed towards the cause. Nutrient vessels course along the medial edge in the middle and distal thirds of the humeral diaphysis. Damage to the soft tissue in this area may disrupt the nutrition of the fracture line and affect the development of nonunion. Fracture union may be negatively affected due to the strong deforming effect of the deltoid muscle and pectoral muscle in proximal fractures, especially in cases followed conservatively. Another factor that may negatively affect union in proximal fractures is the interposition of the biceps tendon.

Routine AP and lateral radiographs are usually used to evaluate nonunion. Delay in the union of fracture ends, hypertrophic callus tissue, and psodoarthrosis formation can be observed on plain radiography. CT imaging may also be requested to see bridge callus formation in detail and for surgical planning. Blood tests such as CBC, CRP, ESR, Vitamin D, PTH, TSH may be requested to exclude infection and metabolic disorders.

The gold standard method in the treatment of nonunion is compression plate and bone graft application (26). It is successfully applied in symptomatic nonunion cases. In studies conducted, treatment success was evaluated as higher than IMN application (26). In the surgical treatment of nonunion, the fracture line should be revived and the intramedullary canal of the humerus should be opened. Fracture lines can be shortened up to 3 cm. After the tissues around the fracture are cleaned, it should be fixed with compression plates and compression should be applied on the fracture line. If necessary, it can be applied for compression if the type of fracture is compatible with the working principle of the lag screw. In diaphyseal fractures, the plate should be stabilized with at least three, preferably four, screws both distally and proximally. While cortical screws are used for compression, locking screws should be preferred for diaphyseal fixation. In cases where stability at the fracture line is suspected, a second plate can be applied anteriorly.

The main problem in hypertrophic nonunions is instability. Bone tissue wants to heal biologically however; pathological movement or deformity in existing fracture lines prevents union. In such cases, union can be achieved with conservative approaches, especially with changes made on the splint or brace. However, in cases where union does not occur, surgical options should be used. In these cases, fixation with external fixator, intramedullary nails or plate screws can be applied. In plate screw applications, the bridge plating method, which aims to provide stability throughout the fracture rather than compression on the fracture lines, should be preferred. Especially in comminuted fractures, very long plates that cover almost the entire humerus should be used and minimal movement should be allowed in the fracture line by fixation with diluted screws. In bridged plate applications, a minimum of 3-4 screws should be applied from the distal and proximal fracture sections.

Conservative follow-up can be planned in limited cases such as patients with high surgical risk, patients with low expectations, and asymptomatic nonunion. Functional bracing is used in conservative treatment. The double plating method is also used in fractures close to the proximal end and near the distal end. Similarly, double plating can be performed in cases of poor bone quality. In cases with severe bone loss, cortical bone grafts may be available. In nonunion surgeries, the radial nerve must be found and explored or neurolysis must be performed. The soft tissue at the fracture ends should be debrided to obtain quality bone tissue. It should be stabilized by ensuring the highest possible contact at the fracture ends. If there is an atrophic nonunion, bone grafts taken from the iliac crest can be placed on the fracture line. Demineralized bone matrix, reaming-irrigator-aspirator (RIA) or local cancellous bones are other options that can be used in grafting. In cases of recurrent nonunion, a free fibular graft can be used.

Malunion

Varus angulation is frequently encountered, especially in humeral shaft fractures that are followed conservatively. However, this angulation rarely causes loss of function. Transverse fractures are more risky in terms of malunion development.

In a study, the results of cases followed with functional bracing were evaluated (27). In this study, eighty-six percent of the cases recovered fully without limitation of shoulder and elbow functions after follow-up. According to subjective criteria, patient satisfaction after treatment was evaluated as ninety-five percent. Sixty-five percent of patients reported that they continued their daily activities without pain. In this study, malposition of more than ten degrees was observed in 10 (12.6%) of the patients followed with functional bracing, but good and excellent functional results were reported in two-thirds of the patients with malposition (27).

Radial nerve palsy

Radial palsy causes deterioration in the motor functions of the hand. The patient loses the ability to extend the wrist, thumb, and other fingers. Since these functions are also important during strong grasping, the strong grasping ability of the hand also decreases. Although radial palsy is very important for the motor functions of the hand, the sensory loss that occurs in radial palsy is mostly well tolerated.

Radial nerve palsy is one of the most common complications of humerus fractures. In cases of humeral shaft fracture presenting to the emergency department, radial nerve examination should be performed carefully. The incidence rate in closed fractures is evaluated as 8% to 15% (28). Among humeral shaft fractures, the probability of occurrence increases in fractures in the distal third region. It is evaluated to be approximately 22% in distal third region fractures (28).

In closed fractures, radial nerve damage is more likely to occur in the form of neuropraxia, whereas in open fractures the injury mechanism is more likely to be neurotmesis (29). Iatrogenic radial palsy is most often seen after open reduction and internal fixation. This rate is observed to be higher in the lateral approach. The lateral approach radial palsy rate was evaluated as 20%. In the posterior approach, this rate is evaluated as 11% (29).

An 85%-90% improvement in radial palsy cases is observed after 3 months of follow-up (30). Studies show that the average spontaneous recovery time is 7 weeks. However, the average time for full recovery is considered to be 6 months (30).

The first treatment option for radial palsy in closed humerus fractures is observation. It is recommended to perform electromyography (EMG) in the

2nd month. EMG is useful in assessing the extent of nerve damage, defining its current function, and monitoring recovery. The first function expected to improve during the healing process is wrist extension in radial deviation (31). Among the muscles innervated by the radial nerve, the brachioradialis muscle is considered to be the first to recover, while the extensor indicis muscle is the last to recover (31).

Radial nerve damage accompanying open fractures is usually neurotmesis type injuries. In a study on this subject, in 14 cases of open humerus fractures and radial plasy, laceration of the radial nerve or interposition between the fracture ends was detected during surgical exploration in 9 cases (64%) (32). For this reason, surgical exploration is recommended in cases of radial sprain associated with open fractures. In closed fractures, if no improvement is observed in the radial deficit during the 4-6 month follow-up period, surgical exploration is again recommended. Observation of fibrillation or denervation findings in EMG also constitutes an indication for surgical exploration.

However, tendon transfer surgeries can be planned in cases of permanent radial palsy that do not improve despite other treatments. The timing of tendon transfer is controversial. Among tendon transfer procedures, the most appropriate method for restoring wrist extension function is the transfer of the pronator teres muscle (PT) to the extensor carpi radialis brevis (ECRB) tendon. If recovery of the nerve is expected later, the ECRB tendon is preserved and the PT tendon is tied together in an end-to-side manner to provide wrist extension and if the radial nerve recovers, the ECRB function will also be preserved.

Many tendons can be transferred to the extensor pollicis longus (EPL) tendon to restore thumb extension. The most commonly used of these are the palmaris longus (PL) and the flexor digitorum superficialis (FDS) tendon of the 4th finger. When the FDS tendon is used, the tendon is generally divided into two and used to provide extension of both the thumb and index finger.

For the extension function of the other fingers, usually the flexor carpi ulnaris (FCU) or flexor carpi radialis (FCR) tendons are transferred to the extensor digitorum communis (EDC) tendon. However, FCU transfer has a disadvantage. After FCU transfer, the ulnar deviation function of the wrist is lost. This function means that important movements of the wrist such as throwing and using a hammer cannot be performed. For this reason, EDC transfer of the FCR tendon is applied more prominently.

After tendon transfer surgeries, the wrist is splinted at 30 degrees of extension, the elbow is at 90 degrees, and the forearm is in pronation, and the splint is renewed every two weeks. The splint is used for approximately eight weeks, but exercises are started gradually during this period. The transition to full activity occurs after the 3rd month.

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Chapter 18

GASTROINTESTINAL STROMAL TUMORS

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Introduction

Within the realm of oncology, a select group of tumors stands out as captivating entities due to their unique biological characteristics and clinical intricacies. Among them, gastrointestinal stromal tumors (GISTs) have emerged as a subject of profound scientific interest and medical significance.[1] These neoplasms, while relatively rare in comparison to other malignancies, present a fascinating and complex set of challenges that require a multidisciplinary approach for understanding and management.

GISTs, originating primarily within the gastrointestinal tract, offer a window into the intricacies of tumorigenesis, molecular signaling, and therapeutic advancements in oncology. In this chapter, we embark on an exploration of GISTs, delving deep into the scientific fabric that underlies these tumors, while also examining their epidemiology, pathogenesis, clinical manifestations, diagnostic strategies, therapeutic interventions, and the latest breakthroughs in GIST research.

The journey begins with a comprehensive analysis of the epidemiological factors surrounding GISTs, shedding light on their incidence, distribution, and demographic patterns. From there, we plunge into the molecular and cellular depths of GIST pathogenesis, where mutations in key genes like KIT and PDGFRA drive tumorigenesis, offering insights into the underlying molecular biology.

Clinical presentations of GISTs are multifaceted, often presenting a diagnostic challenge. Our exploration extends to the clinical landscape, where we dissect the symptoms, signs, and imaging modalities essential for accurate diagnosis. With histopathological examination as the cornerstone, we unveil the intricate diagnostic criteria and immunohistochemical markers crucial for GIST identification.

While surgery remains the primary treatment for localized GISTs, the therapeutic landscape for advanced and metastatic cases has been revolutionized by targeted therapy. We meticulously dissect the pharmacological interventions, such as tyrosine kinase inhibitors (TKIs) like imatinib, sunitinib, and regorafenib, that have reshaped the management of these tumors. The importance of personalized medicine, guided by the molecular profiling of individual tumors, emerges as a pivotal theme in GIST therapeutics.

In the ever-evolving field of oncology, GIST research continually pushes boundaries. We present the latest scientific advances, highlighting emerging therapeutic agents, strategies to overcome resistance, and the promise of precision medicine in GIST management. These cutting-edge developments offer a glimpse into the future of GIST care, raising optimism for patients and healthcare professionals alike.

In this chapter, we invite you to delve into the intricate world of gastrointestinal stromal tumors, a realm where scientific discovery converges with clinical application, striving for improved outcomes and enhanced understanding. Join us on this scientific odyssey as we unravel the complexities of GISTs, aiming to contribute to the broader body of knowledge surrounding these enigmatic tumors.

Epidemiology of Gastrointestinal Stromal Tumors (GISTs)

Understanding the epidemiology of gastrointestinal stromal tumors (GISTs) is essential for assessing the prevalence, distribution, and risk factors associated with this rare type of cancer. Here are key epidemiological aspects of GISTs:

1. Incidence:

• GISTs are relatively rare tumors, accounting for approximately 1-2% of all gastrointestinal malignancies.[2]

• The annual incidence of GISTs is estimated to be around 10 to 20 cases per million people.

• GISTs can occur at any age, but they most commonly affect adults, with a median age at diagnosis of around 60 years.

2. Anatomical Distribution:

• GISTs can develop throughout the gastrointestinal tract, with the majority occurring in the stomach (60-70%) and the small intestine (20-30%).

• Less commonly, GISTs may arise in the esophagus, colon, rectum, or in extraintestinal locations, such as the omentum, pancreas or mesentery.[2]

3. Gender and Racial Disparities:

• GISTs are slightly more common in men than in women, with a male-to-female ratio of approximately 1.5:1.

• There is no significant racial predilection, and GISTs can occur in people of all ethnic backgrounds.

4. Familial and Genetic Factors:

• A small percentage of GISTs are associated with familial syndromes, such as neurofibromatosis type 1 (NF1) and Carney-Stratakis syndrome.

• Familial GISTs are often linked to germline mutations in genes like KIT and PDGFRA.

5. Risk Factors:

• The exact causes of sporadic (non-familial) GISTs are not well understood. However, certain risk factors may be associated with their development, including older age and male gender.[3]

• Exposure to environmental factors or dietary habits is not a known risk factor for GISTs.

• A small percentage of GIST cases are associated with genetic syndromes such as neurofibromatosis type 1 (NF1) and familial GIST syndrome, which are characterized by an increased risk of developing GISTs.

6. Incidental Findings:

• Many GISTs are discovered incidentally during imaging studies or surgeries performed for unrelated conditions.

• Routine screening for GISTs is not performed in the general population due to their relative rarity.

7. Geographic Variation:

• GIST incidence rates may vary regionally and globally, with some areas reporting higher or lower rates than others. Geographic variation may be influenced by genetic, environmental, or healthcare-related factors.

8. Survival Rates:

• The prognosis for GISTs depends on various factors, including tumor size, location, mitotic index, and mutational status.

• With the advent of targeted therapies, such as tyrosine kinase inhibitors (TKIs), the overall survival and progression-free survival of GIST patients have improved significantly.

Pathogenesis

The pathogenesis of gastrointestinal stromal tumors (GISTs) involves a complex interplay of genetic and molecular events that lead to the development and progression of these tumors. The central components of GIST pathogenesis revolve around mutations in specific genes and the subsequent aberrant signaling pathways. Here's an overview of the pathogenesis of GISTs:

1. Origin from Interstitial Cells of Cajal (ICCs): GISTs arise from a type of mesenchymal cell called interstitial cells of Cajal (ICCs). ICCs play a vital role in regulating peristalsis and gastrointestinal motility by generating electrical signals that coordinate smooth muscle contractions.[4]

2. KIT and PDGFRA Mutations: The hallmark of GIST pathogenesis is the presence of activating mutations in key genes, most commonly in the KIT (c-KIT) and PDGFRA genes.[5]

• **KIT Mutations:** These mutations occur in approximately 85% of GISTs. Mutations in KIT lead to the constitutive activation of the KIT receptor, a tyrosine kinase receptor, resulting in uncontrolled cell growth and division.

• **PDGFRA Mutations:** About 5-10% of GISTs have mutations in the PDGFRA gene. These mutations lead to the activation of the PDGFRA receptor, another tyrosine kinase receptor, also causing uncontrolled cell proliferation.[6]

• **Wild-Type GISTs**: In a subset of GISTs (around 5-10%), no mutations are found in either the KIT or PDGFRA genes. These tumors are often referred to as wild-type GISTs.Wild-type GISTs may have distinct genetic alterations, such as mutations in other genes like SDH (succinate dehydrogenase) or NF1 (neurofibromin 1). Understanding the genetic landscape of wild-type GISTs is an area of ongoing research and can influence treatment decisions.

3. Aberrant Signaling Pathways: The mutations in KIT and PDGFRA genes drive the development of GISTs by activating downstream signaling pathways, such as the RAS/RAF/MEK/ERK and PI3K/AKT pathways. These pathways regulate cell survival, proliferation, and growth.

4. Cellular Proliferation: The uncontrolled activation of signaling pathways in GIST cells leads to increased cell proliferation and a lack of apoptosis (programmed cell death), contributing to the formation of tumor masses.

5. Tumor Growth and Progression: GISTs can vary in size and aggressiveness. Larger tumors and those with higher mitotic rates tend to have a worse prognosis. Some GISTs may remain localized, while others can metastasize to distant sites, such as the liver or peritoneum, further complicating the disease.

6. Molecular Subtypes: GISTs can exhibit different molecular subtypes based on the location and type of mutations. For example, exon 11 mutations in KIT are common in gastric GISTs, while exon 9 mutations are more prevalent in GISTs of the small intestine.

7. Secondary Mutations and Resistance: Over time, GISTs may develop secondary mutations, particularly in response to treatment with tyrosine kinase inhibitors (TKIs) like imatinib. These secondary mutations can render the tumors resistant to therapy, leading to disease progression.[7]

Understanding the pathogenesis of GISTs, particularly the role of KIT and PDGFRA mutations and their downstream signaling, has been pivotal in the development of targeted therapies. Tyrosine kinase inhibitors (TKIs), such as imatinib, sunitinib, and regorafenib, specifically target these aberrant signaling pathways, providing effective treatment options for patients with GISTs and improving outcomes. Ongoing research into GIST pathogenesis continues to uncover new insights and potential therapeutic targets for this rare but clinically significant group of tumors.

Locations

Gastrointestinal stromal tumors (GISTs) can develop in various anatomical locations along the gastrointestinal (GI) tract, as well as in extraintestinal sites[8,9]. The primary anatomical locations where GISTs are commonly found include:

1. Stomach (Gastric GISTs):

• Gastric GISTs are the most common type, accounting for approximately 60-70% of all GISTs.

• They can occur anywhere within the stomach, including the fundus, body, antrum, or cardia.

2. Small Intestine (Small Bowel GISTs):

• Small bowel GISTs are the second most common type, representing around 20-30% of GISTs.

• They can arise in various parts of the small intestine, such as the duodenum, jejunum, or ileum.

3. Esophagus (Esophageal GISTs):

• GISTs can occur in the esophagus, though they are less common in this location.

• Esophageal GISTs may present unique challenges due to the proximity to critical structures like the airway and the need for surgical expertise.

4. Colon and Rectum (Colorectal GISTs):

• GISTs can develop in the colon and rectum, though they are relatively rare in these locations.

• Colorectal GISTs may present with symptoms related to bowel obstruction or bleeding.

5. Extraintestinal Sites:

• In some cases, GISTs can occur outside of the GI tract in extraintestinal sites.

• Extraintestinal GISTs can be found in locations such as the omentum (a fold of peritoneum), mesentery (the tissue that attaches the intestines to the abdominal wall), or the retroperitoneum (the area behind the abdominal cavity).

6. Rare Locations:

• Although less common, GISTs have been reported in other rare locations, including the liver, pancreas, gallbladder, and urinary bladder.

The location of a GIST can influence its clinical presentation, behavior, and management. Tumor size and proximity to vital structures also play a role in determining the extent of surgical resection and the choice of treatment options, including the use of targeted therapies. Therefore, accurate diagnosis and precise characterization of the anatomical location are crucial in the evaluation and management of GISTs.

Histology of Gastrointestinal Stromal Tumors (GISTs)

The histology of gastrointestinal stromal tumors (GISTs) is characterized by specific microscopic features that help pathologists identify and diagnose these tumors. GISTs can exhibit varying histological patterns, but they generally share certain common characteristics:

1. Spindle Cells or Epithelioid Cells: GISTs are typically composed of either spindle-shaped cells or epithelioid cells, or they can have a mixed pattern with both cell types. These cells make up the bulk of the tumor tissue and are the primary focus of histological examination.[10]

2. Cellular Atypia: GIST cells can vary in size and shape, but they often exhibit cellular atypia, which refers to irregularities in cell size, shape, and nuclear features. This atypia is one of the key features that distinguish GISTs from non-malignant gastrointestinal lesions.

3. Mitotic Activity: The mitotic index, determined by counting the number of actively dividing cells (mitotic figures) in a given area of the tumor, is an important factor in assessing the aggressiveness of GISTs. A higher mitotic index is associated with a more aggressive tumor and may influence treatment decisions.

4. Stromal Component: GISTs often have a prominent stromal component, which includes connective tissue elements. This stromal component can vary in appearance, and the stroma itself may be fibrous or myxoid (containing a gel-like substance).

5. Immunohistochemical Staining: Immunohistochemistry is a crucial tool in diagnosing GISTs. The most important immunohistochemical marker for GISTs is CD117 (KIT), a cell surface receptor. The majority of GISTs express CD117, and its positivity is a key diagnostic criterion. Other markers, such as CD34, may also be positive in GISTs but are not as specific as CD117.[11]

6. Staining for S100 Protein: Some GISTs, particularly those with epithelioid morphology, may stain positively for the S100 protein. However, S100 staining alone is not sufficient for the diagnosis of GISTs, as it can also be seen in other tumor types.

7. Tumor Size and Borders: Pathologists assess the size of the GIST and examine its margins to determine if it has been completely removed during

surgical resection. The relationship between the tumor and surrounding normal tissue is important for evaluating the success of surgery.

8. Risk Stratification: Based on histological and clinical factors, GISTs are often categorized into risk groups (low, intermediate, or high risk) that help guide treatment decisions and predict the likelihood of tumor recurrence.

In summary, the histology of GISTs is characterized by the presence of spindle-shaped or epithelioid cells with cellular atypia, a variable stromal component, and immunohistochemical markers like CD117 and sometimes CD34 and S100 protein. The specific histological features, mitotic index, and molecular characteristics of the tumor are essential for diagnosing GISTs, determining their prognosis, and guiding treatment strategies.

Symptoms

The symptoms of gastrointestinal stromal tumors (GISTs) can vary widely depending on several factors, including the tumor's location, size, and stage of development. Some individuals with GISTs may remain asymptomatic, while others may experience a range of symptoms.[12] Common GIST-related symptoms and signs include:

1. Abdominal Pain or Discomfort: Many individuals with GISTs, especially those with larger tumors, may experience abdominal pain or discomfort. The pain can vary in intensity and may be localized to the area where the tumor is located.

2. Gastrointestinal Bleeding: GISTs can lead to gastrointestinal bleeding, which may manifest as symptoms such as:

• Dark, tarry stools (melena): Indicative of upper gastrointestinal bleeding.

• Blood in the stool (hematochezia): Indicative of lower gastrointestinal bleeding.

• Vomiting blood (hematemesis): More severe bleeding from the upper GI tract.

3. Anemia: Chronic gastrointestinal bleeding from GISTs can lead to iron-deficiency anemia, resulting in symptoms like fatigue, weakness, and pallor.

4. Early Satiety: Some individuals with GISTs in the stomach or upper gastrointestinal tract may experience a sensation of early satiety (feeling full quickly after eating), which can lead to reduced food intake and weight loss.

5. Nausea and Vomiting: GISTs located in the stomach or other parts of the gastrointestinal tract may cause nausea and occasional vomiting, particularly after meals.

6. Palpable Abdominal Mass: In cases where GISTs grow to a significant size, they may be palpable as a mass or lump in the abdominal area during a physical examination.

7. Difficulty Swallowing (Dysphagia): GISTs in the esophagus can cause difficulty in swallowing, especially when the tumor obstructs the passage of food or liquids.

8. Change in Bowel Habits: GISTs located in the lower gastrointestinal tract, such as the colon or rectum, may lead to changes in bowel habits, including diarrhea, constipation, or alterations in stool appearance.

9. Obstruction: Large GISTs may cause bowel obstruction, resulting in severe abdominal pain, bloating, nausea, and vomiting.

10. Generalized Symptoms: In advanced or metastatic GIST cases, individuals may experience generalized symptoms such as unintentional weight loss, fatigue, and malaise.

It's important to note that some GISTs are discovered incidentally during imaging studies or surgery for unrelated conditions and may not cause noticeable symptoms. Additionally, the severity and combination of symptoms can vary widely from person to person, and not all individuals with GISTs will experience all of these symptoms. If GIST is suspected based on symptoms or clinical findings, further evaluation and diagnostic tests are typically necessary to confirm the diagnosis and determine the appropriate treatment plan. Early detection and intervention can lead to better outcomes for individuals with GISTs.

Diagnosis

The diagnosis of Gastrointestinal Stromal Tumors (GISTs) involves a combination of clinical evaluation, imaging studies, histopathological examination, and molecular analysis.[12] Here is an overview of the steps and methods involved in diagnosing GISTs:

1. Clinical Evaluation:

- The diagnostic process often begins with a thorough clinical evaluation by a healthcare provider.
- The clinician will take a detailed medical history, inquire about symptoms, and perform a physical examination.
- It is essential to discuss any gastrointestinal symptoms, such as abdominal pain, bleeding, early satiety, or palpable abdominal masses, that may suggest GISTs.

2. Imaging Studies:

• Imaging studies play a crucial role in detecting and assessing GISTs. Common imaging modalities include:

• **Computed Tomography (CT) Scan:** CT scans provide detailed cross-sectional images of the abdomen and pelvis and can help locate and characterize GISTs.

• **Magnetic Resonance Imaging (MRI):** MRI scans are useful for evaluating the extent and characteristics of GISTs and may be employed when CT scans are inconclusive or for specific purposes.

• **Endoscopy:** Upper gastrointestinal endoscopy or colonoscopy may be performed to visualize GISTs located in the esophagus, stomach, or colorectal region.

• **Positron Emission Tomography (PET) Scan:** PET scans can help assess the metabolic activity of GISTs and detect potential metastases.

3. Histopathological Examination:

• The definitive diagnosis of GISTs relies on histopathological examination of a tissue sample obtained through biopsy or surgical resection.

• Biopsy may involve endoscopic or image-guided techniques to collect tissue samples for analysis.

• Histopathological evaluation assesses the tissue's microscopic features, including the presence of spindle cells or epithelioid cells, cellular atypia, and mitotic activity.

• Immunohistochemical staining for CD117 (KIT receptor) and sometimes CD34 and S100 protein is crucial for confirming the diagnosis of GISTs.

4. Molecular Analysis:

• Molecular analysis, including mutation testing, may be performed to identify specific mutations in genes such as KIT and PDGFRA.

• Molecular profiling can help determine the genetic characteristics of the GIST and guide treatment decisions, particularly the use of targeted therapies like tyrosine kinase inhibitors (TKIs).

5. Risk Assessment and Staging:

• Once a GIST is diagnosed, it is essential to assess its risk of recurrence and metastasis.

• Risk stratification is based on factors such as tumor size, mitotic index, and location, and it helps classify GISTs into low, intermediate, or high-risk categories.

• Staging may also involve evaluating the extent of tumor spread, including the presence of lymph node involvement or distant metastases.

6. Multidisciplinary Team Collaboration:

• Diagnosing and managing GISTs often involves a multidisciplinary team, including surgeons, pathologists, oncologists, radiologists, and gastroenterologists.

• This collaborative approach helps ensure accurate diagnosis, appropriate staging, and tailored treatment plans for patients with GISTs.

In summary, the diagnosis of GISTs requires a comprehensive approach that includes clinical evaluation, imaging studies, histopathological examination, molecular analysis, risk assessment, and the involvement of a multidisciplinary healthcare team. Accurate diagnosis is critical for determining the most suitable treatment options and optimizing the care of individuals with GISTs.

Prognostic factors

Prognostic factors in gastrointestinal stromal tumors (GISTs) are critical indicators that help predict the likely behavior of the tumor and the patient's overall outcome. These factors guide treatment decisions and provide valuable information for clinicians and patients.[13,14]

1. Tumor Size: The size of the GIST is one of the most important prognostic factors. Larger tumors are associated with a higher risk of malignancy and a worse prognosis. Tumors are often categorized by size:

• Small (<2 cm)

• Intermediate (2-5 cm)

• Large (>5 cm)

2. Mitotic Index: The mitotic index is a measure of the rate of cell division within the tumor. It is determined by counting the number of actively dividing cells (mitotic figures) in a specific area of the tumor under a microscope. A higher mitotic index indicates a more aggressive tumor and is associated with a poorer prognosis.

3. Tumor Location: The anatomical location of the GIST within the gastrointestinal (GI) tract can influence its behavior. Generally, GISTs in the stomach have a better prognosis compared to those in the small intestine or other locations. Rectal GISTs also tend to have a worse prognosis.

4. Tumor Rupture or Perforation: If a GIST ruptures or perforates through the gastrointestinal wall, it is considered an adverse prognostic factor. This event can lead to tumor spillage into the abdominal cavity, increasing the risk of recurrence and metastasis.

5. Tumor Morphology: GISTs can have different histological morphologies, including spindle cell, epithelioid, or mixed patterns. Some studies have suggested that the epithelioid subtype may have a worse prognosis compared to the spindle cell subtype.

6. KIT and PDGFRA Mutation Status: The specific mutations present in the KIT or PDGFRA genes can influence the behavior of GISTs. Certain mutations may be associated with a higher risk of recurrence or resistance to treatment.

7. Risk Stratification Systems: Various risk stratification systems, such as the National Institutes of Health (NIH) criteria and the modified Fletcher classification (commonly used), combine several of these prognostic factors to categorize GISTs into risk groups:

- Low risk
- Intermediate risk
- High risk

8. Tumor Growth Pattern: The growth pattern of a GIST, such as exophytic (growing outward) or endophytic (growing inward), may also influence prognosis and management decisions.

9. Age and Performance Status: Patient-related factors, including age and overall health (performance status), can play a role in prognosis and treatment decisions. Younger age and good performance status are generally associated with better outcomes.

10. Metastasis: The presence of metastatic disease at the time of diagnosis is a significant adverse prognostic factor. GISTs that have spread to distant organs, such as the liver or lungs, often have a poorer prognosis.

It's important to note that risk assessment and prognostication in GISTs are typically based on a combination of these factors. Risk stratification systems are commonly used to categorize GISTs into risk groups, which guide treatment decisions. Treatment approaches, including surgery and the use of tyrosine kinase inhibitors (TKIs), are tailored based on the assessed risk. Regular follow-up and monitoring are also essential to assess treatment response and detect any recurrence or metastasis.

Treatment

The treatment of gastrointestinal stromal tumors (GISTs) typically involves a combination of approaches that may include surgery, targeted therapy with tyrosine kinase inhibitors (TKIs), and, in some cases, other interventions.[15,16,17] The specific treatment plan depends on various factors, including the tumor's size, location, mutation status, risk category, and the

patient's overall health. Here's an overview of the main treatment modalities for GISTs:

1. Surgery: The most common initial treatment for non-metastatic gastrointestinal stromal tumors (GISTs) is surgical resection. The primary goal of curative surgery is to achieve a margin-negative resection, also known as a complete surgical removal, of the tumor. This approach aims to completely eliminate the GIST from the affected organ or tissue, providing the best chance for a successful outcome. Here are key aspects of surgical treatment for GISTs:

• Resectability Assessment: Before surgery, a thorough evaluation is conducted to determine the resectability of the GIST. This assessment includes factors such as the tumor's size, location, and its relationship with adjacent structures.

• Surgical Approach: The choice of surgical approach depends on various factors, including the tumor's site within the gastrointestinal (GI) tract and its size. Surgical options may include:

Wedge Resection: In cases where the GIST is small and localized, a wedge resection is performed. This involves removing the tumor along with a margin of healthy tissue, sparing the rest of the organ.

Partial Gastrectomy or Bowel Resection: For larger or more complex GISTs, a partial gastrectomy (for stomach GISTs) or bowel resection (for GISTs in the small or large intestine) may be necessary to remove the tumor while preserving the function of the remaining GI tract.

Lymph Node Dissection: Unlike many other types of cancer, GISTs do not typically metastasize to lymph nodes. Therefore, lymph node dissection is generally not required during GIST surgery.

R0 Resection: The primary objective during surgery is to achieve an R0 resection, also known as a margin-negative resection. This means that the entire tumor, along with a surrounding margin of healthy tissue, is removed without any visible tumor remaining.

Adjuvant Therapy: In cases of high-risk GISTs, adjuvant therapy with tyrosine kinase inhibitors (TKIs), such as imatinib, may be recommended following surgery to reduce the risk of recurrence.

Postoperative Monitoring: After surgery, regular follow-up and monitoring are essential to assess the patient's recovery, evaluate for any potential complications, and detect any signs of recurrence.

2. Imatinib (Gleevec) Therapy:

• Imatinib mesylate, a tyrosine kinase inhibitor (TKI), is the standard first-line treatment for advanced, unresectable, recurrent, or metastatic GISTs.[18]

• Imatinib targets the abnormal signaling pathways activated by mutations in KIT or PDGFRA genes, effectively inhibiting tumor growth.

• It is also used as adjuvant therapy after complete surgical resection of high-risk GISTs to reduce the risk of recurrence.

3. Sunitinib (Sutent) and Regorafenib (Stivarga):

• Sunitinib and regorafenib are TKIs used in cases of GISTs that have become resistant to imatinib or have secondary mutations.

• Sunitinib is often used as a second-line therapy for GISTs that progress on or are intolerant to imatinib.

• Regorafenib is a third-line treatment option for GISTs that have progressed despite prior treatment with imatinib and sunitinib.

4. Molecularly Targeted Therapies:

• For GISTs with specific mutation profiles, selecting the appropriate TKI is essential. The choice of therapy may depend on the mutation type, location, and the tumor's response to previous treatments.

• Molecular profiling helps identify the most suitable targeted therapy for individual patients.

5. Clinical Trials:

• Participation in clinical trials may be considered, especially for individuals with advanced GISTs that do not respond to standard treatments.

• Clinical trials investigate new therapies, combination approaches, and treatments for rare mutations or resistant GISTs.

6. Radiation Therapy and Other Interventions:

• Radiation therapy is generally not effective for GISTs because they are often resistant to radiation.

• Other interventions, such as embolization or ablation, may be considered for specific cases, such as managing symptoms related to tumor bleeding or obstruction.

7. Multidisciplinary Care:

• Managing GISTs often involves a multidisciplinary team, including surgeons, medical oncologists, radiologists, pathologists, and specialized nurses.

• Regular follow-up and monitoring are essential to assess treatment response, detect recurrence, and address potential side effects.

The treatment of GISTs is highly individualized, and decisions regarding therapy are based on a thorough evaluation of the tumor's characteristics and the patient's overall health. Advances in molecular profiling and targeted therapies have significantly improved the outlook for individuals with GISTs, leading to more personalized and effective treatment approaches.

Locally advanced and metastatic gastrointestinal stromal tumors (GISTs

Locally advanced and metastatic gastrointestinal stromal tumors (GISTs) are more advanced stages of the disease in which the tumors have either grown extensively within the gastrointestinal (GI) tract or have spread to distant organs or tissues. Treatment approaches for these stages of GIST differ from those for early-stage, non-metastatic GISTs.[19]

Locally Advanced GIST:

Locally advanced GISTs are tumors that have grown extensively within the GI tract or have invaded surrounding tissues or structures. Treatment for locally advanced GISTs may include:

1. Surgical Resection: Surgical removal of the tumor is often attempted when it is deemed feasible. However, the extent of surgery may be more extensive, and the primary goal remains achieving an R0 resection (complete removal with clear margins).

2. Neoadjuvant Therapy: In some cases, neoadjuvant therapy with tyrosine kinase inhibitors (TKIs) like imatinib may be administered before surgery to shrink the tumor, making it more amenable to resection.

3. Adjuvant Therapy: After surgical resection, adjuvant therapy with TKIs may be recommended to reduce the risk of recurrence, especially for higher-risk locally advanced GISTs.

Metastatic GIST:

Metastatic GISTs are tumors that have spread beyond the primary site to distant organs or tissues. The treatment of metastatic GISTs is primarily aimed at controlling the disease and improving the patient's quality of life. Approaches may include:

1. Tyrosine Kinase Inhibitors (TKIs):

• Imatinib (Gleevec): Imatinib remains the first-line treatment for metastatic GISTs. It inhibits the activity of KIT and PDGFRA receptors, controlling tumor growth.[20,21]

• Sunitinib (Sutent) and Regorafenib (Stivarga): These TKIs are used as second- and third-line treatments for GISTs that have become resistant to imatinib or have developed secondary mutations.

• The choice of TKI depends on factors such as the tumor's response to prior therapy, mutation status, and individual patient considerations.

2. Monitoring and Response Assessment: Regular imaging studies (e.g., CT scans) are conducted to monitor the tumor's response to therapy and assess disease progression.

3. Clinical Trials: Participation in clinical trials is often considered for individuals with metastatic GISTs to explore novel therapies or combination treatments.

4. Palliative Care: Palliative care is an essential aspect of the management of metastatic GISTs. It focuses on relieving symptoms, managing side effects of treatment, and improving the patient's overall quality of life.

Treatment decisions for locally advanced and metastatic GISTs are highly individualized, taking into account factors such as tumor size, location, mutation status, response to prior therapies, and the patient's overall health. The emergence of targeted therapies like TKIs has significantly improved the prognosis and management of advanced-stage GISTs, leading to more personalized and effective treatment approaches.

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Chapter 19

INFLAMMASOME BIOLOGY: MOLECULAR STRUCTURE, ACTIVATION MECHANISMS, AND CLINICAL IMPLICATIONS

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1. Inflammasome Structure

1.1. Definition of Inflammasomes and General Function

An inflammasome is a molecular complex considered a component of the body's immune system [1]. Inflammasomes are protein complexes that can detect various threats at the cellular level, such as pathogens, damaged cells, or foreign substances, and regulate the immune system's response to these dangers [2]. Additionally, when inflammasomes detect environmental threats, they initiate a series of cellular responses. These responses include the production of proinflammatory cytokines, such as IL-1β and IL-18, and the regulation of cellular death processes. These reactions are essential for protecting the body against infections, injuries, and other threats [3].

1.2. Inflammasome Components

-Pattern Recognition Receptors (PRRs): Inflammasomes' main components, play a crucial role in the detection of dangers. PRRs are receptors that help the body recognize foreign molecules (such as microbial cell walls) or endogenous (coming from within the body) dangers (like released DNA). These PRRs represent the initial step in initiating the inflammasome [4].

-Detection Receptors: These are the main components of the inflammasome and serve as receptors that detect danger signals. NLR and AIM2 are part of this group. Proteins belonging to the NLR family can detect pathogens or other hazardous substances within the cell. Examples include NLRP3 (NLR family, pyrin domain-containing 3) and NLRC4. AIM2, on the other hand, senses the presence of DNA within the cell. The release of DNA within the cell can lead to inflammasome activation [5, 6].

-Adapter Proteins: Adapter proteins process the dangers detected by the detection receptors into signals and initiate the activation of the inflammasome. An example is ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) [7].

-Inflammatory cytokines are crucial molecules produced as a result of inflammasome activation. These cytokines regulate inflammatory processes and enhance the activity of the immune system. For example, IL-1β and IL-18 are cytokines produced as a result of inflammasome activation. These cytokines coordinate immune responses and strengthen defense against pathogens [8,9].

-Caspases represent another important group of components that initiate inflammasome activation. These enzymes are found in an inactive form called pro-caspases and become activated as a result of inflammasome activation. Activated caspases lead to the cleavage and release of cytokines, which, in turn, increases inflammation [10].

The specific components of inflammasome structure can vary depending on the type of danger-sensing mechanism and the triggering signals that activate the inflammasome. For example, the NLRP3 inflammasome can be activated by components of bacterial cell walls, cytokines, or various other factors. The structure and components of inflammasomes play a critical role in regulating inflammation and immune responses in the body. The activation of inflammasomes assists in controlling pathogens, clearing damaged cells, and regulating immune system responses. Therefore, inflammasomes are significant in understanding and treating immune system function and inflammatory diseases.

2. Inflammasome Activation

The activation of inflammasomes begins with the detection of specific dangers. For instance, the NLRP3 inflammasome can be activated by cellular stress, crystals, toxins released by microorganisms, and other dangers. The AIM2 inflammasome, on the other hand, detects free DNA within the cell and initiates its activation [11]. However, in the absence of these dangers or when dangers are within normal limits, inflammasomes remain inactive. In other words, inflammasomes are in a passive state under normal physiological conditions and become active in the presence of dangers or cellular stress conditions. This allows the immune system to regulate appropriate responses to threats.

2.1. Danger-Sensing Capabilities of Inflammasomes

The dangerous situations that activate inflammasomes typically encompass various conditions that threaten the body. These conditions include:

Microbial Infections: Bacterial infections (such as *Salmonella* and *Pseudomonas*), viruses (like *influenza*), and fungi (such as *Candida*) can trigger inflammasome activation [12, 13,14].

Cellular Stress: This includes conditions like damage to the cell membrane or the presence of abnormalities within the cell, such as oxidative stress [15].

Toxins: Toxins produced by certain bacteria or microorganisms can lead to inflammasome activation. For example, the botulinum toxin produced by *Clostridium botulinum* may contribute to inflammasome activation [16].

Crystals: Certain crystals, especially uric acid crystals (associated with gout), can trigger inflammasome activation [17].

Intracellular DNA: DNA released within the cell, as a result of cellular damage or viral infections, can lead to inflammasome activation, especially in the case of AIM2 [18].

2.2. Impact of Inflammasome Activation on Inflammation

Inflammation is a process initiated as a response by the immune system to protect the body against dangerous pathogens, toxins, or cellular stress conditions. Inflammasome activation plays a significant role in initiating this process and is a key factor in regulating inflammation [19].

The effects of inflammasome activation on inflammation occur through various pathways. Firstly, the release of proinflammatory cytokines initiates inflammatory responses and triggers the body's defense mechanisms against threats. Cytokines like IL-1β and IL-18 can activate inflammatory signaling pathways necessary for controlling infections and clearing pathogens. These signaling pathways coordinate cellular responses and optimize the functioning of the immune system.

Excessive inflammation or the transition to a chronic state of inflammation can result from uncontrolled inflammasome activation. This can contribute to the development of chronic inflammatory diseases, such as rheumatoid arthritis or inflammatory bowel diseases. Regulating and controlling inflammasome activation is important for the treatment of such diseases [20]. However, it's essential to note that the effects of inflammasome activation on inflammation are not solely negative. Inflammasomes are necessary to initiate defense mechanisms against infections and other threats. Therefore, inflammasome activation plays a critical role in protecting the body against pathogens.

3. Types of Inflammasomes

Inflammasomes come in various types that respond to different triggering signals and mechanisms. Among the best-known types of inflammasomes are:

NLRP3 Inflammasome: This is a type of inflammasome that can respond to various stimulants and plays a significant role in the pathogenesis of many inflammatory diseases. NLRP3 inflammasome can be activated by cellular stress, toxins released by microorganisms, crystals, and various other threats [21].

NLRC4 Inflammasome: This inflammasome responds to various bacterial pathogens. It detects attacks by bacteria and initiates the immune system's response [22].

AIM2 Inflammasome: It initiates activation by detecting free DNA within the cell. This DNA can be released under conditions of viral infections or cellular stress caused by intracellular pathogens [23].

Pyrin Inflammasome: Associated with autoinflammatory diseases like Familial Mediterranean Fever (FMF). The pyrin protein detects abnormalities

in the cell membrane and initiates inflammasome activation [24].

NLRP1 Inflammasome: It can be activated by different pathogens or under cellular stress conditions. It is particularly associated with *Bacillus anthracis* (the causative agent of anthrax) infections [25].

IFI16 Inflammasome: It detects infections caused by DNA viruses and can respond to cellular stress conditions, similar to the AIM2 inflammasome [26].

4. The Role of Inflammasomes in Immune Responses

4.1. Inflammasomes and Inflammatory Diseases

The relationship between inflammasomes and inflammatory diseases is particularly important due to the irregularity and overactivation of inflammation. Uncontrolled inflammasome activation can contribute to the development of autoimmune diseases, autoinflammatory diseases, and chronic inflammatory diseases. These diseases can involve the initiation of autoimmune responses where the body's immune system targets its own tissues or cells. For example, diseases like rheumatoid arthritis, lupus, and FMF are influenced by the pathophysiology of inflammasome activation [27,28].

The role of inflammasomes in inflammatory diseases is not limited to autoimmune diseases. They also play a significant role in the pathogenesis of various conditions, including neurological diseases, metabolic syndrome, cardiovascular diseases, and cancer. In particular, the NLRP3 inflammasome can contribute to the development of many inflammatory diseases [29].

-Rheumatoid Arthritis: Rheumatoid arthritis is an autoimmune disease resulting from the overactivation of inflammation. Inflammasomes play a critical role in the pathophysiology of inflammation in joint tissues [30].

-FMF: It is an autoinflammatory disease characterized by symptoms such as recurrent fever attacks, abdominal pain, and joint pain. Mutations in the pyrin protein and overactivation of inflammasomes are among the fundamental causes of FMF [31].

-Inflammatory Bowel Diseases (IBD): Conditions like Crohn's disease and ulcerative colitis, known as IBDs, are characterized by chronic inflammation in the intestines. The NLRP3 inflammasome plays an important role in the pathophysiology of these diseases [32].

-Type 2 Diabetes (T2D): It is a disease that emerges as part of metabolic syndrome. The metabolic effects of inflammation can contribute to the development of diabetes. Inflammasomes can also contribute to the increased insulin resistance and the development of T2D [33].

-Cardiovascular Diseases: Inflammation plays a significant role in the development of atherosclerosis and the progression of cardiovascular diseases. Inflammasomes are active in the pathophysiology of atherosclerosis and can increase the risk of cardiovascular diseases [34].

-Cancer: Inflammasomes have been associated with cancer. Specifically, increased inflammasome activation has been observed in some cancer types such as colorectal cancer, breast cancer, and lung cancer [35].

-Neurological Diseases: Inflammation and inflammasomes can play a significant role in the pathogenesis of neurological diseases like Alzheimer's disease and Parkinson's disease. In these diseases, inflammasome activation can contribute to neuron loss and neurodegeneration [36].

Additionally, diseases like Cryopyrin-Associated Periodic Syndromes (CAPS) have a strong connection with inflammasomes. CAPS results from mutations in the NLRP3 inflammasome, leading to continuous inflammasome activation. This disease is characterized by recurrent fever episodes, urticaria-like rashes, and joint pain. Inflammasomes also have a role in other autoinflammatory diseases like Behçet's disease, Schnitzler syndrome, and Deficiency of the IL-1 Receptor Antagonist (DIRA). Understanding the function and regulation of inflammasomes is crucial for the treatment and prevention of these diseases. Targeting inflammasomes holds promise as an approach for the treatment of inflammatory diseases [37, 38, 39, 40].

5. Approaches to Treatment

Inflammasomes have a significant impact on drug development and treatment in various fields of medicine. These molecular complexes hold great promise in offering new treatment options for inflammatory diseases, control of autoimmune diseases, cancer therapy, and the management of metabolic disorders. As a result, inflammasomes have garnered substantial interest and potential in the medical world.

-Treatment of Inflammation: Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids can be used to alleviate inflammation and reduce symptoms [41].

-Biologic Drugs: In certain diseases associated with inflammasomes, biologic drugs can be used to control inflammation. For example, IL-1 blockers (such as Anakinra or Canakinumab) are used in the treatment of autoinflammatory diseases like gout, FMF, and CAPS [42].

-Immunomodulatory Drugs: Immunosuppressive drugs can be used in the treatment of autoimmune diseases associated with inflammasomes. Monoclonal antibodies and immunosuppressants can be employed for this purpose [43].

-Infection Treatment: When an infection is the cause of excessive inflammasome activation, treating the infection can help control inflammation [44].

-Diet and Lifestyle Changes: Some inflammatory diseases can be managed through dietary and lifestyle modifications. For example, patients with gout can make dietary changes to lower uric acid levels [45].

-Gene Therapy: Some autoinflammatory diseases are associated with genetic mutations, so gene therapy or genetic modifications may be potential treatment options [46].

5.1. Drugs Targeting Inflammasomes

There are several different approaches and treatment options for inflammatory diseases associated with inflammasomes. Treatment can vary depending on the type of disease, its severity, and the specific needs of the individual.

5.1.1. Herbal Remedies

-Turmeric: It contains a compound called curcumin, known for its anti-inflammatory properties. Turmeric can be helpful, particularly in managing inflammatory joint diseases such as osteoarthritis [47].

-Ginger: It has anti-inflammatory and analgesic properties. Ginger tea or ginger supplements can help alleviate inflammation [48] .

-Chamomile Tea: It possesses anti-inflammatory properties and can aid in relieving inflammatory stomach issues, such as gastritis [49].

-Fennel: It can be used as a natural remedy for stomach discomfort. It may be beneficial for stomach inflammations and gas problems [50].

-Lavender: It has anti-inflammatory properties and is also known for its relaxing effects. It can particularly help alleviate inflammation associated with stress [51] .

-Green Tea: It has antioxidant properties and may have mild anti-inflammatory effects [52].

5.1.2. Other Drugs Targeting Inflammasomes

-IL-1β Inhibitors: IL-1β is a key driver of inflammation and impacts the pathophysiology of many inflammatory diseases. Therefore, drugs targeting the inhibition of IL-1β have been developed. For example, drugs like anakinra and canakinumab block the effects of IL-1β and are used in the treatment of diseases such as rheumatoid arthritis, FMF, and CAPS [53].

-IL-18 Inhibitors: IL-18 plays a significant role in regulating inflammation. Therefore, the inhibition of IL-18 can help in controlling inflammation.

While drugs of this kind are still in the experimental stage, they are being investigated as a potential treatment option [54].

-NLRP3 Inhibitors: The NLRP3 inflammasome plays a critical role in the development of many inflammatory diseases. Developing drugs that target this inflammasome is of great importance. In particular, drugs like MCC950 have the potential to control inflammation by inhibiting the NLRP3 inflammasome [55].

-Gasdermin D Inhibitors: Gasdermin D initiates cell death as a result of inflammasome activation and leads to the release of proinflammatory cytokines. Therefore, drugs targeting the inhibition of gasdermin D can help regulate inflammation [56].

5.2. Clinical Applications of Inflammasome Drugs

Anakinra (Kineret): It is used for conditions like rheumatoid arthritis, FMF, CAPS and Behçet's disease. Anakinra targets the IL-1 receptor antagonist and, therefore, inhibits the effects of IL-1β [57].

Canakinumab (Ilaris): It can be applied for FMF, CAPS, and Muckle-Wells syndrome. It is used as a monoclonal antibody targeting IL-1β. It is effective in the treatment of inflammatory diseases such as Familial Mediterranean Fever and Cryopyrin-Associated Periodic Syndromes [58].

MCC950 (CRID3): Suitable for diseases associated with the NLRP3 inflammasome. It targets the NLRP3 inflammasome and inhibits inflammasome activation. It is used in the treatment of diseases related to the overactivation of the NLRP3 inflammasome [59].

Rilonacept (Arcalyst): It is used for CAPS. It targets the IL-1 receptor antagonist [60].

These drugs are some examples used in the treatment of diseases associated with inflammasomes. However, research is ongoing to develop new inhibitors and to use existing ones for more diseases. Better understanding of inflammasomes and the development of specific inhibitors for these molecular complexes have significant potential in the treatment and management of inflammatory diseases.

6. Molecular Regulation of Inflammasomes and Genetic Connections

6.1. Genetic and Epigenetic Regulation of Inflammasomes

Genetic regulation of inflammasomes refers to the process in which an organism's genetic makeup influences the function and activation of inflammasomes. This may involve genetic variations in the components of inflammasomes. For example, mutations in the NLRP3 gene, which is involved in the NLRP3 inflammasome, can lead to overactivation of the inflammasome

and the development of inflammatory diseases. Similarly, mutations in the protein pyrin can trigger autoinflammatory diseases such as FMF. Genetic variations, particularly in the promoter regions of inflammasome components, can lead to uncontrolled activation of inflammasomes. Therefore, genetic regulation of inflammasomes is important for understanding the genetic predisposition to inflammation-related diseases and identifying new targets for the treatment of these diseases [61].

Epigenetic regulation involves chemical changes that alter gene expression and activation. This occurs through mechanisms such as DNA methylation, histone modifications, and microRNAs. Epigenetic regulation of inflammasomes affects the expression and activation of inflammasome components. For example, DNA methylation involves modifying DNA with chemical groups in the promoter regions of inflammasome components. This methylation regulates the expression of inflammasome components and affects their activation. Histone modifications refer to chemical changes that alter gene activity and can influence the function of inflammasomes. Specifically, drugs targeting epigenetic modifications can contribute to the development of new treatment strategies aimed at controlling inflammasome activation [62,63] .

6.2. Genetic Mutations Related to Inflammasomes and Diseases

Overactivation of the NLRP3 inflammasome, particularly, leads to autoinflammatory diseases triggered by cold, known as Familial Cold Autoinflammatory Syndrome (FCAS), Muckle-Wells Syndrome (MWS), and Neonatal Onset Multisystem Inflammatory Disease (NOMID). These conditions are associated with mutations in the NLRP3 gene and are exacerbated due to uncontrolled activation of the NLRP3 inflammasome [64, 65].

MEFV Gene and FMF: This disease often leads to symptoms such as recurrent peritonitis, pericarditis, pleuritis, and joint pain. The MEFV gene encodes a protein called pyrin, and the function of this protein is to regulate the activation of inflammasomes. Mutations in the MEFV gene hinder pyrin from functioning correctly, increasing inflammasome activation [66].

NLRC4 Inflammasome and Macrophage Activation Syndrome (MAS): This condition, characterized by symptoms such as high fever, hepatosplenomegaly, and overactivation of macrophages in children. Overactivation of the NLRC4 inflammasome contributes to the development of MAS [67].

Pyrin and Behçet's Disease: Behçet's disease is an autoimmune disease characterized by recurrent mouth ulcers, genital ulcers, eye problems, and skin lesions. Behçet's disease is associated with mutations in the pyrin gene.

Pyrin functions as a protein that regulates inflammasome activation, and mutations in the pyrin gene can increase inflammasome activation [68].

7. Future Potential Research Areas Related to Inflammasomes

A more detailed understanding of the molecular mechanisms of inflammasome activation will be a central focus of future research. Understanding how inflammasomes are activated by specific signals and how these signals are regulated will enable the development of better targets to prevent uncontrolled inflammasome activation and to treat inflammatory diseases. Furthermore, gaining a better understanding of how different inflammasome complexes are associated with different diseases and immune responses will require further exploration of inflammasome diversity. New findings are needed to elucidate the differences between NLRP3 inflammasomes and NLRP1 or AIM2 inflammasomes, for instance. Additionally, the role of epigenetic regulations in inflammasome activation and suppression requires further investigation. Understanding how DNA methylation, histone modifications, and microRNAs interact with inflammasomes will provide opportunities for the development of new therapeutic approaches to regulate these molecular complexes.

A better understanding of how inflammasome activation is associated with neurological diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis will enable the development of new strategies for treating these conditions. Moreover, it is essential to comprehend how inflammasomes may contribute to the recognition and elimination of cancer cells by the immune system in cancer research.

Inflammasome inhibitors and drugs hold significant potential for the treatment of inflammatory diseases. Future research will offer opportunities for the development of more effective inflammasome inhibitors and medications.

In conclusion, the potential areas of future research related to inflammasomes are extensive. These investigations are vital for a better understanding of the functions of inflammasomes and for the development of new approaches to treat diseases associated with inflammation. Inflammasomes will continue to be a key element in the progress of medicine and biomedical sciences.

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Chapter 20

TEA, KOMBUCHA AND FERMENTATION

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1. Introduction

Alternative therapy refers to any method of treatment that deviates from accepted medical norms. It differs in its foundations and methodology, is not based on scientific facts, but rather on historical and cultural traditions. These techniques may draw inspiration from conventional medicine, folklore, spirituality, or recently developed healing philosophies. In order for such historically used drugs to be accepted on a global scale, their efficacy must be scientifically proven. People from every aspect of life have drank tea ever since the beginning of time. According to legend, tea is a form of medicine (Chakravorty ve ark., 2016). Chinese people have been drinking tea for more than 5000 years because of its supposed energizing and removing effects, which include increased blood and urine flow by removing alcohol and toxins, relief from joint problems, and increased disease resistance. As a result, tea had a quick rise in popularity and importance and was included into a variety of social rituals, primarily in China, Japan, and England. As a result, tea is considered to be the second most consumed beverage in the world, behind water.

When bacteria and yeasts collaborate in symbiosis to ferment sweetened black tea, they produce a "tea fungus" or Kombucha pellicle, which is the fermented beverage known as Kombucha. Kombucha is easy to create at home and is quite valued on its own. Depending on how long the fermenting process occurs, the flavor might range from sparkling apple cider to sour, vinegary (Jayabalan ve ark., 2014).

Literature claims that the pleasant beverage kombucha, which is naturally carbonated, has been known by a variety of names in many civilizations. The Japanese word for kombucha has been Germanized for worldwide use. Several of the well-known and widespread names include: Kombucha Fungus, Gout Jelly-fish Manchurian Tea and Kargasok Tea (Chandrakala ve ark., 2019). Kombucha is referred to as "Bio-tea" in Karnataka, India, particularly in the Malnad and coastal districts.

2. History of Kombucha

The "Divine Che," also known as "Remedy of Immortality" or "Tea of Immortality," was a valued beverage during the Tsin Dynasty and was widely renowned for its revitalizing and purifying powers. It is believed that this is where kombucha got its start (Coelho ve ark., 2020). According to legend, kombucha was first consumed in China or the Middle East and then spread along trade channels. According to legend, a doctor by the name of Kombu brought the tea fungus to Japan from Korea in the year 414 AD in order to treat the Emperor Inkyo's stomach problems (Laureys ve ark., 2020). Later, in the late 19th century, kombucha tea made a comeback in Japan under the names "Tea Mushroom" or "Tea Kvass." Later, "Tea Kvass" made its way to Russia and

India thanks to the expansion of trade channels. In the 1800s, kombucha tea spread throughout Russia and became well-known as a reliable folk remedy in many rural areas. As a result, Russia is possibly where the current Kombucha was born. The Kombucha culture is known as tea mushroom in Russia, and the beverage is known as grib (mushroom), "tea kvass," or simply "kvass."

Thus, this fermented beverage was utilized and became well-liked throughout China, Japan, and Korea before being introduced in Russia and other European nations before being made available in Asia and other parts of the world. Towards the end of the 19th century, the first comprehensive history of kombucha from Russia and the Ukraine was first written down. During World War I (WWI), German and Russian prisoners of war assisted in making Kombucha more widely available. By the 1920s, Kombucha was well-liked as a DIY and folk cure all throughout Germany. The Westphalian industrial region of Germany had the greatest prevalence of it, and it extended to France and the North African territory that was administered by the French (Blanc, 1996). Kombucha was, in the words of Dr. Harms, "eagerly sought after in certain circles, and gladly passed on to others." Additionally, it was offered for sale in pharmacies under the names "Mo-Gu" and "Fungojapon." It then expanded all across the world from there. A Polish pharmacist who shared a room with a Polish doctor named Waldeck during World War I demonstrated a Russian folk cure known as "Miracle mushroom," "Volga mushroom," or "Tea-Kvass mushroom" to the doctor (Jayabalan ve ark., 2014).

3. Biological Composition of Kombucha

3.1 Symbiotic Colony of Bacteria and Yeast (SCOBY)

The Kombucha culture, also known as a SCOBY (symbiotic colony of bacteria and yeast), has the shape of a large pancake or pellicle. It is a zoogleal mat made up of several types of bacteria and yeast. The symbiotic relationship that develops between the bacteria and fungus in Kombucha successfully prevents the formation of harmful microorganisms (Martínez Leal ve ark., 2018). Acetobacter and Gluconacetobacter are the two most common bacterial species. *Acetobacter xylinum* is the primary acetic acid bacterium in the tea fungus, according to analysis of the microbial population in several cultures. Others include *Bacterium gluconicum* and *Acetobacter xylinoides* (Chakravorty ve ark., 2016). They are able to make the acetic acid that gives vinegar its taste. New bacterial strains, such Gluconacetobacter sp., have been discovered recently (Wang ve ark., 2010). Unexpectedly, the Kombucha pellicle has shown a lot of lactic acid bacteria in Ireland (Marsh ve ark., 2014).

The sequence-based examination of several cultures revealed a larger fungal diversity in the Kombucha microbiota (Marsh ve ark., 2014). The common yeast species associated with Kombucha are *Saccharomyces ludwigi, S. cerevisiae*; others varyingly include *Brettanomyces bruxellensis*, *B. lambicus*,

B. custersii (Ramadani & Abulreesh, 2010). Acetic acid bacteria have been identified as *Komagataeibacter saccharivorans* and yeast have been identified as *Z. bailii* in kombucha made with 5% tea leaves and fermented for 14 days (Pure & Pure, 2016). As the yeast and bacterial populations vary throughout fermentation, a number of biochemical changes also take place. These changes revealed that Komagataeibacter was the biggest single bacterial species and Candida was the most dominant yeast genus (Chakravorty ve ark., 2016).

3.2 Standard Requirements for Making Kombucha

A kombucha pellicle is a gelatinous mass of yeast and bacteria that is flat, stiff, smooth, and gelatinous. It transforms into a pancake-like blob that covers the tea's surface, becoming a creamy beige tint. The Kombucha pellicle's metabolic processes require fresh air. To sustain an aerobic condition, the container should have a large opening. Sucrose must be available as a carbon source for the Kombucha to flourish and is essential as a nutritional medium (Moreno-Jiménez ve ark., 2018). It is possible to employ sugar substrates with different concentrations (5, 7.5, 10, 15, and 20 g/L) (Jayabalan ve ark., 2008). Additionally, sugar is crucial to the Kombucha culture's metabolic processes. The culture consumes sugar and gets its energy from it as well as from the minerals and nitrogen that the tea leaves have leached into the liquid (Moreno-Jiménez ve ark., 2018).

The type of tea used also affects kombucha fermentation. The recommended substrate for the manufacturing of Kombucha beverage is either black tea or green tea (Jayabalan ve ark., 2008). Tea waste, Japanese green tea, jasmine tea, oolong tea, mulberry tea (Talawat ve ark., 2006), sage, thyme, and peppermint teas (Velićanski ve ark., 2007), among other substrates, have all been researched. Tea substrate has been employed at concentrations ranging from 4 to 37 g/L w/v (Malbaša ve ark., 2006). Purines, nitrogen, and important minerals are nutrients found in tea. Black tea has a smoother taste and more nitrogen and purine than green tea. Green tea has additional tannins that give it a somewhat bitter flavor.

Yeast, which is classified as a facultative anaerobe, can metabolize glucose both with and without oxygen. According to Chen ve Liu (2000), the yeast cells generate enzymes like invertase that are released into the tea infusion and break down the sucrose into glucose and fructose. Under aerobic conditions, all of the sugar is transformed to energy, CO_2, water, and either no alcohol is created at all or a very little quantity of it.

This energy is sufficient for maintaining and promoting internal processes. When conditions are anaerobic, yeast converts to fermentation, which uses only approximately 5% of the energy in glucose and results in the production of ethanol as a byproduct. By converting ethanol and glucose into acetic acid and gluconic acid, respectively, acetic acid bacteria provide a

sour smell. Bacteria may now access alcohol thanks to the yeasts' generation of ethanol as a result of the creation of acetic acid. Both ethanol and acetic acid have antibacterial qualities, which they both use to combat harmful germs and keep Kombucha from being contaminated (Liu ve ark., 1996). Bacteria polymerize the glucose, resulting in the production of cellulose and hemicellulose. According to Iguchi ve ark. (2000), an additional cellulosic gelatinous pellicle is produced by the aerobic bacteria on top of the liquid tea surface. The Kombucha pellicle expands during germination until it covers the solution's surface and then thickens. As a result, the top surface will have the freshly produced pellicle (Blanc, 1996). According to the fermentation duration, the thickness of the ultrafine microfibrils that make up the bacterial cellulose network structure ranges from 0.1 to 0.5 in.

Within 6 to 10 days, the generated beverage will begin to smell fermented, and a few gas bubbles will occur as a result of carbonic acid generation. Black tea gradually transforms from sweet to moderately acidic, much like cider. The tea will develop a more sour (vinegar-like flavor) flavor with prolonged fermenting (Chen & Liu, 2000). Alcohol is eventually present in extremely tiny amounts (0.5%–1%) in the finished product. An alcoholic beverage that contains 0.5% alcohol is referred to as "nonalcoholic" or "alcohol-free." Numerous diabetics use tea that has undergone lengthy fermentation, resulting in a reduction in sugar content and a fructose-based residual sugar. Studies on the bacterial cellulose of Kombucha pellicle have shown that as surface area and broth depth rise, so does production. Another crucial component for the development of pellicle is temperature. The production of pellicle is suppressed at higher temperatures (Abuduaibifu & Tamer, 2019).

3.3 Kombucha Preparation Methods

Different methods can be used to prepare kombucha. Black tea and white sugar are the ideal Kombucha brewing ingredients, according to Jayabalan ve ark. (2008). Green tea, however, can also be employed. One technique is adding cold, sweetened black tea to some Kombucha that has already been made. The initial fraction of the Kombucha is ready after around 8 to 12 days; some of it is withdrawn for consumption while additional tea is added to fill the jar up. The mature Kombucha pellicle may generate a measure of the beverage every day since it is several centimeters thick. Occasionally, pieces of the pellicle can be pulled off to create fresh cultures.

Another technique is bottling Kombucha for later use. In a sizable glass jar, it is made similarly to the earlier approach. The jar has to be covered for the next 7-8 days with either a paper towel or a coffee filter that is tied with threads. While another portion is used to begin a fresh batch, some of the beverage is drained off into glass jars and chilled.

An further "mushroom" layer, zoogleal mat, or pellicle will emerge on top of the first while the Kombucha culture ferments. The liquid will become sour and taste like vinegar once three to four layers have built up. Another batch of pellicles can be started from the newly created ones. Most people choose to throw away the older cultures and utilize the most recent pellicle for the subsequent batch, even though the cultures may be used again.

According to Blanc (1996), kombucha's pH value lowers as its organic content rises. According to Dufresne ve Farnworth (2000), it is unclear exactly how the components of tea are affected during Kombucha pellicle fermentation.

The fermentation with Kombucha pellicle starts after around 7–10 days. A regulated environment with a temperature range of 22–30°C is used for this hygienic operation. Below 18°C, the fermentation process slows down, and beyond 30°C, Kombucha becomes slimy. Each time the Kombucha culture is fermented, a new culture is added to the pot. As the ingredients infuse and ferment, the sugar is broken down and turned into billions of organic acids, enzymes, vitamins, and minerals. As a consequence, the sweet tea is transformed into a tasty beverage with several health advantages. The outcome is a gently carbonated, acidic beverage with a flavor of vinegar. Filtered, then placed in a container with a new top, the fermented tea has to be chilled. Filtered, fresh-capped, and chilled bottles must be used for the fermented tea. In order to avoid contaminating the pellicle or the fermented tea with harmful bacteria or mold, care must be taken when handling Kombucha because it is a living culture. Equipment and utensils must be completely sterilized before use.

3.4. Kombucha Metabolites

The kombucha beverage has a significant number of functional components, which gives it a product with complex biochemistry. Such compounds have been demonstrated to have the ability to speed up human metabolism. It is recognized that tea is the source of the majority of the metabolites in kombucha. Tea is a complex system with potentially high antioxidant properties since it includes a number of polyphenols, flavonols (theaflavins and thearubigins), catechins, caffeine, catechin gallates, adenine, theobromine, theophylline, gallic acids, tannins, and gallotannin. A molecule's capacity to scavenge free radicals is measured by its antioxidant activity. It also depends on the flavonoid rings' level of polymerization, replacement groups connected to them, and isomeric structure (Loganayaki ve ark., 2013). During the kombucha tea's acid-alcoholic fermentation, the structures of several of these metabolites change and new ones are created. Acetic acid, lactic acid, gluconic acid, and glucuronic acid are the distinctive new functional chemicals created in the fermented brew. Yeasts in the SCOBY release invertase enzymes

that hydrolyze the sugar substrate to its monomers, glucose and fructose, and then use glycolysis to turn those monomers into ethanol. The partly aerobic state causes the acetic bacterial strains to become active and act upon glucose and ethanol to make, respectively, gluconic acid and acetic acid. Lactic acid is created when lactic acid bacteria react with carbohydrates. Jayabalan ve ark. (2014) have published in depth on their metabolic genesis. Ascorbic acid, amino acids, biogenic amines, purines, antibiotics, B complex vitamins, malonic acid, tartaric acid, oxalic acid, succinic acid, pyruvic acid, and important minerals are also said to be produced. Their formation's precise method is yet unknown. Variations in bacteria dispersion in the SCOBY, tea leaf composition, sugar content, and fermentation pace have all been implicated in the variable synthesis and percentage of these characteristic and noncharacteristic metabolites. There are several metabolites in kombucha tea that have been found to have health-promoting properties. Despite being presumed to exist and providing consumers with nutraceutical assistance, several of these metabolites have not yet been discovered. It is still unknown exactly how all of the kombucha metabolites are processed in the physiological pathways of tea drinkers. However, several general nutritional traits and advantages connected to the bioactive potential of the kombucha beverage have been investigated.

4. Literature about Kombucha's Health in Science

Due to extensive usage of kombucha in several nations over a long period of time, numerous health advantages have been identified based on firsthand experience and testimonies. However, only a few number of these have been demonstrated via research and experimentation. Since 1852, kombucha beverage has undergone considerable research, mostly in Europe, and has been evaluated (Dufresne & Farnworth, 2000).

The "Russian secret home remedy," also known as "Wonder drink," was reportedly used in Russia at the turn of the 20th century and during World War I to treat stomach ailments, headaches, and, most importantly, regulate intestinal functions that had become disturbed by the army lifestyle. Between 1925 and 1950, medical professionals carried out a number of studies that supported the traditional claims made about Kombucha. They noted beneficial effects such as the regulation of glandular, gastric, and intestinal activities, relief from hemorrhoids, gout, and joint rheumatism, reduction of cholesterol levels and arteriosclerosis, blood purification and toxin excretion, diabetes, and aging issues . But it's still unclear what techniques were employed (Laureys ve ark., 2020).

The "Central Oncological Research Unit" and the "Russian Academy of Sciences in Moscow" conducted a significant population survey in 1951 and discovered a correlation between daily Kombucha drinking and high cancer

resistance. Researchers suggested that long-term use of Kombucha improved immune system function and enhanced interferon production in the 1960s, when the anticancer capabilities of Kombucha and its detoxifying benefits were reinforced. Germany, Switzerland, and the Netherlands all provided additional support for these Russian results (Allen, 2000). Steinkraus ve ark. (1996) showed that Kombucha possesses antibiotic action against *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus aureus*, and *Agrobacterium tumefaciens*. They came to the conclusion that acetic acid generation during fermentation was primarily responsible for the antibiotic resistance. The same concentration of tea extracts showed no beneficial benefits.

The majority of Kombucha's qualities are a result of its acidity. The ability of glucuronic acid to bind to toxin molecules and increase their excretion by the kidneys or intestines is thought to be the cause of its detoxifying properties. As a result of the buildup of toxins, kidney stones, gout, arthritis, and rheumatism may be eased. Following glucuronidation, environmental contaminants, particularly heavy metals, can be eliminated by the kidneys (Dufresne & Farnworth, 2000). However, there is substantial disagreement over the glucuronic acid present in Kombucha and the glucuronide complex formation allegedly brought on by its ingestion. The B-complex vitamins included in Kombucha may have a protective impact on the neurological system, while the presence of lactic acid is thought to have a laxative effect (Reiss, 1994). The lactic acid bacteria in Kombucha have been shown in certain trials to have immunostimulatory effects on the host, however it is still unknown if these microbes can colonize the human gut.

5. The Health Implications of Kombucha's Bioactive Ingredients

Sucrose and the components of black tea gradually change throughout the making of Kombucha thanks to the Kombucha pellicle's action. Acetic acid, lactic acid, gluconic acid, glucuronic acid, ethanol, and glycerol are among the most significant metabolites in the fermented beverage (Blanc, 1996). Folic acid and the B-complex vitamins were synthesized, according to Xia et al.. The tea fungus's yeasts and bacteria utilize the substrates in various and complimentary ways (Xia ve ark., 2019). Yeasts hydrolyze sucrose into glucose and fructose, as well as ethanol, by employing fructose as a substrate of preference, according to (Zubaidah ve ark., 2020). While ethanol is turned to acetic acid by ethanol-producing bacteria, acetic acid bacteria use glucose to make gluconic acid (Wang ve ark., 2010). Both acetic acid and ethanol have been shown to have antibacterial effects on harmful microorganisms. This guards against tea fungus and Kombucha contamination (Liu ve ark., 1996).

The source of the Kombucha pellicle, the amount of sugar present, and the degree of fermentation all affect the metabolite composition and concentration of the various Kombucha components. According to Reiss

(1994), the ideal concentration of sucrose for ethanol and lactic acid synthesis is 50 g/L. Additionally, kombucha is a source of several bioactive substances, including the catechins epicatechin, epigallocatechin gallate, epigallocatechin (Jayabalan ve ark., 2007), gallocatechin, and gallocatechin gallate (Lobo ve ark., 2017), as well as vitamins B_1, B_2, B_6, B_{12}, and vitamin C (Bauer-Petrovska & Petrushevska-Tozi, 2000).

According to Malbaša ve ark. (2006), kombucha is a potent source of antioxidants, probiotic acids, active enzymes, vitamins, amino acids, and tea polyphenols. The primary factors affecting the composition of Kombucha products include the amount of sugar added, the type of tea used, the amount of time spent fermenting, the location and season, and the bacteria present in the area.

According to an in vitro investigation, the total polyphenols, total flavonoids, and gallic acid content of Kombucha ferment are all gradually higher than those of black tea (Lobo ve ark., 2017). The enzymes generated by the bacteria during fermentation that break down complex polyphenols into low-molecular-weight components might be the cause of the rise in polyphenols and flavonoids in Kombucha (Jayabalan ve ark., 2007). Polyphenolic substances known as flavonoids have strong biological and pharmacological effects. Antioxidant, anti-inflammatory, anticarcinogenic, free radical scavenging, apoptosis, activation of antioxidant enzymes, and may even alter several significant cell signaling pathways are just a few of the positive benefits that have been proven (Hosseini ve ark., 2016). Low-density lipoprotein (LDL) is not oxidized by flavonoids, and endothelial dysfunction is not caused by flavonoids (Watawana ve ark., 2015).

Recent research has shown that flavonoids have antibacterial, anti-inflammatory, antiallergenic, and antioxidant properties. Flavonoids function as insulin secretagogues or insulin mimics, promote glucose absorption by peripheral tissues, and control enzyme activity in the carbohydrate metabolism pathway. To help diabetics, they also have an impact on the pleiotropic system of insulin signaling (Cazarolli ve ark., 2008). In the heart of diabetic rats, gallic acid showed to reduce lipid peroxidation and boost antioxidant characteristics (Patel & Goyal, 2011).

6. Possible Health Risks of Kombucha Consumption

The Kombucha culture defends itself against dangerous microbes. The development of foreign microorganisms like molds and bacteria is inhibited by an acidic pH caused by acetic acid and lactic acid. Underdeveloped Kombucha may potentially include pathogens in addition to not developing valuable nutrients. This is due to the fact that when infected, the concoction's acidity is insufficient to prevent the growth of hazardous germs. In four cases of Kombucha poisoning described by Srinivasan et al. in 1997, the patients

had a variety of symptoms, including nausea, vomiting, dizziness, headaches, neck discomfort, and shortness of breath. All of these symptoms were linked to consuming Kombucha that had been poorly made or kept or to metal leaching brought on by acids from certain fermentation-related equipment (Bolle ve ark., 2011). Kombucha that has been too fermented will be quite acidic and might overtax the digestive system. This is because the acetic acid in Kombucha, when consumed, combines with minerals to produce alkalis. As a result, too much acid will deplete the body's supply of vital ionic minerals including calcium, sodium, potassium, and magnesium (Kasper, 2023). Because the different biochemical and physiological parameters evaluated were within therapeutic limits, oral toxicity experiments conducted by Pauline ve ark. (2001) revealed that Kombucha exhibited no significant harm. Additionally, according to the US Food and Drug Administration's 1995 microbiological and biochemical studies, Kombucha is safe for ingestion by humans (Jayabalan ve ark., 2014).

7. Conclusion

Making kombucha at home often entails fermenting black tea that has been sweetened with sugar with a SCOBY. Numerous health-promoting and antibacterial properties of the fermented beverage have been claimed for a very long time in many parts of the world. Kombucha is always being researched for its natural qualities because of its long history of usage as a beverage that promotes health across the world. These investigations have validated a number of widely held misconceptions about it. The therapeutic and health-promoting effects of kombucha beverages, including their anti-inflammatory, anti-cancer, antidiabetic, hepatoprotective, and antibacterial capabilities, have previously been studied and scientifically proven.

Although kombucha drink's many health benefits have been scientifically verified in animal models, its constituent parts' similar processes, their function in consumers' physiological pathways, and their interactions in people are still unknown. The use of kombucha has also been linked to a number of potential health issues. Despite being a well-known folk cure for many illnesses, the product has to be scientifically verified.

The microbial makeup of fermented beverages and SCOBY are now among the least understood and researched topics. Before employing the microfloral community to produce kombucha, it has to be precisely identified and characterized with information on each species' percentage. This will make it easier to comprehend the likely metabolites that they are producing and, in turn, provide a window into mapping out a potential mechanism of action in the physiological pathways of kombucha drinkers. Conducting human trials will enable full investigation and scientific validation of the profiling of all kombucha metabolites, their production, and bioactive interactions.

Consumer awareness of natural and chemical-free goods is growing with the popularity of herbal medicines and supplements like kombucha tea, etc. on a global scale. As a result of growing worries about the risks and safety issues involved, it is now urgent to adopt a standardized production process as well as approved regulations for raw materials, unit operations, quality control, packaging, storage, serving criteria, intake range, etc. from internationally renowned authorities. In this regard, the United States Food and Drug Administration (USFDA) surveyed commercial manufacturers but discovered no evidence of dangerous microorganisms or unsanitary conditions in kombucha tea. Additionally, the Pennsylvania Department of Agriculture's Bureau of Food Safety and Laboratory Services has provided a few rules for the manufacturing and packaging of kombucha. To create a well-standardized process and safety guideline for the commercial manufacturing of kombucha beverage, authors consider that the currently published standards are not complete and that more research is necessary.

8. Future Research Considerations

The study of proteins involved in many processes and their susceptibility to free radical damage is opened up by the research done so far on Kombucha. Bioactive Kombucha molecules may be tested in silico against a variety of proteins, and further computational work can be done to demonstrate their efficacy as therapeutic agents. The bioactive substances that perform well in the docking investigation can also be taken into consideration for the prevention of different oxidative stress-related disorders. Additionally, kombucha may be thought of as a potential source of probiotics and as having an impact on the flora of the stomach. Overall, this work builds a deeper knowledge of the underlying processes and mediators and provides glimpses of prospective health advantages of this traditional beverage/medicine. Microbiomics, which aims to link local microbiota with metabolic, immunological, and developmental processes, has recently received a lot of interest. A fascinating hypothesis would be that Kombucha culture, in addition to its high antioxidant content, influences the gut flora in a way comparable to dietary probiotic supplements.

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Chapter 21

CYTOKINES IN THE PATHOGENESIS OF OSTEOARTHRITIS

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1. Osteoarthritis

Osteoarthritis (OA) is a disorder that results from a variety of traumatic, biomechanical, developmental, metabolic, and genetic variables that alter the biochemistry and morphology of cartilage, bone, and synovial tissues as a result of the disruption of the balance between cartilage formation and destruction (Neogi, 2013; Sharma, 2006).

2. Osteoarthritis classification

Different classifications are used for OA, depending on the joint involved, etiology, and specific features (Dennison, 2003; Martel-Pelletier, Lajeunesse, & Pelletier, 2005). Traditionally, there are two categories of OA: primary (idiopathic) and secondary. If the cause of joint degeneration is unknown, it is called primary OA and is the most common form of OA. Secondary OA is a condition in which the underlying factor is evident. Idiopathic OA is rare before the age of 40. Secondary OA due to reasons such as trauma, infection, avascular necrosis, and hemophilia is mostly seen in young adults(Dennison, 2003). It is seen that the disease particularly affects some joints and does not affect some joints. For example, older interphalangeal joints are usually the distal interphalangeal joints, the first carpometacarpal joints, and less commonly the proximal interphalangeal joints. Primary OA is not seen in other joints of the hand. Similarly, primary OA is rare in the ankle, wrist, elbow and shoulder joints (except the acromioclavicular joint). On the other hand, OA is quite common in the knee, hip, first metatarsophalangeal joint, cervical and lumbar spine facet joints (Dennison, 2003; Hooper, Holderbaum, & Moskowitz, 2005). Primary OA can be local or widespread (Sharma, 2006). Cases involving three or more joint groups are called generalized OA (Dennison, 2003).

3. Incidence and Prevalence of Osteoarthritis

Osteoarthritis is a condition that affects 42.1% of women and 31.2% of men, according to statistics (Roos & Arden, 2016) . Another study claims that symptomatic osteoarthritis affects 9.6% of men and 18% of women over the age of 60 worldwide (Allen & Golightly, 2015). When evaluated radiographically and symptomatically, the prevalence of hip osteoarthritis was 19.6% and 4.2%, respectively (Kim et al., 2014); the prevalence of knee osteoarthritis was 25.4% and 15.4% (Johnson & Hunter, 2014; Neogi & Zhang, 2013); The prevalence of radiographic foot osteoarthritis has been reported to be 0.1%-61% (Kalichman & Hernández-Molina, 2014).

4. Pathogenesis of Osteoarthritis

Although OA affects all elements of the joint such as cartilage, subchondral bone, synovial tissue, ligaments, capsule and muscles that make up the synovial joint, primary changes include loss of articular cartilage,

subchondral bone remodeling and the development of osteophytes (Dicesare & Abramson, 2005). OA is considered a process different from aging cartilage and is considered a pathology that negatively affects the dynamic, biomechanical and cellular functions of the the whole joint. Therefore joint cartilage, subchondral bone and synovium are at the center of the pathological process (Lohmander, 2000).

4.1. The Role of Articular Cartilage

The specialized component known as articular cartilage that reduces friction and supplies effective load distribution. It consists of an extracellular matrix with high fluid content, including chondrocytes, collagen fibers and proteoglycans. Chondrocytes, the only cellular component of hyaline cartilage, are metabolically active and constitute 1-2% of the cartilage. Articular cartilage has no blood vessels and innervation. Tissue repair is weak and occurs only with fibrocartilage tissue (Evcik & Babaoglu, 2007). 65-80% of the extracellular matrix is water. In addition, there are collagen, proteoglycans, lipid and phospholipid molecules (Dicesare & Abramson, 2005). The main collagen of articular cartilage is type 2 collagen, which is found in 90-95% (Dicesare & Abramson, 2005). Type 2 collagen gives tensile properties to cartilage and immobilizes proteoglycans in the extracellular matrix (Goldring, 2000). Proteoglycans fill the spaces between collagen fibers. It consists of glycosaminoglycans (GAG) attached to a proteoglycan core protein. GAGs are present in hyaluronic acid, chondroitin sulfate, keratan sulfate, dermatan sulfate, and cartilage structures (Dicesare & Abramson, 2005). Significant changes occur in the articular cartilage with aging. The number of cells decreases and the chemical structure of the joint cartilage changes, water content and proteglycans decrease. Chondroitin-4-sulfate concentration decreases, keratan sulfate rate increases. The hyaluronate content of cartilage increases, but the chain length decreases. Collagen fibrils also thin with age, forming thinner bundles. As a result of these changes, the structure of the cartilage deteriorates, splits and wears called fibrillation occur on its surface, and tensile strength decreases (Wolheim, 2003). Early on in the OA process, a temporary proliferative response, an increase in extracellular matrix synthesis, and an increase in cytokine and proteinase enzyme activities are observed in chondrocytes. This increase in activity observed in chondrocytes is considered as the "tissue repair response" that occurs in the early period. In the advanced stages of OA, the tensile properties of cartilage deteriorate, especially with the decrease in type 2 collagen and aggrecan synthesis, bringing cartilage degeneration into an irreversible phase (Goldring, 2000). In the first stage, the total proteoglycan content of the articular cartilage decreases in proportion to the OA's degree. As the disease progresses, proteoglycan concentration falls below 50% of normal and GAG chains shorten. Collagenase thins the collagen fibers, loosens the collagen network and causes swelling in the matrix. These

changes cause the cartilage to become weakened against mechanical stress and compression and lead to progressive cartilage loss (Martel-Pelletier et al., 2005). In the second phase, chondrocytes detect tissue damage and changes in osmolarity and charge density and rapidly secrete mediators that stimulate the cellular response. In the second stage, chondrocytes detect changes in osmolarity and charge density as a result of tissue damage and rapidly secrete mediators that stimulate the cellular response. Anabolic and mitogenic factors are significant in the synthesis of matrix macromolecules and the proliferation of chondrocytes. Chondrocytes produce nitric oxide (NO), a free radical, in response to some mechanical and chemical stresses. NO diffuses rapidly and induces the release of Interleukin-1 (IL-1), which leads to the degradation of matrix macromolecules (Dicesare & Abramson, 2005). The production of matrix components is decreased, the production of degradative enzymes is increased and chondrocyte proliferation is inhibited by IL-1. Other cytokines that affect chondrocyte activities are TNF-α (tumor necrosis factor alpha) and IL-6 (Van der Kraan & van den Berg, 2000; Westacott & Sharif, 1996). In addition to cytokines, some growth factors released from chondrocytes and synoviocytes function by inhibiting proteolytic enzymes, stimulating proteoglycan and collagen synthesis, and repairing and protecting damaged cartilage tissue (Goldring, 2000).

The enzymes that break down the extracellular matrix are proteinases, and their activities are controlled by proteinase inhibitors. Proteinases consist of metalloproteinase, aspartic proteinase, cysteine proteinase and serine proteinase enzymes that are involved in extracellular matrix degradation. (Martel-Pelletier et al., 2005). In the second stage of OA development, the repair response can counteract the catabolic effect of proteases and sometimes enable tissue repair. The repair response can last for years, sometimes stopping the course of the disease even temporarily. Failure of stabilization or repair attempt leads to the formation of the third stage of the disease. The anabolic and proliferative responses of chondrocytic cells are reduced, and there is a gradual loss of cartilage. This decrease may result from mechanical damage and death of chondrocytes that are not protected by a functional and stabilized matrix, or it may result from a decrease in the response of chondrocytes to anabolic cytokines (Dicesare & Abramson, 2005; Martel-Pelletier et al., 2005; Wolheim, 2003).

4.2. The Role of Subchondral Bone Tissue

Subchondral bone is viscoelastic and is better shock absorber than cartilage tissue. In case of sudden overload, cartilage protects the tissue and takes part in load distribution and absorbs the load at much higher rates than cartilage (Evcik & Babaoğlu, 2007). In OA, with overload on the joint, the density and rigidity of subchondral bone have increased, but the load joint's distribution capacity decreases. It has been shown that the increase in subchondral bone

density develops early and then progresses to cartilage loss throughout the entire thickness. As OA progresses, the formation of microfractures in weight-bearing joints may be facilitated by mechanical stress in the layer of cartilage and subchondral bone. As microfractures heal, an increase in bone stiffness occurs. As cartilage damage increases, subchondral sclerosis and stiffness progress. Subchondral bone responds to these repetitive stimuli with restructuring and hardened bone tissue. However, the shock absorbing properties of this new bone have decreased. Research has demonstrated a strong correlation between the severity of osteoarthritis and the thickness of the subchondral bone. (Evcik & Babaoglu, 2007; Hough, 2005).

4.3. Osteophyte Formation

The discomfort and restriction of joint motion in OA are partially brought on by bone proliferations that form along the margins of the joints and at the bottom of cartilage lesions. According to one theory, osteophytes develop following the penetration of blood vessels into the basal layer of deteriorating cartilage or perhaps due to stress fractures in the subchondral bone close to the joint edge healing abnormally. In the experimental OA model, it has been shown that osteophytes can develop even when the articular cartilage as a whole is normal. This is because osteophytes increase the joint surface and may contribute to the reversal of cartilage changes in early OA. Human OA joint osteophytes synthesize cartilage rich in type 1 collagen and non-clumping proteoglycans (Dicesare & Abramson, 2005).

4.4. Synovial Changes

Even though OA is often believed to be a non-inflammatory disease, recent studies have demonstrated that low-grade inflammation and synovitis contribute to the etiology of OA (Pelletier, Martel-Pelletier, & Abramson, 2001; Saxne, Lindell, Månsson, Petersson, & Heinegård, 2003). Synovial inflammation is controlled by soluble biochemical factors, prostanoids, cytokines and reactive oxygen species made by synoviocytes and chondrocytes (Henrotin, Bruckner, & Pujol, 2003). Alterations in enzymes involved in cartilage metabolism alone cannot explain the destruction in OA. There are various cytokines and growth factors in increased amounts in the osteoarthritic synovium, which have direct effects on cartilage and chondrocyte functions (Van der Kraan & van den Berg, 2000). Cytokines are substances with a peptide or glycopeptide structure that play a role in chemical communication between cells. They play a role in cell growth, differentiation, inflammation, tissue repair and immune response (Kokuludağ, 1999). Mediators involved in cartilage metabolism are divided into three main groups; 1. Destructive Cytokines: Interleukin 1α and β (IL-1α and β, Tumor necrosis factor (TNF-α and β), Leukemia inhibitory factor, Transforming growth factor (TGF- β) 2. Regulatory Cytokines: IL-6, IL- 10, IL-13, TGF- β 3. Growth Factors: TGF-

β, Insulin-like growth factor (lGF), Fibroblast growth factor (FGF), Platelet-derived growth factor (PDGF) (Van den Berg, 1999; Van der Kraan & van den Berg, 2000).

5. Laboratory Findings

There is no specific diagnostic test for osteoarthritis. In patients with primary OA, erythrocyte sedimentation rate, complete blood count, urinalysis and blood biochemistry are within normal limits. Erythrocyte sedimentation rate may be slightly high in some patients. These tests are used in differential diagnosis to rule out other diseases. Nonspecific changes of mild inflammation are observed in the synovial fluid. It is clear, viscous and the white blood cell count is less than 2000/mm^3 (Atay, 2000; Punzi, Oliviero, & Plebani, 2005). OA affects the metabolism of bone, cartilage and synovial membrane. Biochemical markers that reflect changes in these tissues can be used in the diagnosis of OA, assessment of prognosis, disease activity, and efficacy of treatment (Ödemiş Güngen, 2009).

5.1.CTX-II

Almost all type 2 collagen is found in cartilage. Therefore, measurement of degradation products may be specific for cartilage degradation (Reijman et al., 2004). With N and C propeptides at the ends, type 2 collagen is created as procollagen. During synthesis, propeptides are removed by specific proteases. During cartilage destruction, neoepitope-bearing triple helix fragments (COL2-3/4 long mono and COL 2-3/4C short), nitratable triple helix fragments, and C and N terminal cross-linking telopeptides are released into synovial fluid, serum, and urine (P. Garnero & Delmas, 2003; Rousseau & Delmas, 2007). The C-terminal portion of type 2 collagen (CTX-II) is different from other collagen molecules by metalloproteases. It can be detected in joint fluid and urine. It is specific for mature fibrillar collagen. It does not indicate the destruction of newly synthesized cartilage (Chevalier & Conrozier, 2005). Although patients with OA were found to have considerably higher levels of CTX-II, it was also reported to be at normal levels in a group of patients. For this reason, it is thought to be not sensitive enough as a diagnostic tool on a patient basis (P. Garnero, Rousseau, & Delmas, 2000; Rousseau & Delmas, 2007). It has been reported that high urinary CTX II levels are associated with rapid joint destruction determined by plain x-ray or arthroscopy (Patrick Garnero et al., 2002). It is important in predicting disease progression. It is also used in treatment effectiveness monitoring. A study showed that urinary CTX II excretion decreased with ibuprofen treatment in OA exacerbation in patients with gonarthrosis (Gineyts et al., 2004).

5.2. YKL-40

There are similarities between chitinases and YKL-40 (human cartilage glycoprotein 39) but it is a glycoprotein with no enzymatic activity. While it is synthesized by synoviocytes in the normal adult joint, osteoblasts and chondrocytes do not take part in the synthesis. In osteoarthritis, it is synthesized by chondrocytes almost in direct proportion to the degree of fibrillation in the cartilage. YKL-40 is also synthesized by osteoblasts and primary osteocytes in osteophytes. When chondrocytes and osteoblasts are stimulated, they synthesize YKL-40 during growth or repair (Vignon, 2001). Immunohistochemical analyzes showed that YKL-40 was found in high amounts in chondrocytes in the superficial and middle layers of osteoarthritic cartilage. Volck et al. reported that the inflammatory synovial membrane contains YKL-40, and the level of synovial inflammation is correlated with YKL-40 positive cells. (K. Ø. Volck, Julia Johansen, Charly Garbarsch, PA Price, Birgitte, 1999). YKL-40 from neutrophils in the articular cartilage and synovial fluid outside the synovial membrane contributes to synovial fluid YKL-40. Serum YKL-40 level was found to be proportional to the amount in synovial fluid (B. Volck et al., 2001). It was used to monitor the effectiveness of glucosamine/chondroitin treatment in osteoarthritis (Nakamura & Nıshıoka, 2002) However, it is not specific to the joint alone; it is also synthesized by many other tissues. It can also increase in many conditions such as cirrhosis, metastatic breast cancer, colorectal cancer and pneumonia (Vignon, 2001)

5.3. hsCRP

Acute phase protein C reactive protein (CRP) is created by hepatocytes in response to the stimulation of cytokines such as IL 6, TNF alpha, IL during the acute phase response (Hanna et al., 2008) CRP has traditionally been used to distinguish inflammatory from non-inflammatory events. Recently, the high-sensitivity CRP (hsCRP) marker, which can detect lower levels of CRP, has begun to be used (Pearle et al., 2007) Many studies have shown that hsCRP is increased in the serum of patients with OA (Th Conrozier et al., 2000; Sowers et al., 2002; Wolfe, 1997) Since it is a nonspecific inflammation marker, it cannot be used as a diagnostic marker in OA. However, it is stated that it is associated with disease progression and clinical severity (Th Conrozier et al., 2000; T. Conrozier et al., 1998; Sharif, Shepstone, Elson, Dieppe, & Kirwan, 2000; Wolfe, 1997) It has been reported that the increase in hsCRP is associated with radiographic progression (Sharif et al., 2000). It has been shown that systemic hs CRP levels are related to the degree of OA-related synovial inflammation (Pearle et al., 2007).

6. The Role of Cytokınes in Osteoarthritis

It has been reported that chondrocytes obtained from OA patients actively produce inflammatory mediators, including NO, IL-1β, TNF-α, IL-

6, and IL8, and that these mediators increase the catabolic state within the cartilage, which causes progressive cartilage damage, either autocrine or paracrine (Pelletier et al., 2001).

6.1. Interleukin-1 and Interleukin-1β

Interleukin-1 (IL-1) is a polypeptide cytokine that is effective in almost all tissues and organ systems. The main source of IL-1 is the monocyte-macrophage system. It is also released from connective tissue cells such as fibroblasts, synovial cells and chondrocytes. It is a potent proinflammatory cytokine that has a significant impact on the deterioration of cartilage in degenerative joint disorders (Martel-Pelletier, Alaaeddine, & Pelletier, 1999). The role of IL-1 in articular cartilage is multifaceted. While IL-1 prevents the production of collagen and aggrecan specific to hyaline cartilage, it has an effect on the production of collagen types specific to fibroblasts. Although chondrocytes enter the repair process, the repair made under the influence of IL-1 is not of high quality because it will be fibrous instead of hyaline cartilage (Martel-Pelletier et al., 1999). IL-1 induces the release and synthesis of many matrix degradation enzymes through synoviocytes and chondrocytes. It regulates the synthesis of many cytokines with similar biological activities, such as IL1, IL-6 and IL-8 (S. R. Goldring & Goldring, 2004). One of the factors that play an important role in the effect of IL-1 is the age of the cartilage. Aged cartilage has a lower response to IL-1, but its repair rate is also lower (Fernandes, Martel-Pelletier, & Pelletier, 2002). The IL-1 cytokine family consists of IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra) cytokines (Kokuludağ, 1999). IL-1α and IL-1β have agonist activity. However, IL-1β is synthesized at 10-50 times higher levels than IL-1α and has stronger proinflammatory properties. IL-1β is produced in inactive precursor form. The inactive precursor form of IL-1β is activated by IL-1 convertase enzyme from serine protease family. The expression of this enzyme is increased in both synovium and cartilage in OA (Martel-Pelletier et al., 1999; Martel-Pelletier et al., 2005). IL-1 is an activator of cyclooxygenase-2 (COX-2), phospholipase A2, and iNOS. In vitro studies have shown that it increases MMP enzyme expression in human cartilage chondrocytes and increases NO formation, a catabolic factor in cartilage. (S. R. Goldring & Goldring, 2004). TNF-α and IL-1β cause synovial cells and chondrocytes to release inflammatory mediators such as IL-8, IL-6, NO and prostaglandin E2 (PGE2), which increase bone resorption. Their catabolic activity is further enhanced because IL-1β and TNF-α stimulate their own production autocrine or paracrine (Dicesare & Abramson, 2005). Certain IL-1 receptors on the cell surface facilitate the biological stimulation of chondrocytes and synovial cells by IL-1 (IL-1R). There are two types of IL-1Rs. IL-1α binds better to type I and IL-1β binds better to type II (Martel-Pelletier et al., 1999). IL-1 is the only cytokine with a natural inhibitor. This inhibitor, released from mononuclear phagocytes, is

structurally similar to IL-1 and competes with IL-1 to bind to the receptor of IL-1. This compound is known as an IL-1 receptor antagonist (IL-1Ra), and it functions as a competitive inhibitor of IL-1 (Kokuludağ, 1999). IL-1Ra has physiologically antagonistic activity with the IL-1 molecule. IL-1Ra and IL-1 levels regionally influence the physiological and pathophysiological balance of IL-1 in tissue

(Pelletier et al., 2001). IL-1Ra has been shown to increase in circulation in a wide variety of inflammatory, infectious and post-surgical conditions through its synthesis in the liver. Where inflammatory events occur, the proportion of IL-1 to IL-1Ra levels establishes the shape of the inflammatory response (Arend, 2002). Due to the effect of IL-1Ra on the receptor, only IL-1Ra 100-fold or more is sufficient to suppress the effects of IL-1 on target cells. Therefore, IL-1Ra must be synthesized and released in excessive amounts in order to suppress the effects of IL-1 in tissue. A relative deficiency in the IL-1Ra/IL-1 ratio in OA synovial tissue may allow increased IL-1 activity

(Martel-Pelletier et al., 1999). IL-1 activity is very important in disease progression. It has been noted that adding IL-1Ra to OA cells inhibits synthesis of collagenase, prostaglandin E2 (PGE2) and NO, which have destructive effects (Dicesare & Abramson, 2005).

6.2. Tumor Necrosis Factor and Soluble Tumor Necrosis Factor Receptors

TNF-α, also known as cachectin, is a potent proinflammatory cytokine and a chemotactic agent for monocytes and neutrophils. Its effects are similar to IL-1. It has two different forms, TNF-α and TNF-β. TNF-α is released mainly from activated macrophages under stimuli similar to those of IL-1. TNF-β is made by activated T lymphocytes and is called lymphotoxin. TNF-α plays an important role in physiopathologic conditions related to cachexia, endotoxic shock, inflammation, tissue remodeling, infection, immunity and cytotoxicity. It stimulates mononuclear phagocytes and vascular endothelium to synthesize IL-1 and IL-6 and hepatocytes to synthesize acute phase proteins (Kokuludağ, 1999). Although there is ample evidence that IL-1 is the most important destructive mediator for articular cartilage, the most important driver of IL-1 production is TNF-α. Both IL-1α and IL-1β are present in significant amounts in osteoarthritic synovium, whereas TNF-α is less abundant (Martel-Pelletier et al., 1999). Activated macrophages and monocytes secrete TNF-α and, similar to IL1, is in charge of articular cartilage degradation and in late stages of the disease, subchondral bone. It also induces collagenase synthesis from fibroblasts and is associated with connective tissue destruction and bone resorption. TNF-α stimulates the synthesis of PGE2, the production of IL-6 by chondrocytes and the release of superoxide derivatives (Martel-Pelletier et al., 1999). The bioactivation of chondrocytes and synovial

cells by TNF-α is mediated through specific cell surface TNF-receptors (TNF-R). Type 1 TNF-R affects TNF-mediated cytotoxicity, antiviral effect and fibroblast proliferation, while type 2 TNF-R mediates enhancement of T lymphocyte proliferation (Martel-Pelletier et al., 1999). Increased TNF-R1 expression has been reported in OA chondrocytes and synovial fibroblasts (Martel-Pelletier et al., 1999; Martel-Pelletier et al., 2005). TNF-α is synthesized as a membrane-bound precursor and then proteolytically cleaved by TNF-α converting enzyme (TACE, adamalysin). Studies have shown that TACE expression is increased in osteoarthritic cartilage. TACE also cleaves the extracellular domains of cell surface TNF receptors to form soluble TNF receptors (sTNFRs) (Martel-Pelletier et al., 1999; Martel-Pelletier et al., 2005). sTNFR is thought to act as a receptor antagonist, regulating TNF-α activity or stabilizing TNF-α. The trimeric structure of TNF-α is stabilized by these soluble receptors at low concentrations and prolong the half-life of bioactive TNF-α, whereas at high concentrations they reduce the bioactivity of TNF-α by acting as a cognate for cell-associated binding receptors. (Penninx et al., 2004; Westacott & Sharif, 1996). sTNFRs are more stable in circulation than cytokines and better reflect the inflammatory process. In a study in patients with knee OA, high sTNFR concentration was associated with OA symptoms and physical dysfunction (Penninx et al., 2004).

6.3. Interleukin-2 and Interleukin-2 receptor

IL-2 is a polypeptide also known as T cell growth factor and is among immunoregulatory cytokines. IL-1 and IL-6 released from activated monocyte-macrophages also stimulate IL-2 production. The IL-2 receptor (55-kDa/75-kDa heterodimer) is present on the surface of activated T cells and the 55-kDa chain is called soluble IL-2 receptor (sIL2R). Binding of IL-2 to its receptor causes proliferation of T lymphocytes and release of cytokines. T lymphocytes stimulated with IL-2 increase their cytotoxicity and secretecytokines such as IFN-γ, TNF-β TGF-β, IL-4, IL-6, IL-3, IL-5 (Kokuludağ, 1999).

IL-2R has been reported to be elevated in patients with erosive hand OA (Punzi et al., 2005). In a controlled study conducted by Mabey et al., it was demonstrated that patients with knee OA had significantly higher plasma concentrations of IL-2, IL-4, and IL-6 compared to controls (Mabey et al., 2016). This study has of importance because of having a control group, at the same time has of limitation having a relatively small sample size (32 patients with knee osteoarthritis and 14 healthy controls).

6.4. Interleukin-6 and Soluble Interleukin-6 Receptor

IL-6 is a multifunctional cytokine released by T cells, monocytes, macrophages, macrophages, fibroblasts and endothelial cells and many other cells under the influence of IL-1 and TNF-α (Kokuludağ, 1999). Has synergistic effects with IL-1 and TNF-α (Kokuludağ, 1999; Sanchez et al.,

2004). Cytokines such as TNF, IL-1, platelet-derived growth factor (PDGF), IFN-β, antigens, mitogens and bacterial endotoxins (lipopolysaccharide) stimulate IL-6 formation in different cell types. IL-6 is a major inducer of CRP. It increases the growth and differentiation of B lymphocytes and immunoglobulin production. It acts as a costimulator in the activation of T lymphocytes and increases the activity of cytotoxic T lymphocytes. It also stimulates IL-2 production. With these properties, IL-6 is an important mediator in both humoral and cellular host defense. Increased IL-6 levels have been observed in synovial effusion in inflammatory joint diseases and in non-rheumatic conditions such as pseudogout and traumatic joint disease (Kokuludağ, 1999). By raising the number of inflammatory cells in synovial tissue, promoting chondrocyte proliferation, enhancing MMP synthesis, and inhibiting IL-1's ability to produce proteoglycan, IL-6 contributes to the degenerative process of OA. IL-1 induces the synthesis and secretion of IL-6 in human chondrocytes (Martel-Pelletier et al., 1999; Westacott & Sharif, 1996). In addition, IL-6 induces the production of tissue inhibitors of metalloproteases and thus plays a role in the feed back mechanism that reduces enzyme damage. It has also been reported to play an important role in bone destruction ((Martel-Pelletier et al., 1999; Martel-Pelletier et al., 2005). IL-6 is also involved in the inhibition of IGF-1, an important anabolic stimulant for muscles, leading to a decrease in muscle strength and physical function, especially in the elderly (Ferrucci et al., 2002). In a study by Penninx et al. in 274 patients with knee OA, high serum IL-6 levels were associated with low walking speed ((Penninx et al., 2004). When IL-6 binds to a receptor complex made up of two membrane glycoproteins, its biological action begins (Sanchez et al., 2004; Uson et al., 1997). IL-6 has two receptors, gp130 and IL-6R alpha (Desgeorges et al., 1997). IL-6R is composed of two glycoprotein chains and is present in many target cells (Kokuludağ, 1999). Soluble cytokine receptors can be formed by proteolytic cleavage of the membrane-bound receptor and release in soluble form or by RNA cleavage to produce a soluble receptor (Uson et al., 1997). Free receptors act as cytokine antagonists and compete with membrane receptors to block the association of the cytokine with the target cell. In contrast, the soluble IL-6 receptor (sIL-6R) acts as an IL-6 agonist, binding to IL-6, increasing its potency and helping signal transduction (Sanchez et al., 2004; Uson et al., 1997).

6.5. Interleukin-8

IL-8 is produced by many cells involved in inflammation (blood monocytes, alveolar macrophages, endothelial cells, fibroblasts and epithelial cells). It also has a chemotactic factor role for T lymphocytes, basophils and natural killer cells (Kokuludağ, 1999). It acts on neutrophils in many ways; It stimulates the production of free oxygen radicals and neutrophil degranulation, and increases the expression of integrins and complement

receptor on neutrophil surfaces. The most important stimulants for IL-8 release are IL-1 and TNF-α (Martel-Pelletier et al., 1999). IL-8 levels were investigated in the serum, synovium, and subchondral bone of patients who had OA. It was demonstrated that IL-8 levels were high in all these sample types of OA (Kaneko et al., 2000; Monibi et al., 2015). Further, IL-8 levels of synovial fluid were reported to be linked with the radiographic staging of the disease and the intensity of pain which is felt with motion (Leung, Huebner, Haaland, Wong, & Kraus, 2017; Monibi et al., 2015).

6.6. Interleukin-4

IL-4 is a powerful regulator of the immune system and is a cytokine synthesized by Th2 cells, a subset of CD4+ T lymphocytes, and mast cell precursors (Brown & Hural, 2017). IL-4 is a protein consisting of 129 amino acids and is in the form of a four-helix bundle (Wojdasiewicz, Poniatowski, & Szukiewicz, 2014). IL-4 stimulates B and T cell proliferation, enables CD4+T cells to differentiate into Th2 cells, and plays a key role in humoral and acquired immunity and the development of allergic inflammation. It prevents the formation of IL-1, IL-6, TNF-α, PGE2 (Kokuludağ, 1999). IL-4 has an anti-IL-1 effect on chondrocytes. It increases IL-1Ra production and acts by reducing the amount of NO, which is an important secondary mediator in the inhibition of chondrocyte proteoglycan synthesis. Additionally, enzymatic degradation in the matrix is reduced by IL-4 (S. R. Goldring & Goldring, 2004; Martel-Pelletier et al., 1999; Yorimitsu et al., 2008). Although some pertinent information on the initial intracellular events is available, the precise signaling mechanism of IL-4 is still not completely understood. It is understood that a number of pro-inflammatory genes are expressed as a result of the IL-4R/JAK1/STAT3/STAT6 cascade becoming gradually phosphorylated (Bhattacharjee et al., 2013). There is proof that variation in the active candidate gene IL4R is connected to hand, knee, and hip OA (Forster, Chapman, & Loughlin, 2004). According to Silvestri et al., all OA patients had considerably greater serum concentrations of the soluble interleukin-4 receptor (sIL-4R) than the healthy control group. The levels of IL-4 in the synovial fluid and synovial cells were likewise elevated (Schlaak, Pfers, Meyer Zum Büschenfelde, & Märker-Hermann, 1996; Wagner, Fritz, Einsele, Sell, & Saal, 1997). Noting that IL-4 has a discernible chondroprotective impact is important. It minimizes the variance in the production of proteoglycans that are seen in the course of OA, inhibits the secretion of MMPs metalloproteinases, and, as a result, has an inhibitory effect on the degradation of proteoglycans in the articular cartilage (Nishida et al., 2008; Yeh, Augustine, Lee, Riviere, & Sheldon, 1995). It is not unexpected that IL-4 reduces the production of inflammatory cytokines including IL-1, TNF-, and IL-6 given its chondroprotective action and impact on other cell lineages(Schuerwegh et al., 2003). The release of other inflammatory mediators including PGE-2, COX-2, PLA2, and iNOS is also reduced by IL-4(Wojdasiewicz et al., 2014).

6.7.Interleukin-10 +

IL-10 is a different cytokine with pleiotropic anti-inflammatory qualities. IL-10, which is primarily made by immune cells, is also made by chondrocytes, where it plays a part in the intricate process of cartilage extracellular matrix turnover. IL-10, which shares structural similarities with interferons, starts working by attaching to its receptor, the heterodimer IL-10R, which is made up of the IL-10R1 and IL-10R2 subunits. IL-10 is primarily produced by immune cells, but chondrocytes can also make it, and it plays a part in the intricate system of cartilage extracellular matrix turnover(Kokuludağ, 1999; Schulze-Tanzil et al., 2009).

IL-10 inhibits the synthesis of inflammatory enzymes such as iNOS and COX-2 in macrophages. IL-1Ra increases the expression of many anti-inflammatory proteins such as sTNFR1 and matrix metalloproteinase tissue inhibitör (S. R. Goldring & Goldring, 2004; Martel-Pelletier et al., 1999). It has been shown that IL-10 inhibits the expression of IL-1 and TNF-α in human chondocytes (Iannone et al., 2001). IL-10, a regulatory cytokine, works against the effects of catabolic cytokines and plays a role in the pathogenesis of OA. Like IL-4, it increases the production of IL-1Ra and reduces the level of inducible NO synthetase from chondrocytes (S. R. Goldring & Goldring, 2004; Martel-Pelletier et al., 1999).

In a significant study established by Barker et al., researchers have investigated whether serum interleukin IL-10 and tumor necrosis factor TNF-α concentrations and their ratio (IL-10/TNF-α) are related with the predisposition of knee osteartheritis development in patients with ligamentous injury and in patients with advanced knee osteoarthritis. Researchers have reported that in patients with severe knee osteoarthritis compared to mild (Kellgren-Lawrence grade 4 vs. 3, respectively), serum IL-10 and the serum IL-10/TNF-α ratio were considerably lower, whereas serum TNF-α was not significantly altered. Without changes in serum TNF-α, serum IL-10 was noticeably lower in the group anterior cruciate ligament surgery. Researchers have concluded that people predisposed to developing knee osteoarthritis after ligamentous stress and subjects with radiographic evidence of severe knee osteoarthritis have impaired serum IL-10 concentrations (Barker et al., 2021).

7. Conclusion

The imbalance of proinflammatory and antiinflammatory mediators that causes low-grade inflammation, which is responsible for cartilage degradation, bone remodeling, and synovial proliferation, is a major factor in the etiology of OA. The role of cytokines as biomarkers in osteoarthritis is important in diagnosing the disease, monitoring its progression, and evaluating response to treatment. Cytokines can be used as markers of inflammatory processes,

especially measured in intra-articular fluid and serum. They provide information about the severity and type of inflammation, which can be helpful in assessing the stage of the disease and potential treatment options.

It is clear that inflammatory and pro-inflammatory cytokines have a role in the pathogenesis of OA, but their involvement in clinical applications has not yet occurred. Although, there are promising studies in the studies about the involvement of cytokines in the pathogenesis of OA, we can say that inflammatory and pro-inflammatory cytokines are more useful in evaluating the treatment response in biological treatment methods such as the application of mesenchymal stem cells.

Again, it should be considered that the reduction of one cytokine may not be enough to stop the inflammation and the creation of matrix-degrading enzymes in OA since its effects are not always dependent on the activation of another.

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Chapter 22

BIOCERAMIC BASED ROOT CANAL SEALERS

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Success of Root canal treatment (RCT); elimination of microorganisms root canals (RC) together with the preparation made to ensure that the RCs depends on irrigation and a leak-proof filling (Seltzer, 1965).

Controlling the environment in the canal in terms of microorganisms is an important step for the success of canal treatment. For this reason, it is necessary to remove the smear layer, which provides an ideal area for bacteria. In addition, with the penetration of the canal paste into the dentinal tubules (DT). Bacteria are also neutralized by being trapped here (Nikhil, 2013).

Root canal sealer (RCS) is necessary to fill the space between the surfaces of the main cone filling material and the dentin walls. The paste also fills irregularities in the canal caused by lateral condensation, lateral and accessory canals, and gaps between the cones of the gutta percha. They also have a lubricating effect during the filling process (Kaur, 2015).

Although RCSs are used sparingly to fill the (RC), they have been shown to affect the outcome of RCT (Ørstavik, 2005).

According to Grossman, the qualities sought in a good RCS are as follows: (Grossman, 1982)

1. It must be adhesive when mixed and thus ensure good adhesion between itself and the canal wall when hardened.
2. Provide a hermetic seal in the RC.
3. Radiopaque so that it can be observed on radiographs.
4. It can be easily mixed with the liquid part.
5. It shouldn't show shrinkage during hardening.
6. It shouldn't cause discoloration of tooth tissues.
7. Bacteriostatic, or at least prevent bacterial growth.
8. Slow-hardening properties.
9. It shouldn't dissolve in tissue fluids.
10. It shouldn't have a harmful effect on periapical tissues.
11. Solvent materials so that it can be removed from the RC when necessary.

Some more properties were added to those reported by Grossman. These are: (Ingle et al., 2002)

12. It shouldn't cause an immune response in periapical tissues.
13. It shouldn't have carcinogenic or mutagenic effects.

- **Dentin Tubule Structure and Canal Paste Penetration**

Dentin is a living and dynamic tissue that changes throughout life. Dentin consists mainly of minerals, organic matrix and water. 70% by weight, 70% by volume 45% of the mineral content. The mineral structure is mainly composed of hydroxyapatite. Organic matrix constitutes 20% by weight and 33% by volume of dentin. Organic matrix content is collagen (Ayaz, 2011).

The diameters of dentin tubules are between 0.5 and 0.9 μm at the enamel dentin border. However, as they approach the pulp, the tubules merge and their diameters range from 2- 3 μm (Ayaz, 2011). DT diameters also vary from coronal to It also changes towards the apical region. DT diameter, number, exposure of dentin in physiological course pathophysiological changes and canal preparation caused by the remaining factors The smear layer formed during canalisation affects the canal paste penetration (Carrigan, 1984).

Penetration of the RCS into dentin tubules; the diameter of the paste particles is smaller than the diameter of the tubules. Dentin tubules with advancing age number decreases and tubule diameters shrink. Reduction in tubule diameters peritubular due to mineralisation of the dentin. Even as a result of this mineralisation tubules may be completely occluded (Carrigan, 1984). The number of DT from coronal to apical tubule diameters decrease. Therefore, canal paste penetration is less in the apical region (Mjör, 2001)

The aim of a successful RCT is to fill the RC with an inert, dimensionally stable and biologically compatible canal filling material up to the apical foramen in a sealed three-dimensional manner after the RC has been properly expanded and disinfected. The most commonly used filling material for RCs is gutta-percha/pat. However, the lack of a paste that can provide better sealing and hermetic occlusion today pushes researchers to search for new paste (Kaur, 2015). Bioceramics, which have entered our lives in recent years, have been introduced to the market with features such as osteoinductive effect, hardening in the presence of tissue fluids, long-term antibacterial effect after placement in the canal, impermeability, long working time, filling the RC without gaps by expanding while hardening and simplifying the single cone technique (Ørstavik, 2005). The clinician's knowledge of the properties of the RCS used will contribute positively to the treatment prognosis by selecting the paste according to the case.

Bioceramics have been used in many areas of medicine for a long time and recently It has also entered the field of use of endodontics. Damaged or impaired function of the organism for the repair, reconstruction or replacement of lost organs specially designed ceramics are called bioceramics (Pasinli, 2004).

Although bioceramic-based RC sealers (BRCS) are new in the field of

endodontics, they have many advantages. In this review, different properties of BRCS such as physical properties, biocompatibility, sealing, adhesion, solubility and antibacterial activity are mentioned and due to these properties, the use of BRCS has become quite widespread.

Bioceramics are becoming one of the alternative materials in clinical use due to the healing quality they provide not only as RCS but also as vital treatments such as amputation and direct quotation, apical plugs in teeth with incomplete apex and retrograde filling material after apical resection (Tyagi, 2013).

The main functions of RCSs are to plug cavities, accessory canals and multiple foramina, to form a bond between the core material and the RC wall, to act as a lubricant to facilitate the placement of the core material, and to leave no space for residual bacteria (Kaur, 2015).

Since RC sealants are of biological importance, their chemical and physical properties have attracted great interest since the early twentieth century (Orstavik, 2005). RC sealants are classified according to their main chemical components: zinc oxide eugenol, calcium hydroxide (CH), glass ionomer, silicone, resin and BRCSs. Although RC sealants have been analysed in many studies based on their composition, including zinc oxide eugenol (Markowitz, 1992), CH (Desai, 2009), glass ionomer (Buck, 2002) and resin-based sealants (Kim, 2002), there has been no comprehensive review of BRCS.

Bioceramics are specially produced ceramic materials used in dentistry. Bioceramics include alumina, zirconia, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates (Hench, 1991). The classification of bioceramics as bioactive materials is a function of their interaction with living tissue in the environment (Best, 2008). Bioactive materials such as glass and calcium phosphate interact with surrounding tissues to provide hard tissue formation (Koch, 2009). Bioinert materials such as zirconia and alumina are independent of the surrounding tissues without biological or physiological effects (Best, 2008). The use of bioceramics as RCS provides two important advantages. Firstly, they are not rejected by the surrounding tissues due to their biocompatibility (Koch, 2009). Secondly, bioceramics contain calcium phosphate, which is similar to the chemical composition of hydroxyapatite crystals found in tooth and bone structure. Calcium phosphate enhances the hardening properties of bioceramics and strengthens the bond between root dentin and RCS (Ginebra, 1997). However, the most important disadvantage of these materials is the difficulty in removing them from the canal when preparing the post cavity or when the RCT needs to be renewed.

The exact mechanism of binding of BRCS to root dentin is not known; however, the following mechanisms have been proposed for calcium silicate-based RCSs; Tubular diffusion; Diffusion of paste particles into DT to form

mechanically locked bonds (Zhang, 2007). Denaturation of collagen fibres with a strong alkaline paste to form a mineral infiltration zone by infiltration of the mineral content of the paste into the intertubular dentin (Atmeh, 2012). Formation of hydroxyapatite along the mineral infiltration zone as a result of partial reaction of phosphate with calcium silicate and CH(Zhang, 2009). While there are several brands of BRCSs on the market, many are still experimental and further studies are needed to determine their efficacy.

The biological and physical properties of BRCSs were investigated based on the ideal RC sealant properties defined by Grossman (Al-Haddad, 2016), It should be fluid when first mixed to ensure good adhesion with the canal wall when hardened. It should provide hermetic closure. It should be radiopaque to be visible on radiographs. The powder particles must be very fine so that they can be easily mixed with the liquid. It should not shrink during hardening. It should not discolour the tooth structure. It should be bacteriostatic or at least should not encourage bacterial growth. It should not harden too fast, working time should be long. It should not dissolve in tissue fluids. It should be well tolerated by the periapical tissue. If the root canal filling (RCF) needs to be removed, it should dissolve easily with known solvents.

Classification of Bioceramic Based RC Sealers

1. Calcium-silicate-phosphate

a) iRoot SP

b) Bioseal

c) EndoSequence BC Sealer

d) Smartpaste Bio

e) Appetite RCS

2. Mineral Trioxide Aggregate (MTA)

a) ProRootEndoSealer

b) MTA Obtura

c) Endo CPM Sealer

d) DiaRoot Bioaggreate

e) MTA Angelus

f) MTA-Fillapex

1. **Calcium-silicate-phosphate**

These pads usually contain zirconium oxide, calcium silicate, calcium phosphate, CH, hydroxyapatite filler, and hardening agents. Calcium silicate and hydroxyapatite give the material biocompatibility and bioactive

properties. The material is hydrophilic and absorbs moisture from the dentin tubules hardened by attraction. Thanks to its small particle structure and fluidity, it can penetrate into the lateral canal and DT. The increase in pH during hardening is bactericidal increases its specialty (Çalt, 2010).

a) iRoot SP (Verio Dental Co. Ltd. Vacour, Canada) This paste contains zirconium oxide, calcium silicate, calcium phosphate, CH, fillers and thickeners. The paste in ready-to-use syringes can be applied directly into the RC. Since it hardens with the moisture in the RC, there is no need to dry the canal. In addition to not showing shrinkage during hardening, the hardening time is four hours. In addition to its strong bond to dentine, its very low toxicity and antimicrobial properties give this paste an advantage (Tyagi, 2013).

b) Bioseal (Ogna, Milan, Italy) This paste, which has two components as powder and liquid, contains hydroxyapatite, barium sulphate, diiodothymol, natural resin, zinc oxide, CHand zinc acetate in the powder content and eugenol and oleoresin in the liquid part. Hydroxyapatite increases the biocompatibility of the paste and ensures that it does not change dimensionally (Tyagi, 2013). Working time; two forms, normal and extended, are available in the market.

c) EndoSequence BC Sealer (Brasseler, Sanannah, Geoergia, ABD) It contains zirconium oxide, calcium silicate, calcium phosphate monobasic, CH, fillers and thickeners. Since its main components are calcium silicate and hydroxyapatite, it is both biocompatible and bioactive. Due to the hydrophilic properties of the paste, hardening takes place by absorbing the water in the DT. Working and hardening time is four hours. The pH of 12.9 during the hardening process increases the bactericidal property of the paste. It is produced to be applied directly into the RC with the help of a cannula. It reaches the lateral canals and dentin tubules with its small particles and suitable fluidity (Koch, 2012).

d) Smartpaste Bio (Smart Seal DRFP Ltd, Stamford, England) It is a biocompatible paste that does not resorb, does not show dimensional change and has hydrophilic properties. It shows strong antibacterial properties because it hardens by absorbing water in the RC and CHand hydroxyapatite are released during hardening. It can be applied directly to the RC (Koch, 2012).

e) Appetite RCS (Dentsplay-Sankin Trading Co., Tokyo, Japan) is a biocompatible material containing hydroxyapaptite and tricalcium phosphate. It has three different types;

Type 1. The powder contains tricalcium phosphate, hydroxyapatite and the liquid contains water and polyacrylic acid. This type of paste is indicated for use in non-infected canals due to its lack of antibacterial properties.

Type 2. The powder contains iodoform (30%), tricalcium phosphate, hydroxyapatite; the liquid contains water and polyacrylic acid.

Type 3. The powder contains iodoform (5%), tricalcium phosphate, hydroxyapatite, bismuth subcarbonate, water and polyacrylic acid in the liquid (Tyagi, 2013).

2. Mineral Trioxide Aggregate (MTA)

The paste in this group is used as perforation repair material, retrograde filling material, as well as canal paste in clinics (Koch, 2012). MTA contains tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, calcium sulphate and tetracalcium aluminoferrite. It shows biological and histological properties similar to CH due to its pH of 12.5. It forms a very hard surface that is not resorbed. Due to its hydrophilicity, it hardens in humid environment and hardening time is at least three hours. Due to its excellent biocompatibility, low solubility and impermeability, it is widely used in repairing perforations in RCs and creating apical barrier. It is also used for filling RCs alone (Tyagi, 2013).

In recent years, MTA-RCF pastes have been developed by improving the positive properties of MTA and adding properties such as fluidity, curing time, and adhesion, which should be in RCSs, and have found a wide range of use in endodontics (Camilleri, 2009).

a) ProRoot EndoSealer (Dentsply/Tulsa Dental Specialties, Tulsa, OK, ABD) The powder of this material, which is in two forms as powder and liquid, contains tricalcium silicate, dicalcium silicate, calcium sulphate bismuth oxide and tricalcium aluminate, while the liquid contains water-soluble polymer. As a result of the reaction with water, CHis released. Thus, hydroxyapatite stimulation starts and creates minimum tissue irritation (Tyagi, 2013).

b) MTA Obtura (Angelus, Londrina, Brazil) was produced by mixing white MTA with a special viscous liquid. Due to its low viscosity, it is stated that it is difficult to fill the recesses and resorption areas in the RC system that cannot be reached by shaping (Bayram, 2012).

c) Endo CPM Sealer (EGEO S.R.L. Buenos Aires, Argentina) Although the basic content is the same as MTA, the pH is reduced from 12.5 to 10 after hardening with the added calcium carbonate. The decrease in pH is aimed to prevent necrosis in the surrounding tissues and to maintain alkaline phosphatase activity. It provides biocompatibility and mineralisation (Tyagi, 2013).

d) DiaRoot Bioaggreate (Diadent Group International, BC, Canada) It is a newly developed ceramic-containing repair material produced in Canada. It has been approved for clinical use by the FDA. Recommended

areas of use; repair of root perforations, direct hairdressing, apexification, repair of internal root resorption, retrograde RCF. Working time is 5 minutes. The general properties of Diaroot Bioaggregate are that it does not contain aluminium, is non-toxic, tooth-coloured, easy to apply, shows 20% expansion during hardening, is highly hydrophilic and chemically bonded to dentin (Bayram, 2012).

Properties of the Ideal RC Sealer

Biocompatibility

The materials used in RCT may be directly related to periapical tissues. Therefore, biocompatibility is an important parameter in the choice of materials to be used in RCT. The ideal RC sealer should be a product that is well tolerated by the periapical tissues, does not irritate the apical tissues and does not interfere with the repair process, and even has healing stimulating properties (Candeiro, 2012). Since the RCS comes into contact with vital tissues directly through the apical and lateral foramen of the root or indirectly through the surface restoration, biocompatibility of the RCS is a basic requirement (Orstavik, 2005).

Biocompatibility is defined as the ability of a material to obtain an advantageous host response at the site of application (Williams, 1986). In other words, a material is said to be biocompatible if the material in contact with the tissue does not trigger an adverse reaction such as toxicity, irritation, inflammation, allergy (Sun, 1997). Most studies assess biocompatibility through cytotoxicity, referring to the effect of the material on cell survival (Schmalz, 1994). The cytotoxicity of BRCS has been evaluated in vitro using mouse and human osteoblast cells (Salles, 2012) and human periodontal ligament cells (Bae, 2010).

Most BRCS were found to be biocompatible. This biocompatibility is due to the presence of calcium phosphate in the paste. Calcium phosphate is also the main inorganic component of hard tissues (teeth and bone). As a result, the literature indicates that many bioceramic pastes have the potential to promote bone regeneration when extruded through the apical foramen during RCF or repair of root perforations (Bae, 2010).

Recently, the production of BRCS has also been in this direction. The bioactive, porous, osteoblast differentiation, vascularisation (Willershausen, 2013) and osteoconductive properties of bioceramics provide superior advantages (Willershausen, 2013). Güven et al. (Güven, 2013) found that the cytotoxic effect of BRCS on human tooth germ cells was very low. Bioceramics are known to stimulate periodontal regeneration (Koch, 2012) and increase intercellular connection and cellular exchanges (Lin, 2013). It does not cause inflammatory reaction in case of periapical overflow (Chen, 2015). In the study

conducted by Chang et al. (Chang, 2014), it was determined that the BRCS has more osteogenic potential as well as generating less proinflammatory mediators than the calcium hydroxide-based paste. In another study, the cytotoxicity of iRoot SP and AH Plus was compared, and it was found that the cytotoxicity of AH plus was higher on the 3rd day, while the cytotoxicity decreased at the end of the 2nd week (Zhang, 2010). In the study by Loushine et al. (Loushine, 2011), it was stated that although AH Plus cytotoxicity decreased over time, the BRCS paste was non-toxic and more biocompatible. In the study by De-Deus et al. (De-Deus, 2012), iRoot BP Plus and MTA were found to be biocompatible, while the cytotoxic effect of both was not significant. In the light of these studies, bioceramics can be used as an alternative paste in RCF of teeth with open apex or perforation due to its biocompatibility, stimulation of cementoblastic and osteogenic activity.

Hardening time

The paste used in RCT should provide sufficient working time for the physician and be compatible with the surrounding tissues while hardening. Bioceramics harden by attracting water in DT due to their hydrophilic properties. It can also harden in the presence of fluids such as water, blood, dentin fluid and saliva. The ideal RCS should allow sufficient working time. However, a slow curing time can cause tissue irritation and most RCS produce a small amount of toxicity until fully cured.

According to the manufacturers of EndoSequence BC Sealer or iRoot SP, the hardening reaction is catalysed by the presence of moisture in the DT. While the normal curing time is four hours, this can be considerably longer, especially in patients with dry canals. Although the working time is ideal, the hardening time is approximately four hours (Chard, 2013). As a result of hardening, hydroxyapatite structure is formed4 and shows an expansion between 0.2-6% while hardening (Koch, 2012). In a study, it has been reported that the dimensional change rate of BRCS pads is at the level accepted by ISO 6876/2001. In addition, it was also stated that these paste showed alkaline pH (Zhou, 2013).

The amount of moisture present in the DT may be affected by the use of paper cones (Hosoya, 2000), smear layer or tubular sclerosis (Paque, 2006). Loushine et al. (Loushine, 2011) reported that EndoSequence BC Sealer requires at least 168 hours to fully cure under different humidity conditions, while Zhou et al. (Zhou, 2013) reported a time of 2.7 hours. The curing reaction of EndoSequence BC Sealer is a two-phase reaction. In Phase I, monobasic calcium phosphate reacts with CHin the presence of moisture to produce water and hydroxyapatite. In phase II, water derived from dentin moisture and also water produced by the phase I reaction contribute to the hydration of calcium silicate particles to trigger the calcium silicate hydrate

phase (Loushine, 2011).

The manufacturer of MTA-Fillapex claims that their product will harden in at least two hours and this hardening time has been confirmed in at least two studies (Vitti, 2013). However, shorter curing times (66 minutes) have been reported for MTA-Fillapex (Viapiana, 2014).

Fluency

The fluidity of the canal paste is an important property that enables the filling of hard-to-reach areas such as isthmuses, accessory canals, and irregularities formed on the canal walls after canal widening operations with canal paste (Candeiro, 2012). When the canal paste is not flowable, it is very difficult for the paste to penetrate into these recesses, while when it is too watery, there is a possibility of overflow (Zhou, 2013). According to ISO 6786/2001, the flow rate of a RCS should not be less than 20 mm. Factors affecting the flow rate of a RCS include particle size, temperature, mixing speed and time elapsed since mixing (Desai, 2009).

Flow rates of 23.1 mm and 26.96 mm for EndoSequence BC Sealer and 22 mm, 24.9 mm and 29.04 mm for MTAFillapex were reported variously by rheometer method (Viapiana, 2014). Zhou et al. (Zhou, 2013) reported that MTA Fillapex and Endosequence BC paste had acceptable viscosity and dimensional stability according to ISO standards. Candeiro et al. (Candeiro, 2012) found that the viscosity of BRCS was above the minimum viscosity required for the paste.

Removability

RCF provide a mechanical barrier for the isolation of necrotic tissue or bacteria responsible for the persistence of periapical inflammation or postoperative pain (Schirrmeister, 2006). Wilcox et al. (Wilcox, 1987) observed that most of the debris remaining after retreatment is paste. Therefore, complete removal of the paste is essential to obtain healthy periapical tissues. EndoSequence BC Sealer is difficult to remove from the RC using conventional methods such as heat, chloroform, rotary instruments and hand files. In contrast, Sankin Apatite RCS is said to be easily removed with or without the use of solvents (Erdemir, 2003).

Resolution

Solubility is the loss of mass of a material as it decomposes into its molecules in a liquid. The solubility of the RCS may cause the formation of gaps between the paste and dentin or between the paste and gutta-percha, leading to failure of RCT. (Zhou, 2013). RC sealants should not dissolve or show little or no solubility in the RC. In addition, in contact with periapical tissues, it should dissolve without causing a foreign body reaction (Calt, 2010).

Bioceramics remain chemically stable in the RC and dissolve in the apical overflow. It has been shown that BRCS remain dimensionally stable in the RC and dissolve when overflowed (Shinbori, 2015). In another study, it was reported that these paste were more soluble than resin and silicone based paste (Zhou, 2013).

Borges et al. (Borges, 2012) reported dissolution of calcium and carbon on the surfaces of BRCS when exposed to distilled water. In another study (Zhou, 2013), the solubility of BRCS paste was found to be higher than epoxy resin and silicone-based paste. de Siqueira Zuolo et al. (de Siqueira, 2016) stated that BRCS could not be completely removed from the canals and more time was needed for retreatment. In the light of these studies, bioceramics is an alternative paste for RCF because it does not resorb in the canal, but resorbs without toxicity when in contact with periapical tissues. However, the difficulty of removal in repeated treatments is an issue that should not be forgotten.

Discolouration of Tooth Structure

RCF paste should not cause tooth discolouration. Since the chromogenic effects of paste residues that cannot be removed from the pulp chamber increase, they cause tooth discolouration. Therefore, canal paste or filling material residues must be completely removed from the pulp chamber. MTA-Fillapex has been found to cause clinically imperceptible crown discolouration (Ioannidis, 2013).

Radiopacity

One of the characteristics of an ideal RCS is that it should have more radiopacity than dentin so that it can be easily distinguished from neighbouring anatomical structures on radiography and the quality of the RCF can be evaluated (Erinc, 2012).

The radiopacity value of bioceramics was found to be 3.8 mm Al (Candeiro, 2012). The radiopacity of bioceramics is provided by zirconium oxide, barium sulfate and calcium tungstate. Canderio et al. (Candeiro, 2012) found the radiopacity of EndoSequence BC Sealer to be at the accepted level according to ISO, but lower than AH Plus paste. Due to the sufficient radiopacity of bioceramics, it is accepted as an alternative paste in terms of providing advantage to the physician in the evaluation of the quality of the RCF.

Antimicrobial Properties

RC sealants with antibacterial activity on bacteria and intraradicular infections that cannot be eliminated during RCT or that subsequently infiltrate into the canal through microleakage increase the success rate of

RCTs. According to the literature, the main antimicrobial properties of BRCS lie in their alkalinity and release of calcium ions (Desai, 2009). In this way, repair is provided by mineralised tissue deposition (Okabe, 2006).

Two methods are commonly used to evaluate the antibacterial activity of BRCS: agar diffusion test (Tanomaru-Filho, 2007) and direct contact test (Zhang, 2009). It has also been reported that BRCS have antibacterial activity against E. faecalis and minimal cytotoxicity (Candeiro, 2012).

The main cause of most RCT failures is the coronal/apical leakage of microorganisms between the filled RCF material and the dentin wall. In addition, microorganisms may remain at a certain level in the canal system during RCF. Therefore, the antimicrobial properties of RC sealants contribute to the success of RCT (Haapasalo, 2007).

BRCS provide their biological activity with alkaline pH (pH>12), high Ca+2 ion release and hydroxyapatite formation (Borges, 2012). Candeiro et al. (Candeiro, 2016) reported that BRCS have antibacterial effects against E. faecalis and have very low cytotoxicity. In the light of these studies; it is an option that can be considered when antibacterial activity is expected from BRCS.

Adhesion

The adhesion of RCS is defined as the bonding capacity between the root dentin and the core material (Sousa-Neto, 2005). There is no standardised method used to measure RC sealant adhesion to dentin. Microleakage and bond strength tests are commonly used (Schwartz, 2006). The impermeability of a RC sealant depends firstly on its solubility level and secondly on its bond to dentin and core material (Desai, 2009). The adhesion of BRCS was found to be satisfactory and comparable to other RC sealants.

Although no correlation has been found between the sealing and bond strength of RC sealants (Wennberg, 1990), bond strength testing has received considerable attention due to the development of the -monoblock‖ concept, which improves the sealing and fracture resistance of RC treated teeth by bonding the RC sealant to both the core material and the dentin wall (Teixeira, 2004). BRCS have the ability to form a bond between dentin and core material (Koch, 2009).

The bondability of RCF materials to dentin walls is important both statically and dynamically. In the static state, the connection should eliminate gaps between the canal filling and the walls that may cause microleakage. In the case of dynamic connection, it is expected that the connection of the canal filling to the dentin will not deteriorate during the subsequent applications and will increase the structural function by forming a monobloc structure with the tooth (Calt, 2010). Topcuoglu et al. (Topcuoglu, 2013) reported that

the roots filled with BRCS were more resistant to fracture than those filled with mineral trioxide aggregate-based paste. Ghoneim et al. (Ghoneim, 2011) reported that the fracture resistance of endodontically treated teeth increased when the BRCS was used with gutta percha and Activ GP, but the use of Activ GP increased this resistance even more. In conclusion, this paste, which has the ability to bond to dentin, is an alternative paste for RCF in RCTs of teeth with excessive loss of material and/or in RCTs of teeth with large pulp cavities that have gangrenised at a young age.

CONCLUSION

BRCS are emerging as alternative sealants in RCT due to their biocompatibility, antibacterial properties, ability to harden in moist environment, ideal expansion percentage, impermeability and adhesion to dentin, and RCTs completed with these sealants are promising. However, the difficulty of removal from the RCs and the need for additional time for removal is an issue that should not be forgotten by the clinician. In addition, the differences in the results of the studies conducted reveal that these pastes do not meet all the desired requirements. Further *in-vivo* and *in-vitro* studies are needed to clarify the clinical outcomes associated with the use of BRCS.

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Chapter 23

THE RELATIONSHIP BETWEEN DIABETES MELLITUS AND FOURNIER'S GANGRENE: A COMPREHENSIVE REVIEW FROM PATHOPHYSIOLOGY TO CLINICAL FINDINGS

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Introduction

Fournier's Gangrene (FG) is a rare yet highly severe necrotizing fasciitis characterized by the necrosis of the perineal, genital, or perianal regions. This condition arises due to a synergistic polymicrobial infection (1). The etiology of Fournier's Gangrene is multifactorial, with gastrointestinal, genitourinary, and cutaneous sources accounting for the majority of cases (2). Several risk factors contribute to the development and progression of this condition, and one of the most significant is diabetes mellitus, which is present in approximately 32-66% of FG cases (3).

The focus of this study is to explore the intricate relationship between diabetes mellitus and Fournier's Gangrene, with a particular emphasis on assessing whether diabetes increases the risk of developing FG, and whether it influences important clinical outcomes such as mortality rates and duration of hospitalization. Furthermore, the research aims to examine how diabetes impacts specific laboratory markers and the Fournier's Gangrene Severity Index (FGSI), a prognostic tool utilized to predict disease severity and mortality risk among individuals diagnosed with Fournier's Gangrene.

A point of contention within the medical community is the potential association between Fournier's Gangrene and the use of sodium glucose co-transporter 2 (SGLT-2) inhibitors, which are oral antidiabetic medications commonly prescribed for the management of diabetes mellitus. This research seeks to delve into this controversial aspect, shedding light on whether there is indeed a causal link or if other factors are at play in cases where such inhibitors are involved. By comprehensively examining these facets, this study contributes to a better understanding of the intricate interplay between diabetes mellitus and Fournier's Gangrene, providing valuable insights for both clinical management and further research directions.

General Informatıon

Dıabetes Mellitus

Definition

Diabetes mellitus is a chronic disease characterized by insufficient secretion of insulin from the pancreas or inadequate effectiveness of the secreted insulin. Symptoms and signs of diabetes mellitus can include dry mouth, frequent urination, increased appetite, intense hunger, excessive thirst, weakness, dry skin, tingling sensations, among others. In the context of diabetes mellitus, genitourinary system infections such as vulvovaginitis and urinary tract infections can also occur (4).

Diabetes Mellitus Diagnostic Criteria

Diabetes diagnostic criteria were last updated in 2018, incorporating HbA1c into the criteria. Diabetes mellitus is defined by fasting blood glucose (FBG) ≥ 126 mg/dl, HbA1c ≥ 6.5%, a 2-hour postprandial blood glucose (PPBG) ≥ 200 mg/dl during a 75g oral glucose tolerance test (OGTT), and random blood sugar level exceeding 200 mg/dl in a symptomatic individual with hyperglycemia (5).

Etiological Classification of Diabetes Mellitus

Diabetes is primarily classified into four categories: type 1 diabetes, type 2 diabetes, gestational diabetes, and specific types of diabetes.

Etiological Classification of Diabetes Mellitus (4):

Type 1 DM

A. Immune-mediated

B. Idiopathic

2. Type 2 DM

Gestational diabetes (GDM)

Other specific types of diabetes

A. Genetic defects of beta-cell function (monogenic diabetes forms)

B. Genetic defects in insulin action

C. Diseases of the exocrine pancreas

D. Endocrinopathies

E. Drug or chemical-induced

F. Rare forms of immune-mediated diabetes

G. Diabetes associated with genetic syndromes

H. Infections

Epidemiology of Diabetes Mellitus

The prevalence of diabetes mellitus worldwide increased from 4.7% in adults in 1980 to 8.5% in 2014 (7). In the European Region, around 60 million people have diabetes, with a diagnosis present in approximately 10.3% of males and 9.6% of females aged 25 and older.

Globally, elevated blood glucose levels lead to approximately 3.4 million deaths annually. Nearly 80% of these deaths occur in low- and middle-income countries, and half of these deaths occur in individuals under 70 years of age. According to the WHO, it is projected that deaths attributed to diabetes will double by 2030 (8).

Pathogenesis of Diabetes Mellitus

Multiple factors contribute to the pathogenesis of type 2 diabetes (9). Type 2 diabetes is characterized by elevated blood sugar levels, insulin resistance, and impaired insulin secretion (beta-cell dysfunction). The disease's prevalence increases with rising obesity rates (10). Insulin resistance, often associated with overeating and sedentary lifestyle, leads to decreased insulin sensitivity and secretion, resulting in type 2 diabetes (11). While genetics and aging play a role, inadequate insulin secretion is predominantly influenced by genetic factors (12,13). Type 2 diabetes is often accompanied by cardiovascular risk factors like hypertension, dyslipidemia (elevated triglycerides, high LDL, and low HDL), and central obesity. These clinical conditions occurring together are referred to as metabolic syndrome (14).

Genetic susceptibility is significant in type 2 diabetes. For instance, type 2 diabetes prevalence varies among ethnic groups within the same environment (15). In the United States, African Americans, Native Americans, Pima Indians, and Hispanic Americans have a two- to six-fold higher prevalence of type 2 diabetes compared to White Americans (16,17). Approximately 39% of individuals with type 2 diabetes have at least one parent with the condition (18). The lifetime risk of type 2 diabetes for a first-degree relative of a patient with type 2 diabetes is 5 to 10 times higher compared to individuals without a family history of diabetes and with similar age and weight (15). Although genetic risk is significant, environmental factors still play a crucial role in diabetes development in these groups. For instance, diabetes prevalence among Pima Indians in Mexico is 6.9%, whereas it's 38% among Pima Indians in the United States (19).

Medication-related hyperglycemia can develop. Numerous drugs can impair glucose tolerance by reducing insulin secretion, increasing hepatic glucose production, or creating insulin resistance. Examples include glucocorticoids, oral contraceptives, thiazide diuretics, statins, beta-blockers, protease inhibitors, tacrolimus, and sirolimus (20).

Complications of Diabetes Mellitus

Diabetic ketoacidosis (DKA), hyperosmolar hyperglycemic state (HHS), lactic acidosis (LA), and hypoglycemia are acute complications of diabetes. Chronic complications of diabetes can be divided into macrovascular and microvascular, with microvascular complications including retinopathy, nephropathy, and neuropathy.

Diabetes Mellitus and Infections

Diabetes is a significant risk factor for all types of infections. It has been shown to increase the rates of both hospitalizations and outpatient infections. Respiratory tract, gastrointestinal system, skin and soft tissue infections,

and genitourinary infections are more commonly observed in individuals with diabetes. Furthermore, infections in diabetic patients tend to respond more poorly to treatment and progress to serious infections more rapidly (21). One study found that diabetes is responsible for 6% of infection-related hospitalizations and 12% of infection-related deaths. Conditions such as bone and joint infections, cellulitis, and sepsis are more prevalent (22).

Diabetes involves various dysfunctions within the immune system. Cytokine signaling is affected in both innate and acquired immunity. Diabetes suppresses several cytokines. Diabetic neuropathy increases susceptibility to lesions in the first line of defense, the skin barrier. Additionally, poor vascular flow in infection areas leads to inadequate immune responses, making individuals more prone to secondary infections and complicating the healing process (23).

The complement system plays a role in opsonization, degradation, and phagocytosis of pathogens in both innate and acquired immunity, as well as in the recruitment of immune cells to the infection site. Several studies have shown that hyperglycemia reduces binding of immunoglobulins to complement and inhibits phagocytosis. Moreover, diabetes decreases the chemotaxis of polymorphonuclear leukocytes, reducing their bactericidal capabilities, such as hydrogen peroxide production (24).

Treatment of Diabetes Mellitus

Diabetes treatment should be patient-centered, with glycemic targets tailored to individual patients (28). Lifestyle modifications, including diet, weight loss, smoking cessation, and physical activity, play a crucial role in diabetes management (29). Pharmacological treatments are also used in addition to these recommendations.

In pharmacological treatments, both insulin and oral antidiabetic medications are used. Insulin-independent antihyperglycemic drugs (oral antidiabetics and insulin mimetics) include biguanides, thiazolidinediones, insulin secretagogues, insulin mimetics, alpha-glucosidase inhibitors, and sodium glucose co-transporter 2 (SGLT-2) inhibitors (6).

SGLT-2 Inhibitors (Glucuretics; SGLT-2 Inhibitors): These drugs inhibit SGLT2 in the proximal tubule of the kidneys, reducing glucose reabsorption and increasing glucose excretion through urine (6).

This group includes canagliflozin, dapagliflozin, and empagliflozin. In Turkey, all SGLT-2 inhibitors except canagliflozin are used. SGLT2 inhibitors block the reabsorption of filtered glucose as plasma levels decrease, resulting in reduced glucose levels. They do not cause hypoglycemia due to this effect. They also mildly lower blood pressure (30). Empagliflozin and canagliflozin have been shown to reduce atherosclerotic cardiovascular morbidity and

mortality in individuals with type 2 diabetes and evident cardiovascular diseases (31,32). In primary analysis, dapagliflozin did not demonstrate a reduction in atherosclerotic cardiovascular morbidity or mortality. However, a subanalysis of the primary study revealed favorable cardiovascular outcomes (33). These drugs have been shown to reduce hospitalizations due to heart failure and slow the progression of kidney disease (34). Compared to GLP-1 receptor agonists, SGLT-2 inhibitors are more effective in preventing the progression of kidney disease and hospitalizations due to heart failure (35).

Side effects of SGLT-2 inhibitors include polyuria, fluid loss, hypotension, and genitourinary infections (36). Patients should be monitored for signs and symptoms of Fournier's Gangrene, as cases of this condition have been reported in individuals treated with SGLT-2 inhibitors. Between March 1, 2013, and January 31, 2019, the FDA identified 55 cases of Fournier's Gangrene in patients receiving SGLT2 inhibitors (37).

Contraindications: Patients undergoing dialysis should not be treated with these SGLT-2 inhibitors. Extreme hypersensitivity reactions such as anaphylaxis and angioedema are also contraindications for SGLT-2 inhibitors (36).

Fournier Gangrene

Definition

Fournier's gangrene is a necrotizing fasciitis caused by polymicrobial infection that affects the scrotum, perineum, and perianal regions, sometimes extending to the abdomen and chest(1). The disease is named after Jean Alfred Fournier, a Parisian dermatologist and venereologist, who in 1883 described it as idiopathic fulminant gangrene occurring in the scrotum and penis in five patients(38).

Epidemiology

Up to this point, two significant studies on Fournier's gangrene have been conducted. In 2000, Eke studied 1726 patients, while Sorensen and colleagues examined 1680 patients in 2009. In Eke's research between 1950 and 1999, the mortality rate of Fournier's gangrene was 16%. Furthermore, the incidence of Fournier's gangrene was found to be 10 times higher in males compared to females(2). In the United States, between 2001 and 2004, the diagnosis rate of Fournier's gangrene was 0.02% among hospital admissions, with a mortality rate of 7.5%. According to Sorensen and colleagues, the annual incidence of Fournier's gangrene was 1.6 per 1000 males, and this rate increased to 3.3 per 1000 males over the age of 50. The frequency of Fournier's gangrene increases with age and diabetes mellitus. The mortality rate is higher in females compared to males (12.8% vs. 7.5%)(39).

Etiology

The most common causes of Fournier's gangrene are gastrointestinal system (30-50%), genitourinary system (20-40%), and cutaneous factors (20%) (2). Idiopathic causes account for less than 25% of cases in modern times(26). Colorectal causes include anal fissures, perianal and perirectal abscesses, diverticulitis, rectal carcinoma, and colon perforation(40). Genitourinary causes encompass vasectomy, prostate biopsy, urethral stones, neurogenic bladder, acute epididymitis, malignancies, and urinary tract infections(41,42). Cutaneous factors include pressure sores, furuncles, genital piercings, and superficial skin infections(43,44).

Risk Factors

Diabetes mellitus, obesity, chronic alcoholism, smoking, renal failure, liver failure, malignancy, and HIV infection predispose individuals to Fournier's gangrene(45). It is estimated that 36-56% of patients with Fournier's gangrene have diabetes mellitus(39,45,46). In a study in Germany, 51.5% of Fournier's gangrene patients had a body mass index above 25, and 39.4% had a BMI above 30. Alcoholism is present in 25-50% of cases(45). Liver cirrhosis has been identified as a significant mortality factor in patients with Fournier's gangrene(47). Low socioeconomic status is also a predisposing factor(39,48).

Clinical Presentation

The clinical presentation of Fournier's gangrene can vary based on the stage of infection, patient comorbidities, and overall health. It may resemble milder infections like cellulitis or erysipelas, characterized by tenderness, erythema, swelling, cellulitis, and erysipelas. However, disproportionate pain upon clinical examination should raise suspicion of Fournier's gangrene(49). Cellulitis and erysipelas show limited erythema or inflammation, while necrotizing fasciitis presents with poorly demarcated erythema and inflammation. Cellulitis and erysipelas manifest with general infection symptoms such as fever and malaise, while necrotizing fasciitis can lead to systemic toxicity like multi-organ failure. Blisters and bullae in the later stages are associated with necrotizing fasciitis. Although necrotizing fasciitis typically presents as an acute event, it can also have a subacute onset. During the subacute phase, symptoms like fever and fatigue may appear. As bacteria spread to surrounding structures, the infection can transition into an acute phase. Localized numbness may occur if nerves are damaged before the infection spreads. When deeper fascial layers are affected, thrombosis, ischemia, and necrosis of blood vessels can occur. In the study, the most common symptoms at presentation were swelling (81%), pain (79%), and erythema (71%). Later-stage symptoms such as bullae (26%), skin necrosis (24%), and crepitus (20%) were less common(49).

Laboratory Tests

There is no single laboratory test to differentiate necrotizing fasciitis from other soft tissue infections. However, there are methods developed to assess infection risk, mortality, and morbidity. The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) scoring system is used for this purpose. It involves evaluating age, gender, hemoglobin, platelets, leukocyte count, serum potassium, serum sodium, creatinine, and CRP. It is scored on a scale of 0 to 13. In Chin-Ho Wong's study, patients were categorized into three groups: low risk (≤5), moderate risk (6-7), and high risk (≥8). It was determined that the risk of developing Fournier's gangrene was less than 50% in the low-risk group, 50-75% in the moderate-risk group, and over 75% in the high-risk group(50).

Diabetes Mellitus and Fournier's Gangrene

In Fournier's Gangrene, a common feature of all risk factors is the impairment of cellular immunity, leading to compromised immune resistance (2). Diabetics commonly harbor a variety of bacteria on their skin, increasing the risk of skin infections. In diabetes, immune functions like chemotaxis and phagocytosis are impaired, resulting in increased bacterial spread. Diabetic angiopathy disrupts blood circulation in the affected area, facilitating anaerobic infections (25). Impaired blood flow in small vessels causes tissue ischemia. Diabetic neuropathy increases the risk of urinary tract infections in individuals with urethral obstructions, providing a conducive environment for Fournier's Gangrene (26). Diabetic neuropathy delays the clinical presentation of Fournier's Gangrene (27).

Conclusions

Fournier's gangrene, a severe necrotizing soft tissue infection affecting the genital and perineal regions, poses a significant clinical challenge. Over the years, extensive research has shed light on various aspects of the disease, particularly its strong association with diabetes mellitus. The intricate interplay between diabetes and Fournier's gangrene has garnered substantial attention in the medical literature.

- Diabetes Mellitus as a Predisposing Factor: Extensive evidence underscores the compelling connection between diabetes mellitus and Fournier's gangrene. Patients with diabetes exhibit a significantly increased risk of developing this aggressive infection compared to non-diabetic individuals. The underlying mechanisms are multifaceted and primarily involve the compromised immune response, vascular alterations, and impaired tissue healing seen in diabetes. Hyperglycemia, a hallmark of diabetes, creates a favorable environment for microbial growth and invasion, further exacerbating the risk of necrotizing infections. Moreover, diabetes-

related neuropathy can mask the early signs of infection, leading to delayed diagnosis and more advanced disease stages upon presentation.

- Clinical Implications and Management Challenges: The presence of diabetes in Fournier's gangrene patients has profound clinical implications. Diabetic individuals are not only more susceptible to the disease but also tend to experience more severe manifestations and a higher mortality rate. The complex interplay between diabetes and Fournier's gangrene necessitates a comprehensive approach to management. Timely and aggressive surgical debridement, broad-spectrum antibiotics, and meticulous wound care are cornerstones of treatment. However, managing hyperglycemia in diabetic patients presents an additional layer of complexity. Striking a balance between glycemic control and the metabolic demands of a critical infection is a challenging task.

- Preventive Strategies and Patient Education: Given the substantial contribution of diabetes to Fournier's gangrene, preventive strategies gain paramount importance. Effective glycemic control emerges as a crucial preventive measure to mitigate the risk of infection in diabetic individuals. Healthcare providers play a pivotal role in educating patients about the heightened susceptibility and the importance of early intervention. Regular self-monitoring of blood glucose levels, meticulous hygiene, and prompt reporting of any signs of infection are essential components of patient education.

- Multidisciplinary Collaboration: The intricate relationship between diabetes and Fournier's gangrene underscores the significance of a multidisciplinary approach. Collaboration among endocrinologists, infectious disease specialists, surgeons, and wound care experts is pivotal to ensuring comprehensive patient management. A tailored treatment strategy that addresses both the acute infection and the underlying diabetic condition is essential for favorable outcomes.

In conclusion, the association between diabetes mellitus and Fournier's gangrene is well-established in the literature. Diabetes acts as a potent predisposing factor, magnifying the risk, severity, and challenges associated with this devastating soft tissue infection. Recognizing this intricate relationship, along with implementing effective preventive measures and a multidisciplinary treatment approach, is paramount for improving patient outcomes and minimizing the impact of Fournier's gangrene in individuals with diabetes.

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Chapter 24

PLEXUS LUMBALIS AND IT S BRANCHES

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Introduction

Plexus; It is a word used to mean knitting and network. Plexus lumbosacralis means wide nerve spread in the lumbar and rump region. This neural network consists of the fusion of the plexus lumbalis and the plexus sacralis (Arifoğlu, 2016).

Plexus lumbosacralis carries the signals coming from both the lumbar and sacral parts of the body to the brain and initiates the voluntary movements coming from the brain. The medulla spinalis, which is the origin of the fibers belonging to the parasympathetic system that goes to some regions, especially the sympathetic system, is located in the columna vertebralis (Tunç, 2003). Although there are 33 segments in the spinal cord, there are 31 pairs of spinal nerves that separate from these segments. It has 33 segments, eight cervical, twelve thoracic, five lumbar, five sacral and one coccygeal pair. Spinal nerve fibers emerging from the plexus lumbosacralis innervate the lower extremities and organs of the body. It ensures the realization of various processes such as movement and excretion by transmitting the messages it receives from the brain. Even the slightest damage to the spinal nerves can lead to fatal consequences (Tunç, 2003).

Lumbar plexus

It is the structure formed in the inner lower part of the musculus (m.) psoas major on the outer side of the lumbar vertebrae, mainly with the participation of the branches from the thoracic 12 to the anterior roots of the lumbar 1-2 spinal nerves. After the fibers from the thoracic 12 combine with the lumbar 1, the nerve structure formed is divided into its upper and branches (Tunç, 2003).

It is the nerve network formed by the anterior branches of all the first three lumbar spinal nerves, a large part of the fourth lumbar spinal nerve and a small part of the twelfth thoracic spinal nerve in the posterior abdominal wall, anterior to the processus transversus, behind or inside the musculus psoas major. Although rami communicanstes grisei from truncus sympaticus joins all lumbar spinal nerves, it only joins rami communicantes albi truncus sympaticus from the first two lumbar spinal nerves (Tunç 2003, Alfrei et al.2006).

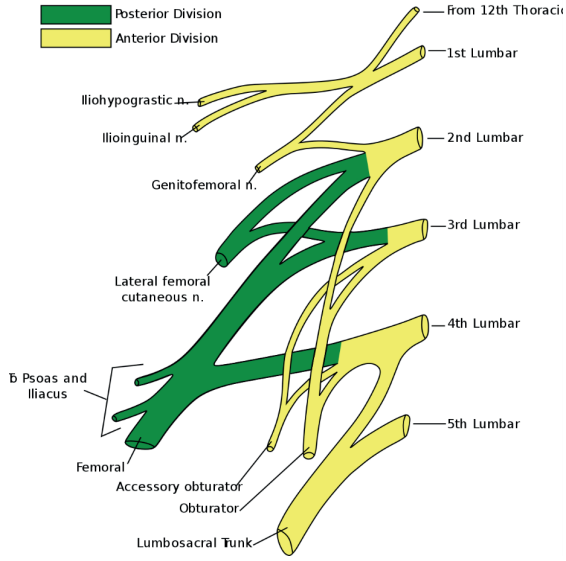


Figure 1: Plexus lumbalis and its branches (Drenckhahn and Waschke 2008)

They arise from the lateral and medial edges of the musculus psoas major and from the anterior surface of the muscle. These branches are;

- Rami musculares
- Nervus iliohypogastricus (nervus iliopubicus)(L1)
- Nervus ilioinguinalis (L1)
- Nervus genitofemoralis (L1 – L2)
- Nervus cutaneus femoris lateralis (L2 – L3)
- Nervus obturatorius (L2 – L3 – L4)
- Nervus obturatorius accessorius (L3 – L4)
- Nervus femoralis (L2 – L3 -L4)

1) Rami musculares: It is the short branch that separates from the plexus lumbalis. It provides innervation of musculus quadratus lumborum, musculus psoas major, musculus psoas minor and musculus iliacus (Alfrei et al. 2006).

2) Nervus iliohypogastricus (nervus iliopubicus- L1): It appears on the posterior abdominal wall in the upper part of the outer edge of the musculus psoas major and extends from the back of the kidney to the crista iliaca by crossing the musculus quadratus lumborum. It innervates musculus obliquus internus abdominis, musculus obliquus externus abdominis, musculus transversus abdominis (Alfrei et al. 2006).

It divides into two branches between the musculus transversus abdominis and the musculus obliquus internus abdominis. With these two branches, it innervates the skin in the lower part of the anterior abdominal wall (Alfrei et al. 2006). It has two branches. These;

a) Ramus cutaneus lateralis: It comes out by piercing the musculus obliquus internus abdominis and musculus obliquus externus abdominis at the level of the crista iliaca. It provides sensitive innervation of the posterior outer side of the gluteal region (Alfrei et al.2006).

b) Ramus cutaneus anterior: It is located between the musculus obliquus internus abdominis and the musculus transversus abdominis. It provides the motor innervation of these muscles. It pierces the aponeurosis of the musculus obliquus externus abdominis 3 cm above the inguinalis superficialis and comes out under the skin. It is distributed over the skin covering the pubis. Nervus iliohypogastricus has connections with nervus subcostalis and nervus ilioinguinalis (Alfrei et al.2006).

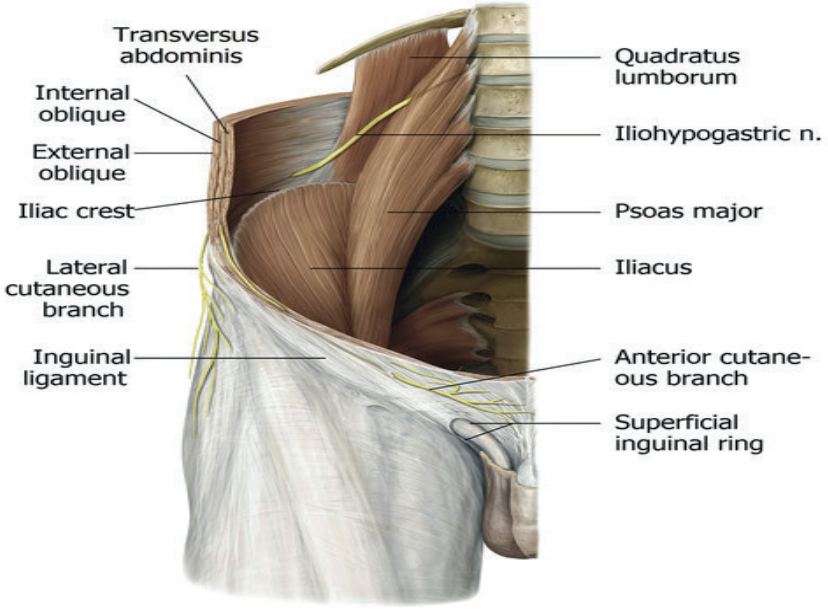


Figure 2: Branches of nervus iliohypogastricus (Heisel, 2007)

3) Nervus ilioinguinalis (L1): It is the nerve that runs just below the nervus iliohypogastricus, thinner than it. It emerges from the outer edge of the musculus psoas major and reaches the crista iliaca. It first pierces the musculus transversus abdominis. It provides motor innervation to the musculus transversus abdominis and musculus obliquus internus abdominis with the branches it gives along its course.

The nerve, which runs together with the funiculus spermaticus, passes through the anulus inguinalis superficialis and provides sensitive innervation of the penis root and the nervi scrotales anteriores of the front of the scrotum in men, and of the pubis and labium majus (nervi labiales anteriores) in women (Arıncı, 2014).

4) Nervus genitofemoralis (L1 – L2):

The nerve running within the musculus psoas major pierces this muscle from the front and extends downwards on the posterior abdominal wall, covered by the peritoneum parietale. It crosses the ureter from behind (Tunç, 2003).

It is the nerve that makes both the afferent and efferent pathways of the cremaster reflex. It is divided into branches as ramus genitalis and ramus femoralis, generally in front of, and sometimes inside, the musculus psoas major (Arıncı, 2014).

a) Ramus genitalis:

It crosses the arteria iliaca externa and vena iliaca externa, passes through the annulus inguinalis profundus and enters the canalis inguinalis. In men, it provides innervation to a part of the skin covering the scrotum by giving off the motor branches of the musculus cremaster (Tunç, 2003).

In women, the ligamentum passes through the canalis inguinalis together with the teres uteri and receives sensation from the skin covering the mons pubis and labium majus pudendi. It stimulates musculus dartos and musculus cremaster (Arıncı, 2014).

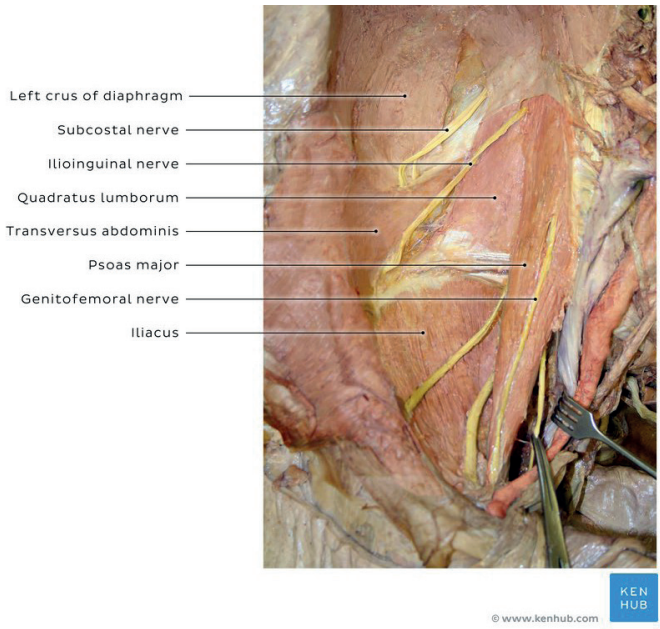


Figure 3: The branches of lumbar pleksus[5]

b) Ramus femoralis:

It descends above the musculus psoas major, on the outer side of the ramus genitalis. It passes through the lacuna vasorum together with the arteria iliaca externa. It is located within the femoral sheath in the lacuna vasorum and on the front outer side of the arteria femoralis (Arıncı, 2014).

The superficial nerve, which pierces the fascia lata slightly below the ligamentum inguinale, provides sensitive innervation to the skin of the upper part of the trigonum femorale. Variation: In some cases, the nervus iliohypogastricus and nervus ilioinguinalis may arise as a single root. Although it is quite rare, the iliohypogastricus nerve may be absent. In this case, nerve branches extend from the ilioinguinalis nerve to the region (Büyükmumcu, 2017).

5) Nervus cutaneus femoris lateralis (L2 – L3):

It originates from the outer edge of the musculus psoas major and crosses the musculus iliacus and gives branches to the peritoneum parietale in the fossa iliaca. The nerve lying on the inner side of the spina iliaca anterior superior on the front side of the musculus iliacus passes deep into the ligamentum inguinale. It gives two branches, anterior and posterior, under the skin on the front of the thigh. The anterior branch provides the sensitive innervation of the skin covering the front and outer part of the thigh up to the knee, and the posterior branch provides sensitive innervation of the skin covering the outer and back part of the upper part of the thigh.

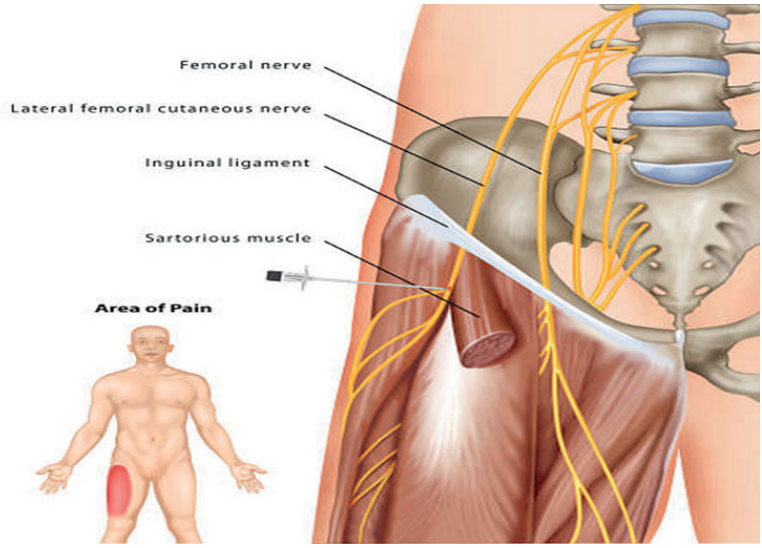


Figure 4: Distribution of the femoral nerve and sacral nerve[17]

6) Nervus obturatorius (L2– L3 – L4):

It emerges from the inner side of the musculus psoas major, at the level of the articulatio sacroiliaca at the entrance of the pelvis. It innervates the anterior part of musculus adductor longus, musculus adductor brevis, musculus gracilis, and musculus adductor magnus. It progresses forward and downward along the outer wall of the pelvis minor and enters the foramen obturatorium. Immediately after passing from the canalis obturatorium to the thigh, it divides into anterior and posterior branches (Büyükmumcu, 2017).

a) Ramus anterior:

It extends downward, passing between these muscles, in front of the musculus obturatorius externus and musculus adductor brevis, and behind the musculus pectineus and musculus adductor longus. It gives motor branches to the musculus gracilis, musculus adductor brevis, musculus adductor longus and sometimes musculus pectineus. The nerve that also provides the sensitive innervation of the articulatio coxae, and the branches of the nervus femoralis that combine with the nervus cutaneus femoris anterior (ramus cutaneus) form the plexus subsartorialis and provide the sensitive innervation of the inner surface of the thigh (Büyükmumcu, 2017).

b) Ramus posterior:

It pierces the musculus obturatorius externus and passes between these muscles, behind the musculus adductor brevis and in front of the musculus adductor longus. It enters the canalis adductorius and follows the arteria femoralis and arteria poplitea before distributing in the articulatio genus. It provides sensitive innervation of the articulatio genus. It gives somatomotor branches to the musculus obturatorius externus, the external part of the musculus adductor magnus, and sometimes to the musculus adductor brevis (Büyükmumcu, 2017).

Injury to only the obturator nerve is extremely rare. Injury to the nerve in the retroperitoneal region occurs through the femoral nerve. Adduction of the thigh becomes difficult (Anolague and Huijbergts, 2009).

7) Nervus obturatorius accesorius (L3-L4):

It descends from the inner edge of the musculus psoas major and passes over the ramus ososis pubis before entering the foramen obturatorum and is inserted deep into the musculus pectineus. It gives somatomotor branches to the musculus pectineus. The nerve giving articulatio coxae sensory fibers connects with the ramus anterior of the nervus obturatorius (Atılhan et al. 2000).

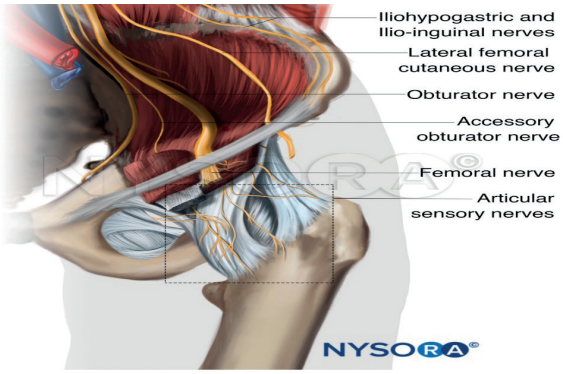


Figure 5: Lumbar nerves and their branches[17]

8) Nervus femoralis (L2 – L3 –L4)

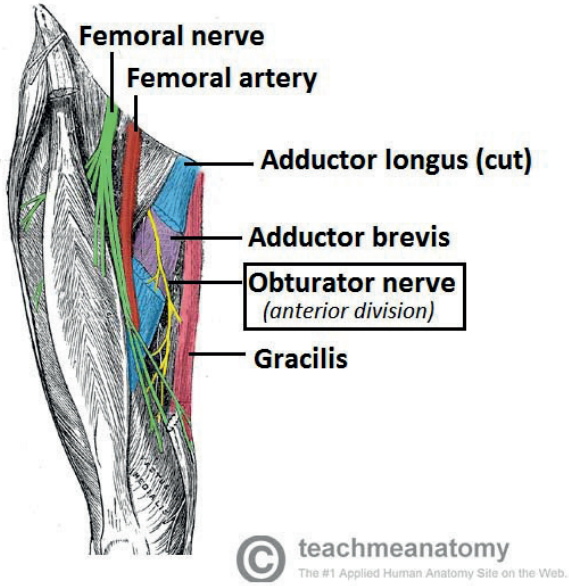
It is the thickest branch of Plexus lumbalis. It progresses down to the outer side in the groove between the musculus psoas major and the musculus iliacus. In this section, the nerve located under the fascia iliaca gives motor branches to the musculus iliacus and musculus pectineus while in the abdominal cavity. It innervates musculus iliacus, musculus pectineus, musculus sartorius, and musculus quadriceps femoris. After passing deep into the ligamentum inguinale, it divides into its branches. It divides into branches within the trigonum femorale, 5 cm below the ligamentum inguinale. Arteria circumflexa femoris lateralis divides these branches into ramus superficialis and ramus profundus (Atilhan et al. 2000).

Ramus superficialis:

It consists of two muscular branches, the ramus cutaneus medialis and intermedius, which innervate the skin of the medial and anterior part of the thigh, and the musculus sartorius and musculus pectineus (Atılhan et al. 2000).

Ramus profundus:

It has four muscular branches and one articular branch that feed the parts of the quadriceps femoris muscle. It then continues as the nervus saphaneus.



Resim 6: The anatomy of femoral nerve[21]

This nerve, which is a cutaneal branch, continues below the knee joint. It pierces the fascia forming the canalis adductorius and extends down the leg, courses together with the vena saphena magna, and innervates the medial skin of the leg and foot (Atılhan et al. 2000).

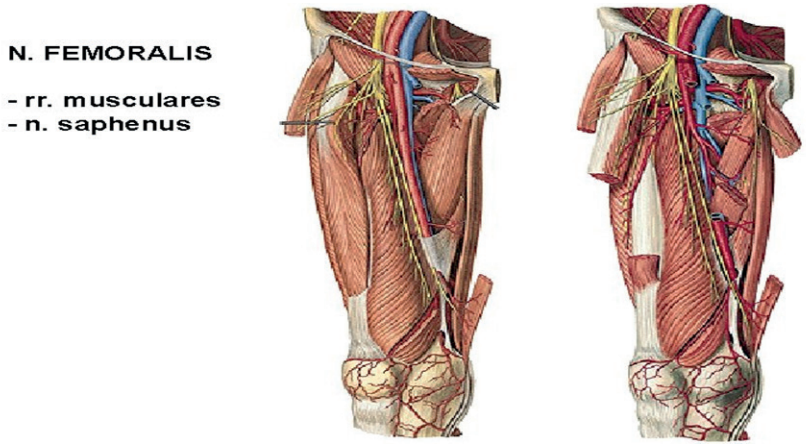


Figure 7: location of femoral artery and nerve[18]

3. Posterior Branch:

The only skin branch of this section, which is mostly formed by the somatomotor branches that it gives to the musculus quadriceps femoris, is the saphenus nerve. It gives muscle branches to the musculus rectus femoris. It also provides sensitive innervation of the articulatio coxae (Alsever, 1996).

Nervus saphenus:

It is the thickest and longest branch of the nerve femoralis. The nerve entering the canalis adductorius crosses the arteria femoralis from the front to the outer side and passes to the inner side of the artery. The nerve, which leaves the canalis adductorius by piercing the lamina vasto adductorius, pierces the fascia lata between the tendons of musculus sartorius and musculus garcilis on the inner side of the knee and emerges under the skin. Then, it extends into the leg together with the vena saphena magna, called the rami cutanei cruris medialis. It divides into two terminal branches in 1/3 of the leg. One of its terminal branches follows the medial edge of the tibia and ends at the ankle, while the other terminal branch extends from the front of the malleolus medialis to the big toe and the medial half of the foot. It carries the sensitive sensation of the skin on the medial surface of the leg (Alsever, 1996).

a) Ramus infrapatellaris:

Immediately after exiting the canalis adductorius, it separates from the nervus saphenus. It becomes superficial by piercing the musculus sartorius and fascia lata. Nervus cutaneus femoris lateralis, rami cutanei anteriores (nervus femoralis) and other branches of the nervus saphenus form the plexus patellaris. It provides sensory innervation to the skin covering the front of the patella (Bardeen, 1901).

b) Rami cutanei cruris medialis:

It is the part of the nerve saphenus after it gives the ramus infrapatellaris branch. The nerve running together with the vena saphena magna provides sensory innervation of the front and inner side of the leg. It divides into two terminal branches in the lower 1/3 of the leg. While one of the branches extends along the inner edge of the tibia and ends at the heel, the other one passes from the front of the malleolus medialis and distributes to the skin area up to the articulatio metatarsophalangea of the big toe. It gives branches to the hip and knee joints (Bergman, 2009).

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Chapter 25

GUT MICROBIOTA IN CARDIOVASCULAR HEALTH AND DISEASES

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CARDIOVASCULAR DISEASES

Cardiovascular diseases (CVDs) are the leading causes of death in the world (Zhou et al. 2018). CVDs are a group of diseases characterized by cardiovascular disorders that affect the flow of blood to the heart, brain, and peripheral parts of the body. CVDs include coronary heart disease, congenital heart diseases cerebrovascular disease and rheumatic heart diseases (Hijová 2023).

CVD is a lifelong disease characterized by the development of subclinical atherosclerosis (Santhakumar, Battino, and Alvarez-Suarez 2018). Inflammation plays an extremely important role in the onset and development of atherosclerosis (Henein et al. 2022). Atherosclerotic cardiovascular diseases are among the chronic inflammatory diseases (Henein et al. 2022; Irwandi et al. 2022).

Traditional risk factors for cardiovascular diseases include dyslipidaemia, hypertension, family history, diabetes, obesity, and lifestyle indicators such as physical inactivity, smoking and alcohol consumption, and inappropriate diet (Mehta et al. 2023).

Studies have shown that a balanced diet plays an important role in preventing CVD risk (Llorente-Cortés et al. 2010; De Lorgeril et al. 1999; Widmer et al. 2015). Diet is one of the most important factors of a healthy life and plays an important role in the development of chronic diseases such as obesity, diabetes and cardiovascular diseases. In addition, gut microbiota is directly related to the foods consumed and plays an important role in these diseases (Asadi et al. 2022; Gomes, Hoffmann, and Mota 2018)

GUT MICROBIOTA AND METABOLITES

The term microbiome refers to the diverse communities of microorganisms that live inside and on the skin of humans and animals. The microbiota is a complex ecosystem that includes commensal, symbiotic and even pathogenic organisms (El-Sayed, Aleya, and Kamel n.d.). Microbiomes are located in the oral cavity, oesophagus, digestive tract, respiratory tract and lungs, skin and vagina. Mainly bacteria, archaea, viruses, phage and fungi constitute the human microbiota (Manos 2022). The external environment, nutrition and lifestyle are factors that affect the composition and vitality of the microbiome. Studies show that the microbiome has significant effects on health and disease. Cancer, cardiovascular diseases, obesity, type 2 diabetes and even psychiatric diseases are affected by the gut microbiota (El-Sayed et al. n.d.).

Trillions of microorganisms colonize the intestines to create a physiologically healthy intestinal environment. This community is called the gut microbiota (Wang et al. 2022).

The human intestinal microbiota contains more than 100 trillion microorganisms, the majority of which live symbiotically with their hosts. The genome of these microorganisms is approximately 150 times larger than the host human genome (Gomes et al. 2018). The human intestinal microbiota mainly consists of *Firmicutes, Proteobacteria, Bacteroidetes* and *Actinobacteria* (Wang et al. 2022).

Gut microbiota has now been accepted as an endocrine organ that plays a role in energy homeostasis and host immunity (Gomes et al. 2018). The metabolism of some bacteria facilitates the provision of calories from the diet, increasing fat metabolism in adipose tissue and obtaining energy for microbial growth and proliferation.

Host-microorganism interaction occurs primarily along mucosal surfaces. One of the largest surfaces is the human intestinal mucosa. There is a bidirectional host-flora exchange in the intestine (O'Hara and Shanahan 2006; Qian et al. 2022).

The gut microbiota has many positive effects on host health. Gut bacteria aid digestion and contribute to the absorption of many nutrients and metabolites such as essential amino acids, lipids, short-chain fatty acids and vitamins (Jin et al. 2019; Nesci et al. 2023; Qian et al. 2022). It also plays an important role in ensuring immune homeostasis and increasing host resistance against infectious diseases(Tang, Kitai, and Hazen 2017). Gut microbiota regulates the nervous system, especially the neuroendocrine system, through the gut-brain axis and contributes to the regulation of glucose, lipid and protein metabolism with the metabolites it produces (Wang et al. 2022). Trimethylamine N-oxide (TMAO), short-chain fatty acids (SCFA), bile acids (BA), ammonium and phenols are among these metabolites (Peng et al. 2018; Tang et al. 2017; Wang et al. 2022).

Trimethylamine N-oxide

Nutrients such as choline, betaine and L-carnitine are converted to trimethylamines by gut microbiota. Trimethylamines is absorbed and reached the liver and, in the liver, it conversed to TMAO by flavin-monooxygenase 3. TMAO plays a role in development of atherosclerosis by causing endothelial cell dysfunction (Nesci et al. 2023), inducing the expression of inflammatory cytokines and adhesion molecules, affecting the cholesterol mechanism, increasing platelet activation and thrombosis (Figure 1) (Canyelles et al. 2023).

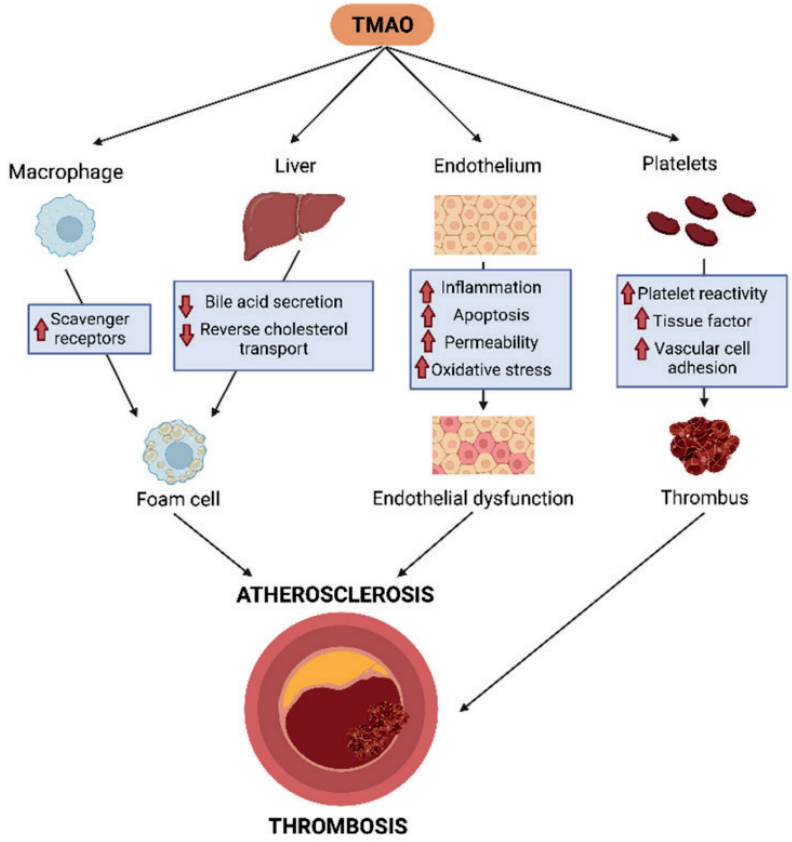

Figure 1. *TMAO and its effects on cardiovascular events (Canyelles et al. 2023)*

Short- Chain Fatty Acids

Short-chain fatty acids are produced in gastrointestinal system that metabolites formed from dietary fibers as a result of the metabolism of intestinal bacteria (Peng et al. 2018). Acetate, butyrate and propionate are classified as SCFA. SCFAs have beneficial effects on the host (Figure 2). They contribute to glycemic control and lipid metabolism, reduce oxidative stress and have anti-inflammatory effects (Nesci et al. 2023).

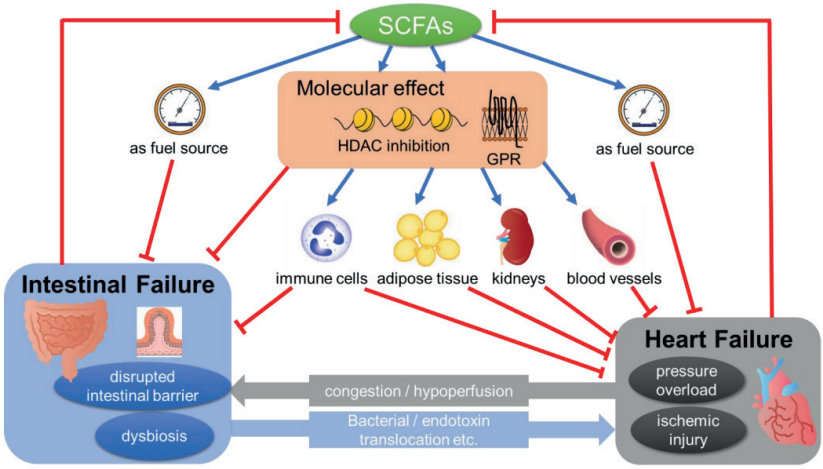

Figure 2. *Short-chain fatty acids as gut microbiota metabolites (HDAC: Hyston deacetylases; GPR: G protein coupled receptor) (Yukino-Iwashita et al. 2022).*

Bile Acids

Bile acids are one of the most important metabolites of gut microbiota. Bile acids are amphipathic molecules synthesized from cholesterol and play a role in the digestion and absorption of dietary lipids. Bile acids synthesized by hepatocytes and stored in the gallbladder are called primary bile acids, and those that are products of bacterial metabolism are called secondary bile acids (Belli et al. 2023; Collins et al. 2023; Rahman et al. 2022).

Bile acids regulate glucose, lipid and protein metabolism and inflammation through nuclear receptors and G protein coupled receptors (GPCRs) (Collins et al. 2023).

GUT MICROBIOTA IN CARDIOVASCULAR DISEASES

Studies have revealed that the gut microbiota and its metabolites play a role in regulating normal physiological functions (Figure 3), and diseases such as, diabetes cardiovascular diseases and obesity are related to gut microbiota.

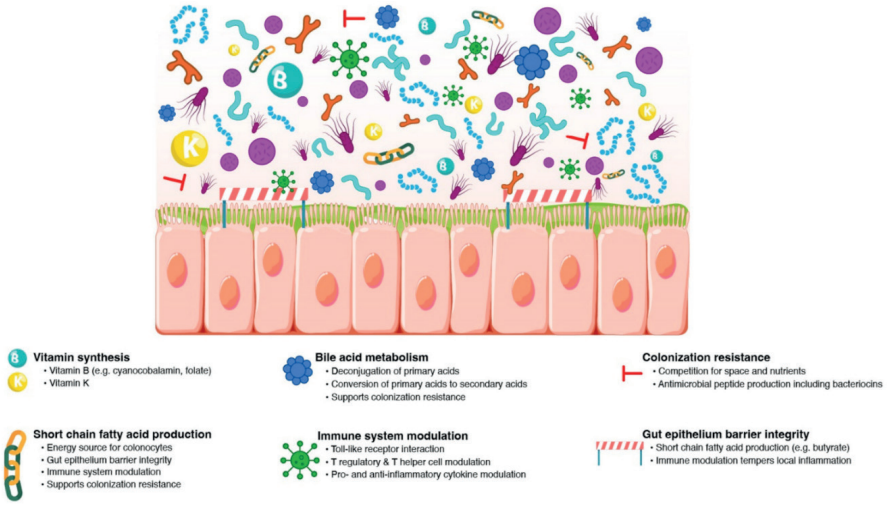

Figure 3. *Gut microbiota and functions (Bidell, Hobbs, and Lodise 2022)*

Disruption of the intestinal microbiota is called dysbiosis. Dysbiosis is characterized by a decrease in the proportion of healthy bacteria and an increase in the proportion of harmful bacteria. In this case, the healthy intestinal ecosystem is eliminated. Dysbiosis is associated with many diseases such as immune diseases, metabolic disorders, cancer and infectious diseases (Belli et al. 2023; Bidell et al. 2022; Rahman et al. 2022).

Dysbiosis promotes the development of cardiovascular disease, especially as it is associated with impaired mucosal barrier, inflammation and immune system dysfunction (Wang et al. 2022).

Gut Microbiota and Hypertension

Hypertension is one of the risk factors for cardiovascular diseases and also global public health problem. Recent studies have shown that abnormal bacterial population are associated with hypertension and also excessive formation of gut microbiota metabolites contributes to hypertension (Qian et al. 2022; Yang et al. 2023).

Gut microbiota regulates blood pressure through various mechanisms. One of these is the intestinal barrier. When intestinal barrier function is normal, intestinal permeability is low and pathogens, toxins and other substances cannot enter the circulation. Dysbiosis causes barrier dysfunction. In this case, as intestinal permeability increases, pathogenic bacteria and lipopolysaccharides enter the circulation and cause systemic inflammation. This causes endothelial dysfunction, vascular sclerosis and hypertension (Yang et al. 2023).

Another mechanism is the structure of the gut microbiota and gut microbiota metabolites. Studies have shown that serum levels of short-chain fatty acids are negatively correlated with blood pressure, and that short-chain fatty acids reduce blood pressure by directly causing vasodilatation(Yang et al. 2023). Another metabolite, TMAO, increases the risk of hypertension. Studies have shown that circulating TMAO concentration is positively correlated with blood pressure (Wang et al. 2022).

Gut Microbiota and Atherosclerosis

Atherosclerosis is a chronic, low-grade inflammatory disease affecting large and medium-sized arteries and is considered the major underlying cause of cardiovascular disease. Atherosclerosis is a chronic inflammatory process manifested by impaired lipid metabolism, arterial stiffness, foam cell formation, and congestion in blood vessels. Endothelial dysfunction has an important role in the development of atherosclerosis. Endothelial dysfunction is defined as the initial step in the pathophysiology of atherosclerosis (Santhakumar et al. 2018; Torres et al. 2015).

In the development of atherosclerosis, disruption of lipid metabolism, arterial stiffness, foam cell formation and occlusion of blood vessels occur (Vesnina et al. 2022). First, atherogenic lipoproteins accumulate in the intimal area and are modified by oxidation. This modification causes endothelial cells to express adhesion and chemotaxis molecules. Monocytes come to this area, bind to adhesion molecules, migrate to the intimal area and turn into macrophages. Foam cells form when macrophages phagocytose modified lipoproteins. The inflammatory process begins with the accumulation of foam cells (Vesnina et al. 2022).

Dysbiosis affects the intestinal barrier, which is considered a risk factor for atherosclerosis, an inflammatory disease. It has been observed that especially *Colinsella* genus are increased in the gut microbiota of atherosclerotic patients, whereas the intestinal microbiota of healthy people is rich in SCFA-producing genus such as *Eubacterium* and *Roseburia*. (Karlsson et al. 2012; Verhaar et al. 2020). Intestinal microbiota metabolites also affect the development of atherosclerosis. While SFAs prevent the development of atherosclerosis, TMAO promotes the development of atherosclerosis through its effect on lipid metabolism and endothelial dysfunction (Figure 4) (Wang et al. 2022).

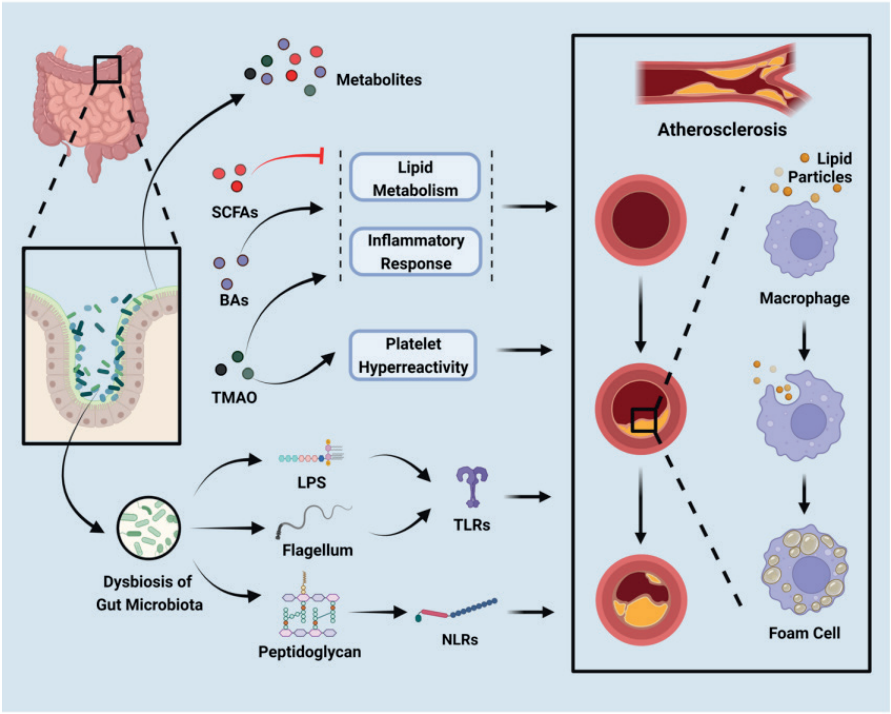

Figure 4. *Gut microbiota and atherosclerosis (SCFAs: Short chain fatty acids; TLRs: Tool-like receptors, BAs: Bile acids; TMAO: Trimethylamine N-oxide; LPS: Lipopolysaccharide; Nod-like receptors) (Wang et al. 2022)*

GUT MICROBIOTA AS A THERAPEUTIC TARGET FOR CARDIOVASCULAR DISEASES

In recent years, the intestinal microbiota has now been accepted as an endocrine organ that plays a role in energy homeostasis and host immunity. The metabolism of some bacteria facilitates the provision of calories from the diet, increasing fat metabolism in adipose tissue and obtaining energy for microbial growth and proliferation (Gomes et al. 2018).

After it was revealed that dysbiosis plays a role in the development of metabolic diseases such as cardiovascular diseases, obesity, type 2 diabetes and metabolic syndrome, regulation of the intestinal microbiota has become a priority for the treatment of these diseases.

Intestinal microbiota is directly related to nutrition. Therefore, diet has an important place in the treatment of cardiovascular diseases by regulating the gut microbiota (Rahman et al. 2022). Including probiotics and prebiotics in diet is important for a healthy intestinal microbiota. Probiotics are live microorganisms that have beneficial health effects on the organisms they

live on. Prebiotics are dietary fibers and source of nutrition for beneficial microorganisms, especially in the intestine (Abenavoli et al. 2019).

Various drugs such as antibiotics and TMA lyase inhibitors are also used to regulate the intestinal microbiota. It has also been reported that drugs used for the cardiovascular system, such as statins, glucose-lowering drugs and antihypertensives, also regulate the intestinal microbiota (Khan et al. 2018; Robles-Vera et al. 2020).

In addition, exercise and fecal microbiota transplantation are methods used to treat cardiovascular diseases by improving the intestinal microbiota (Wang et al. 2022).

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Chapter 26

MICROBIOTA–GUT–BRAIN AXIS: ROLE IN NEURODEGENERATIVE DISEASES

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1. Introduction

Recent research has highlighted the critical link between microbiota and neurodegenerative diseases. The community of microorganisms in the gut, known as the gut microbiota, impacts the central nervous system via the gut-brain axis, contributing significantly to the development and progression of neurodegenerative diseases. Recognizing the role of gut microbiota in both the onset and management of neurodegenerative diseases suggests it could be a promising therapeutic target.

1.1. Neurodegenerative Diseases

Neurodegenerative diseases refer to a variety of disorders that progressively affect the brain and nervous system. These diseases have a significant impact on millions of people globally, severely affecting their quality of life. Common neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS).

Alzheimer's disease is the most common cause of dementia in older adults and is marked by memory loss, language difficulties, and cognitive decline. The development of Alzheimer's is associated with the buildup of amyloid-beta plaques and neurofibrillary tangles in the brain (Selkoe, 2001).

Parkinson's disease manifests through movement disorders such as tremors, stiffness, and bradykinesia. The development of Parkinson's is linked to the degeneration of dopaminergic neurons in the substantia nigra and the accumulation of Lewy bodies in the brain (Poewe et al., 2017).

Amyotrophic Lateral Sclerosis (ALS) involves the progressive degeneration of motor neurons, resulting in muscle weakness and eventually paralysis. Glutamate toxicity, oxidative stress, and inflammation are all contributing factors in the development of ALS (Hardiman et al., 2017).

Huntington's disease, usually manifesting in middle age, is a genetic disorder characterized by chorea, dementia, and psychiatric issues. The mutation of the huntingtin protein and the subsequent death of neuronal cells play a crucial role in the development of Huntington's disease (Walker, 2007).

1.1.1. Pathogenesis of Neurodegenerative Diseases

Multiple factors, including genetics, environment, oxidative stress, and inflammation, contribute to the development of neurodegenerative diseases (Jellinger, 2020).

Genetic predisposition is a key determinant of an individual's vulnerability to neurodegenerative diseases. For example, in Alzheimer's disease, the ε4 allele of the apolipoprotein E (APOE) gene is a risk factor that influences disease onset and progression (Corder et al., 1993). Likewise, mutations in the alpha-synuclein (SNCA) and leucine-rich repeat kinase 2 (LRRK2) genes

are linked to both familial and sporadic Parkinson's disease cases (Paisan-Ruiz et al., 2004). Also, mutations in superoxide dismutase 1 (SOD1), TAR DNA-binding protein (TARDBP), and fused in sarcoma (FUS) genes are implicated in ALS development (Renton et al., 2014). Finally, mutations in the huntingtin (HTT) gene are involved in the development of Huntington's disease (MacDonald et al., 1993). However, genetics is just one piece of the puzzle, as environmental factors are also pivotal.

Environmental factors include lifestyle choices and exposure to toxins. For instance, smoking and heavy alcohol consumption are associated with a heightened risk of developing neurodegenerative diseases (Tyas et al., 2001). Moreover, exposure to environmental toxins like pesticides and heavy metals can also foster the development of neurodegenerative diseases (Baldi et al., 2003).

Additionally, oxidative stress and inflammation are crucial in the development of neurodegenerative diseases. Oxidative stress occurs when there is an accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells, leading to detrimental effects on proteins, lipids, and DNA (Barnham et al., 2004). Inflammation, on the other hand, can cause cell death and neuronal dysfunction (Amor et al., 2010).

1.2. Intestinal Microbiota

Microbiota refers to the vast array of microorganisms that inhabit the human body and significantly affect human health. The gut microbiota, which is the most extensively researched component of the human microbiota, comprises all the microorganisms present in the intestines. It plays a crucial role in various physiological functions, including digestion, metabolism, immune response, and even brain activity (Valdes et al., 2021).

Over the past few years, there has been a surge in research exploring the impact of gut microbiota on human health and diseases. For instance, studies have demonstrated a link between gut microbiota and several conditions such as obesity, diabetes, inflammatory bowel disease, and certain cancers (Tilg et al., 2020). Moreover, the gut microbiota has been found to influence brain function, thereby contributing to the development of neuropsychiatric disorders like depression, anxiety, and neurodegenerative diseases (Sherwin et al., 2020).

1.2.1. Relationship Between Gut Microbiota and Blood-Brain Barrier

The blood-brain barrier (BBB) and gut microbiota dysfunctions are key factors in the pathophysiology of numerous neurological disorders. The BBB is a specialized structure comprising brain endothelial cells, pericytes, and astrocytes, which together control the transfer of substances between the blood and the brain (Sweeney et al., 2019).

BBB dysfunction involves increased permeability, allowing harmful substances to enter the brain, and is linked to several neurological diseases (Montagne et al., 2020). Recent research has also underscored the role of gut microbiota in maintaining BBB integrity. For example, studies have shown that germ-free mice, which lack microorganisms, exhibit heightened BBB permeability compared to conventionally raised mice. However, this permeability can be reversed by introducing a normal gut microbiota (Braniste et al., 2014).

The gut microbiota influences BBB function through various mechanisms, including the production of short-chain fatty acids (SCFAs), immune system regulation, and the gut-brain axis (Erickson & Banks, 2019). SCFAs like butyrate, acetate, and propionate, produced during dietary fiber fermentation by gut microbiota, help reinforce the BBB by enhancing the expression of tight junction proteins. Additionally, gut microbiota regulates the immune system, indirectly affecting BBB function. Lastly, the gut-brain axis, a bidirectional communication system between the gut and brain, is pivotal in controlling BBB function.

1.2.2. Gut Microbiota Metabolites

Metabolites produced by the microbiota are substances created by gut microorganisms, which greatly influence human health. The gut microbiota impacts several physiological functions, such as digestion, metabolism, immune response, and even brain activity (Valdes et al., 2021). These influences are facilitated through a variety of metabolites generated by gut microorganisms. For instance, the gut microbiota generates numerous metabolites, including short-chain fatty acids (SCFAs), bile acids, amino acids, and neurotransmitters (Tilg et al., 2020).

1.2.2.1. Short-Chain Fatty Acids

SCFAs are one of the most crucial metabolites generated by the gut microbiota, formed during the fermentation of carbohydrates that are not digested by gut microorganisms. These fatty acids act as an energy source for the cells lining the intestine and also regulate intestinal barrier function, inflammation, and immune responses (Hill et al., 2021).

1.2.2.2. Bile Acids

Bile acids undergo alteration by gut microorganisms, which impacts their absorption and metabolism. These acids are crucial components of bile salts, necessary for lipid digestion and absorption. The gut microbiota is instrumental in the conversion of primary bile acids, showcasing its role in regulating lipid metabolism and energy balance. Moreover, both bile acids and gut microbiota are involved in the development of metabolic diseases, obesity, and diabetes (Long et al., 2017).

The interplay between microbiota and bile acids has wide-ranging effects on human health and disease. For example, bile acids can alter the composition of the microbiota, and conversely, the microbiota can affect bile acid profiles. Therefore, this interaction is viewed as a potential target in the development and treatment of metabolic diseases (Wahlström et al., 2016).

1.2.2.3. Amino Acids

Gut microorganisms metabolize amino acids, influencing their absorption and metabolism. Amino acids serve as the foundation of proteins, and the microbiota can affect both host and microbial metabolism by decomposing dietary proteins and modifying amino acids.

The gut microbiota is known to have a vital role in the biosynthesis and alteration of amino acids. These acids are used by gut microorganisms for energy production, protein creation, and the synthesis of other biological molecules. Moreover, the involvement of gut microbiota in amino acid metabolism can have implications for the host's health and disease state. For example, it is suggested that the gut microbiota plays a part in the development of specific diseases linked to amino acid transformation (Neis et al., 2015).

1.2.2.4. Neurotransmitters

Neurotransmitters, which are key chemical compounds for nerve cell communication, are also produced by gut microorganisms, and can affect brain functions (Sherwin et al., 2020). The gut microbiota can indirectly influence brain functions and behaviors by affecting the biosynthesis and metabolism of neurotransmitters. For example, the gut microbiota is known to affect the production of neurotransmitters such as serotonin and gamma-aminobutyric acid (GABA) (Yano et al., 2015).

Serotonin, a neurotransmitter found in both the gut and brain, regulates various physiological processes such as mood, sleep, and appetite. The ability of the gut microbiota to influence serotonin production in the gut may, therefore, have downstream effects on brain functions and behaviors. Similarly, GABA is an inhibitory neurotransmitter, and the influence of gut microbiota on GABA levels may play a role in the development of neurological disorders like anxiety and depression.

1.2.3 Manipulation of Gut Microbiota

Altering the gut microbiota may be a potential method for treating various diseases. Approaches like using probiotics, prebiotics, and fecal microbiota transplantation (FMT) are some techniques utilized for modifying gut microbiota (Hill et al., 2021).

1.2.3.1 Probiotics

Probiotics are living microorganisms that can alter the composition and function of the gut microbiota, resulting in positive health effects such as enhancing immune function and preventing pathogens from adhering to the intestinal lining. Common bacteria such as lactobacilli and bifidobacteria are classified as probiotics, but some yeast species also fall into this category (Hill et al., 2014). Probiotics function by changing the gut microbiota, enhancing immune function, and preventing pathogen attachment to the intestinal lining. They have been found to positively impact digestive health, immune function, and even mood (Sánchez et al., 2017).

The effectiveness of probiotics varies based on the type and strain of microorganism ingested, making it crucial to choose the most appropriate probiotic strain for a specific health issue. Probiotics are present in fermented foods, supplements, and even medicinal products. Due to their potential health benefits, probiotics have become increasingly popular in recent years. For instance, they are thought to alter the gut microbiota to aid in preventing and treating chronic diseases such as obesity, diabetes, and cardiovascular diseases (Kechagia et al., 2013). Additionally, probiotics are believed to modulate immune responses, decrease allergic reactions, and potentially help prevent certain cancers (El-Nezami et al., 2006).

1.2.3.2 Prebiotics

Prebiotics are non-digestible food components that are fermented by gut microorganisms, leading to changes in the composition and activity of the gut microbiota. They essentially serve as nourishment for the growth and activity of probiotics, beneficial bacteria in the gut. Common prebiotics are carbohydrates that are not digestible by humans but can be fermented by gut microbiota, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS), and inulin (Gibson et al., 2017). Prebiotics function by enhancing the gut mucosal integrity, modulating immune responses, and providing several health benefits like supporting digestive health, immune function, and metabolic health (Slavin, 2013).

Prebiotics can be found naturally in various foods like onions, garlic, bananas, oats, and wheat, and they are also available as supplements and in functional foods. They alter the gut microbiota by specifically supporting the growth of probiotic bacteria like bifidobacteria and lactobacilli, which in turn improves the composition and function of the gut microbiota, promoting better gut health and overall well-being (Bindels et al., 2015).

1.2.3.3 Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT) is a procedure that involves the transfer of fecal material from a healthy donor to the gastrointestinal tract of

a recipient with a diseased gut, with the aim of restoring the gut microbiota's composition and function. This technique is utilized to alter the gut microbiota and normalize it in individuals with certain diseases, such as Clostridioides difficile infection (CDI). CDI is associated with gut microbiota dysbiosis caused by antibiotic treatment, making the restoration of gut microbiota a key strategy in its treatment (van Nood et al., 2013).

The efficacy of FMT lies in its ability to modify the gut microbiota's composition and function. The fecal material from the donor helps recolonize the recipient's gut microbiota, enhancing its composition and function. This can lead to improved gut mucosal integrity, modulated immune responses, and consequently, better gut health and overall well-being (Khoruts & Sadowsky, 2016). However, additional research is required to fully establish the efficacy and safety of this approach.

1.3. Examples of Studies in the Literature

The potential link between gut microbiota and neurodegenerative diseases has been investigated in various experimental studies:

Hoban et al. (2016) assessed the impact of gut microbiota on the myelination processes in the prefrontal cortex and its association with neurodegenerative diseases. Sampson et al. (2016) analyzed the impact of gut microbiota on motor deficits and neuroinflammation in a mouse model of Parkinson's disease. Sharon et al. (2016) explored the association between gut microbiota and the central nervous system and its potential implications on the pathophysiology of neurodegenerative diseases. Bonfili et al. (2017) examined how modifying gut microbiota in an Alzheimer's disease mouse model affects disease progression and the role of neuronal proteolysis and gut hormones. Burokas et al. (2017) assessed the influence of gut microbiota on anxiety and depression-like behaviors in mice and evaluated the potential of prebiotics to counter these effects. Harach et al. (2017) assessed the effects of depleting gut microbiota on the accumulation of amyloid plaques in a mouse model of Alzheimer's disease. A study on Alzheimer's disease in mice by Vogt et al. (2017) examined alterations in gut microbiota and its potential implications on the disease. Blacher et al. (2019) examined the possible role of gut microbiota in amyotrophic lateral sclerosis (ALS) and discussed novel therapeutic strategies for the condition. Sun et al. (2020) investigated the link between gut microbiota and Alzheimer's disease, evaluating its influence on disease development and treatment.

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