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Theory, Current Researches

and New Trends/2021

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Assoc. Prof. Dr. Savas KARYAGAR

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PREFACE

Dear scientists,

The production of knowledge and the sharing of the produced information within the scientific community, and its contribution to the solution of human health problems are the necessities of the age of science we are in. The book “Current Researches and New trends / 2021” is serving an academic forum for both academics and researchers working in such fields. Scientists, especially those working in clinical branches, are trying to provide health services to their patients during the Covid 19 pandemic days, while on the other hand, they are trying to do scientific research and share scientific data in their field with the scientific community. In this book, the selected articles have been reviewed and approved for publication by referees. I hope this will set an example for the younger generation. I would like to thank all of my colleagues and publishing house for their devotion.

Assoc. Prof. Dr. Savaş KARYAĞAR

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Prof. Dr. Nizami Duran, Hatay Namık Kemal University

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CHAPTER I

THE IMPORTANCE OF KEFIR IN HEALTHY NUTRITION: ANTIOXIDANT AND HYPOCHOLESTEROLEMIC EFFECT

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1. Introduction

For many years, alternative drugs, antibiotics and foods have been used for various specific reasons against harmful structures that adversely affect human health. Today, it has been revealed that these drugs and antibiotics can be harmful for health. This has led people to natural diets and functional food products. In human diet, the desire to reach functional food products that provide additional benefits to health which are effective in preventing the formation of diseases and improving health increases steadily (Ender et al., 2006; Gürsoy and Kınık, 2006; Köroğlu et al., 2015).

Functional foods are those foods or food components that have beneficial effects on human physiology and metabolic functions. As a result of growing consumer preference for natural and functional foods, which leads to an increased demand for probiotic products, it is observed that fermented dairy products have gained a different position and become very important in terms of human health and nutrition. The increase in consumer awareness and the tendency towards healthy foods have also increased the interest in fermented products (Ender et al., 2006; Hacıoğlu and Kurt, 2012; Köroğlu et al., 2015). These products have functions such as preserving beneficial bacteria in the intestinal flora, increasing their numbers and keeping the body's defences strong. Kefir is one of these

products. Different from other fermented dairy products, kefir is a drink originated in North Caucasus with very high probiotic properties. It is a dairy product that has been cultured for many years, obtained by adding kefir granules to the milk of sheep, goats, mares and cows, and in which acidic and alcoholic fermentations occur together (Koroleva, 1988; Gorbach et al., 1996; Güven et al., 2003; Karatepe et al., 2012).

The composition of kefir may vary depending on the chemical content of the milk used, the method of production, the microbial flora of the kefir or kefir granules used, fermentation conditions and techniques and duration of storage. Due to its nutritional value and therapeutic properties, kefir is an important part of people's diet in many regions including Northern Europe, America, Japan, the Middle East and Russia. Previously produced as part of a tradition, it has recently begun to be mass-produced using modern techniques and industrial methods (Güven et al., 2003; Bellekçi-Koyu and Büyüktuncer, 2018).

Obtained from skimmed or low-fat milk depending on preference, kefir contains many organic and inorganic substances, as well as various beneficial microorganisms (bacteria and yeast). As a probiotic, it contains most of the essential nutrients found in milk. Due to the fermentation caused by microorganisms during its production, some vitamins and bioactive substances are synthesized when lactose and proteins are broken down. This makes kefir a product with higher nutritional value than other dairy products (Yetişemiyen, 1995; Güven et al., 2003; Rattray and O'Connell, 2011).

With this rich content, kefir is widely used in many parts of the world today in order to strengthen the body resistance and defence system, prevent the effects of some harmful substances and reduce or treat present or possible diseases. Some studies report that there is a correlation between kefir-supplemented diets and the reduction of diseases, and that kefir has antioxidant, anticarcinogenic, antibacterial, antiallergic and hypocholesterolemic effects in addition to its regulatory action on the immune and digestive systems (Zubillaga et al., 2001; Ahmed et al., 2013; Arslan, 2015; Köroğlu et al., 2015; Rosa et al., 2017; Çevikbaş, 1994; Adiloğlu et al., 2013; Wang et al., 2009; Güven, 2003; Güven, 2005, Tomar 2017).

The purpose of this literature study is to emphasize the antioxidant and hypocholesterolemic effects of kefir, which has many functional importance on human nutrition and health in the light of current scientific studies, and to contribute to consumer health and awareness.

2. Microbial flora of kefir

The microbial composition of a kefir grain includes homofermentative and heterofermentative Lactobacillus species, Lactococcus, Streptococcus and Acetobacter species, as well as Candida and Saccharomyces yeast species (Angulo et al., 1993; Farnworth, 2005). The bacteria and yeast species found in kefir are listed in ***Table 1*** and ***Table 2***.

Table 1 Bacteria found in kefir (Farnworth, 2005).

Lactobacillus	***Lactococcus***
Lactobacillus kefir	*Lactococcus lactis subsp. lactis*
Lactobacillus delbrueckii	*Lactococcus lactis subsp. cremoris*
Lactobacillus kefiranofaciens	***Acetic acid bacteria***
Lactobacillus rhamnosus	*Acetobacter spp.*
Lactobacillus kefirgranum	*Acetobacter pasteurianus*
Lactobacillus casei	***Enterococcus***
Lactobacillus parakefir	*Enterococcus durans*
Lactobacillus paracasei	***Streptococcus***
Lactobacillus brevis	*Streptococcus thermophilus*
Lactobacillus fructivorans	***Leuconostocs***
Lactobacillus plantarum	*Leuconostoc spp.*
Lactobacillus hilgardii	*Leuconostoc mesenteroides*
Lactobacillus helveticus	***Other bacteria***
Lactobacillus fermentum	*Bacillus spp.*
Lactobacillus acidophilus	*Bacillus subtilis*
Lactobacillus viridescens	*Micrococcus spp.*
	Escherichia coli

Table 2 Yeasts found in kefir (Farnworth, 2005).

Saccharomyces spp.	*Candida friedrichii*
Saccharomyces exiguus	*Candida pseudotropicalis*
Saccharomyces cerevisiae	*Candida tenuis*
Saccharomyces unisporus	*Candida inconspicua*
Saccharomyces dairensis	*Candida maris*
Saccharomyces turicensis	*Candida lambica*
Kluyveromyces marxianus	*Candida tannotelerans*
Torulaspora delbrueckii	*Candida valida*
Brettanomyces anomalus	*Candida kefyr*
Issatchenkia occidentalis	*Candida holmii*
Pichia fermentans	

The microbial flora found in kefir grains is used as an example of a symbiotic community. When isolated as pure cultures, kefir bacteria and yeasts are either unable to grow in milk or have a reduced biochemical

activity. This makes it even more difficult to examine the microbial flora of kefir grains (Koroleva, 1991; Margulis, 1995).

The origin of a kefir grain is affected by many factors such as the level of microorganisms in it, the ratio of different microorganism types to each other, the incubation temperature and duration applied in production and storage time of the grains. The hygiene and sanitation practices of a kefir business will also significantly affect microbial flora of kefir (Güzel-Seydim et al., 2005).

What distinguishes kefir from other fermented dairy products is the co-occurrence of lactic acid and alcohol fermentation due to the symbiotic activity of bacteria and yeast species found in kefir grains. Lactic acid, CO2, ethanol and other products formed as a result of fermentation are components that directly affect the formation of taste and aroma. Additionally, free fatty acids produced in milk by lipolysis are responsible for the taste and aroma of various fermented dairy products, including kefir. Fermented milk contains 5 to 10 times more free fatty acids (FFA) than non-fermented milk. In summary, the biochemical events that occur during the fermentation of kefir will influence its taste and aroma characteristics (Wszolek et al., 2001; Beshkova et al., 2003; Leite et al., 2013; Yılmaz et al., 2006; Farag et al., 2020).

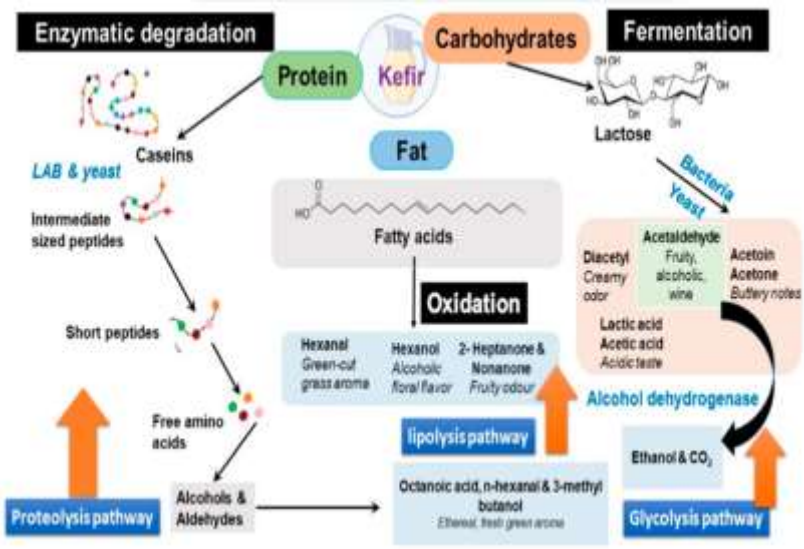

Figure.1 Formation of kefir aroma as a result of biochemical degradation of macronutrients in milk by the activity of various microorganisms (Farag et al., 2020).

3. Nutritional value and biochemical properties of kefir

Kefir's nutritional value is produced by its rich chemical composition of minerals, vitamins, carbohydrates, peptides, proteins and fats. This nutritional value is further increased during the fermentation process thanks to secondary bioactive components such as catechin, vanillin, ferulic acid and salicylic acid. Kefir is rich in essential amino acids, minerals and vitamins B1, B2, B5, and C that are important for wound healing, wellness and homeostasis. Kefir also contains partially digested proteins such as casein, which helps the body to digest and absorb. Essential amino acids found in abundance in kefir make a favourable effect on body weight regulation, energy balance and immune response by regulating protein, glucose and lipid metabolisms (Simova et al., 2006; Grohmann and Bronte, 2010; Farag et al., 2020).

Kefir contains macro elements such as calcium, magnesium, potassium and sodium. These macro elements assist in the utilization of carbohydrates, fats and proteins. Kefir also possesses microelements such as iron, zinc and copper, which are especially valuable in cellular metabolism and blood production. Peptides are a unique and important class of compounds produced during milk fermentation and explain many of the health benefits of fermented dairy products. Fermented sheep milk provides a good source of bioactive peptides that exhibit antioxidant and antimicrobial activities (Ebner et al., 2015; Bakırcıoğlu et al., 2017; Farag et al., 2020).

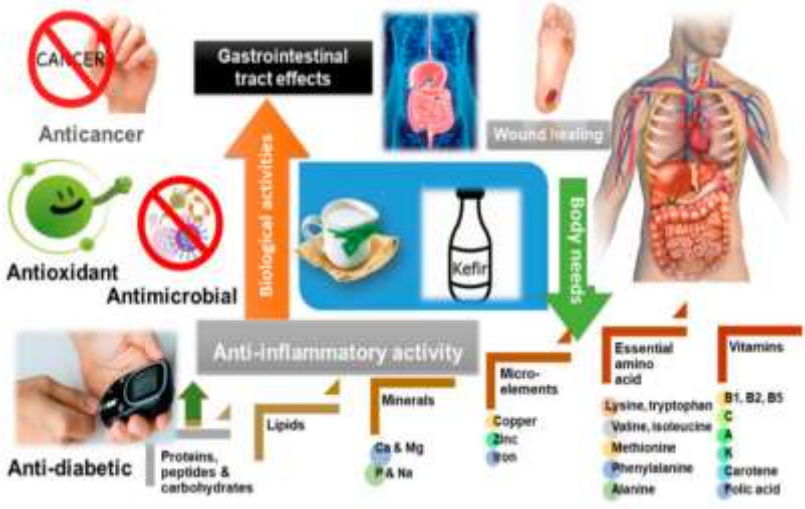

Figure.2 Nutritional value and biochemical properties of kefir (Farag et al., 2020).

4. Antioxidant and hypocholesterolemic effect of kefir

Having a complex microbial flora, kefir contains many microorganisms. It provides good antioxidant activity due to the presence of these microorganisms, especially lactic acid bacteria (Lin and Yen, 1999; Farnworth, 2005). Kefir contains partially broken proteins that can be absorbed by the body and aid its digestion. Bioactive peptides formed as a result of protein fermentation show antimicrobial and antioxidant properties (Ebner et al., 2015; Bulut-Solak, 2020).

Kefir oxidizes free radicals and reduces their adverse impacts on cells and tissues. Since it is a food rich in antioxidants, it also neutralizes free radicals and delays the aging process (Bulut-Solak, 2020).

Lb. plantarum, one of the bacteria found in kefir, rearranges biochemical events that take a role in the formation of free radicals and contributes significantly to kefir's antioxidant effect (Tang et al., 2017). It has been determined that the antioxidant capacity of kefir made with goat's milk is much higher than kefir made with cow's milk. Similarly, the antioxidant capacity of kefir made with kefir grain was found to be higher than that made with kefir starter culture. (Yılmaz-Ersan et al., 2018).

Since kefir contains many antioxidant molecules, it has therapeutic properties against diseases. Among the bacteria contained in kefir, Lactobacillus species play important roles in protecting from pathogenic bacteria, strengthening the immune system, reducing the risk of allergies and cancer, preventing the formation of free radicals, and preventing hypercholesterol and diabetes (Bulut-Solak, 2020).

Cholesterol is an organic substance that has many functions in human metabolism. In the organism, half of the cholesterol is produced by synthesis, while the rest is obtained from a normal diet. The increase in the amount of cholesterol in the blood causes some disorders in the organism. When the cholesterol level in serum increases, there is a significant risk for cardiovascular diseases (CVD) (Wong et al., 2016).

Diets rich in cholesterol can cause oxidative stress and hypercholesterolemia by leading to the formation of free radicals. In other words, oxidative stress is one of the factors associated with hypercholesterolemia and atherosclerosis (Ismail et al., 2010). Oxidative stress plays a critical role in the pathogenesis of atherosclerosis by causing the formation of lipid-loaded macrophages and development of inflammation. The increase in low-density lipoprotein (LDL) production and secretion increases the formation of oxidized LDL (ox-LDL) and oxidative stress. The prolongation of the LDL circulation period is

followed by permanent hypercholesterolemia (Byon et al., 2008; Vazquez-Castilla et al., 2013).

For this reason, recently, studies on nutrients have been emphasized as well as medical research to prevent hypercholesterolemia or atherosclerosis. Kefir is one of these foods. Although the mechanism by which kefir reduces serum cholesterol level is not known exactly, it has been confirmed by research that more than one mechanism is involved.

Kefir helps to lower cholesterol, triglycerides and LDL in the blood. Kefir consumption is beneficial in preventing many cardiovascular diseases such as heart disease, hypertension and stroke (Cho et al. 2017; Rosa et al., 2017). Kefir's inhibitory effect on high cholesterol has been linked to rearrangement of genes in fatty acid oxidation. Bacteria of Lactobacillus species play an important role in reducing heart disease by lowering the level of LDL in the blood (Kim et al., 2017). The presence of Lactobacillus species, one of the bacteria found in kefir, especially *Lb. plantarum*, decreases the cholesterol level in plasma and liver. In a research, triglyceride and LDL levels in the blood of mice with high cholesterol fed with Tibetan Kefir containing *Lb. plantarum* bacteria were found to decrease after 4 weeks (Huang et al., 2013). Table 3 below shows some studies on the hypocholesterolemic and antioxidant effects of kefir-supplemented diet in mice and rabbits.

Table 3 Hypocholesterolemic and antioxidant effects of kefir-supplemented diet in mice and rabbits.

Materiel	Application time	TBARS/MDA		GSH		GSH-Px		CAT		References
		Before	After	Before	After	Before	After	Before	After	
Mice liver	50 days	454.8±0.6	316.6±2.4	184.1±0.04	201±0.08	18.7±4.55	28.1±2.9	28.6±2.64	28.5±2.28	Güven et al., 2003[a]
Mice blood	84 days	3.27±0.5	2.02±0.1	3.49±0.9	2.83±0.9	56.80±9.65	31.33±7.18	42.2±4.14	36.3±3.29	Güven et al., 2009
Mice blood	50 days	5.84±0.04	2.62±0.04	0.90±0.04	1.65±0.04	29.22±1.47	35.54±0.98	27.4±2.14	28.8±2.75	Güven et al., 2004
Mice blood	60 days	3.70±2.1	2.13±0.3	1.02±0.4	1.29±0.2	26.80±1.3	29.82±2.2	28.16±1.8	29.22±2.7	Güven et al., 2003[b]

Materiel	Application time	Total cholesterol (mg/dl)		HDL (mg/dl)		LDL (mg/dl)		Triglyceride (mg/dl)		References
		Before	After	Before	After	Before	After	Before	After	
Rabbit blood	56 days	88.6±5.4	71.5±3.0	12.8±3.2	9.6±1.4	50.1±5.4	31.1±3.0	102.5±8	86.4±2.2	Güven and Güven 2005

Materiel	Application time	HDL (mg/dl)		LDL (mg/dl)		References
		Before	After	Before	After	
Mice blood	30 days	42.16±2.13	57,71 ± 5,90	78.12±10.99	64.71±5.90	Güven and Alkış 2018

for tissue	for blood
MDA: nmol/g (dry weight)	nmol/ml
GSH: nmol/g (dry weight)	nmol/ml
GSH-Px: U/mg prot (dry weight)	U/gHb
CAT: k/mg prot (dry weight)	k/mg Hb

5. Conclusion

It is known that the correct consumption of fermented milk products for a long time acts as a shield against diseases in the body. In this context, kefir today is a food source which has dozens of benefits for health in terms of both the variety of nutrients it contains and the richness of microbial flora, and whose use by consumers is increasing day by day. In studies on kefir, researchers agree that this traditional drink can have positive effects on human health. This review is a blend of many studies that consistently reveal the importance of diets supplemented with kefir in terms of its effects on liver and other tissues degeneration. More in vivo studies are required for the biochemical mechanism of action of kefir, which is effective on cholesterol and has antioxidant properties. In these studies, we believe that it will be important to include volunteer human subjects with different diseases instead of experimental animals in terms of revealing how influential kefir is in human diet and health preservation.

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CHAPTER II

DIGITAL PHOTOGRAPHY IN ORTHODONTICS

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1. Introduction

1.1 Significance and Areas of Use of Photography

Photography, derived from the Greek words of *photos* (light) and *graphs* (letter), is a physical and chemical process where the visible shapes are captured on a light-sensitive surface with the help of some chemical substances (1). The process is carried out by a camera which is a sealed box consisting of various setups and mechanisms. In 1839, the adventure of modern photography started at the time William Henry Fox Talbot captured the first permanent images in a dark box (2). In 1840, the photographs were considered as the complementary part of the patient treatment plans in the first dental school of Baltimore College of Dental Surgery. The process beginning with the first use of clinical photography in orthodontics by Dr. Edward Angle has pioneered the use of photography in the diagnosis and treatment planning in dentistry (3,4).

The basic operational principle of a camera is the "light". We can consider a camera as an eye thanks to this principle. Just as in the case of an eye needing a light to see, a camera also needs the same to operate. The pupil in an eye corresponds to an "objective" in a camera. The objective is usually a "convergent" lens or group of lenses. It enables the light coming from a subject, i.e. any object to be photographed, to fall entirely onto a light-sensitive film without being scattered. The retina in an eye where the objects appear corresponds to a light-sensitive film in a camera. The light passing through the lens falls onto this film inversely and there leaves a mark (5).

Today, in addition to the ease of transportation and use, compact cameras have a wide area of use, since their prices are quite low compared to other types of cameras. The single-lens reflex cameras, where the interchangeable lenses and external flashes can be used, offer

many alternatives to a photographer to shoot landscape, portrait, and macro images. The era of digital photography has begun with the use of electronic light sensors that detect the light instead of the light-sensitive films used in the compact and SLR cameras (6).

Two different types of light sensor systems are used in digital cameras, namely the CCD and the CMOS. The CCD and the CMOS sensors have many advantages and disadvantages when compared to each other. The CCD sensors are more sensitive than the CMOS sensors. The CMOS sensors last longer than the CCD sensors, consume less battery, give better output in bright light, and are cheaper. The digital images captured can be recorded on the in-built memory cards in the camera (7).

1.2. Parts of a Camera (8,9)

On/Off Button: When the On/Off button is <ON> or <OFF> position, the sensor cleaning runs automatically.

Shutter Button: The shutter button has two grades. The shutter button can be half or full pressed.

Half Press: This activates the autofocus (AF) and the auto exposure (AE) features, which set the shutter speed and the aperture.

ISO Speed Setting Button: This button adjusts the ISO speed (light-sensibility of the image sensor) to the intensity of light in the media.

Hot Shoe: This is the part, where an external flash can be attached when necessary and provides contact through the connections to the camera.

Lens Release Button: This button is used when inserting or removing a lens and pressed to remove the lens from the camera by rotating the lens clockwise or counterclockwise (depending on the brand).

Depth of Field Preview Button: This button is pressed to stop the existing aperture setting.

Viewfinder: This is the camera part that helps us to correctly frame the object we want to photograph and contains the exposure information as well. Since the viewfinder is positioned 2-3 cm above or at the edge of the lens, the object we look at appears at a slightly different angle than the lens sees. The difference between the image that passes through the lens and falls onto the film and the image seen through the viewfinder is called the parallax error. It can be overcome by using the LCD screen instead of looking through the viewfinder.

Camera Setting Display: The screen is turned on and off by pressing the <DISP> button. The existing camera settings can be controlled when

the menu thereof is displayed.

Menu Button: Different settings such as image recording quality, LCD screen brightness, etc. can be controlled via the menu.

Dioptric Setting Button: 9 AF points appear clearly in the viewfinder when the handle is rotated to the right or left.

AV Aperture/Exposure Compensation Button: In the case of a black or white object (i.e. clothes), the white may be darkened, and the black may be brightened. The exposure compensation setting is done, and the image is retaken to obtain the color shade desired. The exposure compensation may also be used to darken or brighten the pictures as we wish.

AE Lock/FE Lock Button/Index/Reduction Button: The AE lock allows us to lock the exposure in a place apart from the focus point. After locking the exposure, we can recreate and shoot the picture with the help of the exposure setting we want.

Delete Button: This button is used to delete the images individually or in a batch.

Playback Button: When this button is pressed, the last picture captured appears on the screen.

Metering Mode/Image Skip Selection Button: The objects in foreground and background can be focused automatically. All the AF points are put into the function to scan the object, and the depth of field needed by the aperture is automatically adjusted. After pressing this button, the desired modes of evaluative metering, partial metering, spot metering, center-weighted metering can be chosen.

Picture Style Selection Button: This button helps to get the image effects we want.

Advance Mode Selection Button: The auto timer can be selected by pressing this button. We can also shoot about 3.5 shots per second in a row.

AF Mode Selection Button: This button is used to select the appropriate mode among the One-Shot AF, AI Focus AF, and AI Servo AF modes.

One-Shot AF: This feature is ideal for the fixed objects and snapshots. The focus gets locked when the shutter button is kept pressed.

AI Focus AF: This feature is used to shoot objects that move suddenly such as animals. When the shutter button is half-pressed, the

focus gets locked as in One-Shot AF. If the object starts to move, the camera switches to the AL Servo AF mode and focuses continuously.

AL Servo AF: This feature is provided for the shooting of sports events and other moving objects. When the shutter button is kept half-pressed, the focus and the exposure are adjusted continuously.

1.3. Shooting Settings Display (10.11)

Shutter Speed: For an image to appear on the sensor, a dark box, a sensitive surface, and light are required. Besides, the beams reflected from the object must remain on the sensor for a certain period. The light reception period should be neither more nor less. Therefore, there is a need for a mechanism (an opening and closing door) to adjust this period. This system is called the shutter.

Aperture: This is the part of a digital camera where the intensity of the light falling onto the sensor is set. The aperture is the first door in a camera before the shutter. If we want more light on the film, we must keep the aperture interval large, and *vice versa*. The aperture interval is measured in f numbers and is more commonly referred to as f-stops. Contrary to what one might expect, the larger the number of f-stops, the smaller the aperture, and the less light entering into the camera. Each f-stop setting allows the incoming light up to half of the subsequent smaller f-stop number.

ASO (ISO) Setting: In some cameras, we have the number options ranging from 200 to 800 and in some cameras ranging from 100 to 1600. These numbers are the sensitivity setting of a sensor.

Flash Exposure Compensation: The flash exposure compensation is used when the flash exposure of the main object is not realized as desired.

WB White Balance: The white balance (WB) makes the white areas appear white without mixing the color. Usually, the AWB setting provides the automatic white balance setting.

Apart from these, the remaining exposure amount, the battery control, the image-recording quality, the metering mode, the exposure level display can be demonstrated on the camera screen.

1.4. Basic Shooting Methods (12)

 Automatic Mode:

In this shooting mode, everything is done by our camera. All that needs to be done is just set the frame and press the shutter button.

 Portrait Mode:

In this shooting mode, our camera opens the aperture as much as possible (small f values). In this way, the foreground becomes clear and the background becomes blurred, which is indispensable for the portrait shots.

 Landscape Mode:

In this shooting mode, our camera shuts the aperture as much as possible (large f numbers). In this way, more space becomes clear.

 Macro Mode:

In this shooting mode, our camera keeps the aperture at a medium number. In this way, high definition photos are captured, and the background becomes blurred.

 Sports Shooting Mode:

Our camera tries to keep the shutter at the highest number in the sports shooting mode. In this way, it can freeze the motion.

 Night Shooting Mode:

Our camera tries to keep the shutter at the lowest number in the night shooting mode. In this way, a long exposure period is attained.

 No Flash Shooting Mode:

As can be guessed, this is the shooting mode without flash, where the shooting mode does not require the flash, no matter how dark the media is.

 Shutter Priority Shooting Mode:

This is an automatic exposure mode, where the ISO and the shutter speed are personally set, while the aperture number is set automatically

by the camera for the correct exposure. This mode is used to freeze the motion or attain the feeling of continuity in the motion, that is to say, to control the shooting period. The shutter priority shooting mode is often used to freeze the motion and attain a long exposure period. It is not usual for normal shootings.

Aperture Priority Mode:

This is an automatic exposure mode, where the ISO and the aperture number (f-value) are personally set, and the shutter number is set automatically by the camera for the correct exposure.

Full Manual Mode:

This is the manual exposure mode. When you select the ISO, the aperture, and the shutter speed, there will be no camera control on the exposure. You can use this mode to take the exposure control just like the manual flash use.

Depth of Field Mode:

This shooting mode can easily provide the depth of field effect in the photographs.

:

This is a fully automatic exposure mode, where except for the exposure setting, all the settings like the AF mode, the metering mode, etc. are decided by the camera. The only thing you need to do is to press the shutter button and take the photo.

Program Auto:

This is an automatic exposure mode. In this program mode, you control the ISO number during the exposure, while other variables are under the camera control. Its advantage over the AUTO mode is that you can make many settings except for the exposure.

1.5. Parts of a Lens

Focus Ring: The focus is achieved by rotating the ring until the object is clear in the viewfinder.

Focus Mode Setting Button: There are two modes as <AF> (autofocus) and <MF> (manual focus). If the focus is set to <MF> mode, the auto setting is no more possible.

Zoom Ring: Zoom ring on the lens is rotated for zooming (13).

1.6. Dental Digital Systems

There must be a modified lens and a ring flash for the close-up photography and excellent object lighting. Digital technology advances fast. There were only a handful of SLR cameras in 1998. Today, there are highly expensive digital SLR models enabling high-quality images. For instance, Canon and Nikon brand digital SLR cameras with 2 inputs (Canon XSi and Nikon D60) provide satisfactory outputs in clinical photography.

The cameras equipped with sensors of 6 megapixels or above and appropriate accessories may provide acceptable image quality. The authorities in the past have agreed on the sensors of a minimum of 3 megapixels for clinical photography; whereas they nowadays think that sensors between 6 and 21 megapixels are needed (14).

1.6.1. Macro Lenses:

Dental photography has been taken into consideration together with macro photography. In clinical photography, the macro lenses having an aperture interval between 22 and 32 and a depth of field between 60 and 100 mm are needed to achieve a clear focus on an object. The lenses affect the image quality (color, image sharpness), magnification rate, operational distance, depth of field, and image distortion. The 60 mm macro lenses have some advantages over the 100 mm or above lenses. In terms of object proximity, the 60 mm lenses on the APS-C cameras allow closer proximity. Usually, these lenses can focus at a distance of 8-9 inches away from the object with a magnification rate of 1:2, whereas the 100 mm lenses have a smaller coverage area with the same magnification rate and focusing distance. They are therefore ideal for small hands to use. The 60 mm lenses have some disadvantages. While choosing a lens, the lens numbers and quality should be evaluated more carefully than the camera casing. The lenses retain their value more than the camera casing over time. It is likely that when new model cameras are launched in the market, such lenses can be transferred to these new cameras (15).

1.6.2. Flash Accessories:

Intraoral photography is based on flash photography which provides an adequate amount of object lighting. A ring flash system needs to be used inside the mouth which is very dark and where the lens can only provide the appropriate focus with the depth of field having an aperture interval of 22-32. The intraoral ring flash systems provide the light stretching to the darkest points of the mouth. Some clinicians prefer

single- or double-barrel mounted point flash systems so that they can well-provide the color and tissue details of the surface tissue (16).

1.6.3. Retractor and Mirrors:

The clinicians need a good clinical camera, lens, and flash system besides the lip reactors and the mirrors for the demonstration of anatomical structures. A typical set of plastic or metal lip retractors and special double-shaped mirrors (buccal and occlusal) are preferred. The surfaces of mirrors are usually coated with chromium, rhodium, and titanium to obtain maximum reflection and avoid image distortion. Special care should be taken against any possible damage to the sensitive coated surfaces. The use of automatic microfiber towels during cleaning and maintenance shall extend the lifetime of the mirrors (17).

1.7. Photograph Shootings in Orthodontics:

1.7.1. Face Photographs:

For each case report, the face photographs include profile photograph, front photograph, and smiley photograph, respectively. The photographs are recommended to be shot with loose lips, but those with lips slightly touching each other are also acceptable. It is evaluated that the face photographs shot at levels A (pre-treatment), A1 (mid-treatment), and B (post-treatment) are needed. Frankfurt should be routed according to the horizontal plane. These photographs should comply with the photo mounting paste, otherwise, the photo mounting paste is not needed if they are already set on the computer, provided that they are on the same plane. The photographs should be at the closest proximity of ¼ of the natural dimension, covering the lowest point of the chin and the highest point of the head (18). The photographs can be analyzed when they are vertically at ¼ of the natural dimension measured from the lower edge of the chin to the hairline of the patient. For instance, a natural dimension of 8 inches corresponds to a dimension of 2 inches or ¼ of the natural dimension in a photograph. The photographs can be printed either colorless or colorful. The photographic method used in level A should also be repeated on photographic levels of A1 and B.

The requirements for the images set on the computer are below the requirements for the digital recording. The digital photographs should be printed on glossy paper. This review recalls that all the records are official documents and therefore should not be altered (19).

1.7.2. Intraoral Photographs:

The main purpose of the intraoral photograph is to enable the orthodontist:

To review hard and soft tissue in clinical examinations.

To record the condition of hard and soft tissue before treatment

(Required to document patients with enamel white spot lesions, hyperplastic areas, and cleft gum) (20).

• It is important to get permission from the patient before taking a photo.

• The patient should sit on the chair, slightly leaning back.

• The height of the chair should be adjusted so that the patient's head is lower than that of the photographer.

• All standard views should be made in a horizontal frame.

A stable photographer position is mandatory (because the camera is held and not placed on a tripod)

Upper arm is held towards the upper body while the left hand supports the front of the lens

• The eye is pressed gently behind the eye, not the cup. The other eye is open

• To find a safe and comfortable position, the photographer's foot should be supported by the outer edge of the patient chair.

• Select the magnification according to the desired frame and focus by moving the camera back and forth.

1.7.3. Frontal View

The frontal view is important as it details the appearance of teeth as seen by patient, parent and general public.

This view is preferable in particular for general purpose and orthodontics; this is taken in landscape orientation with the teeth in occlusion filling the frame with the occlusal frame horizontal and bisecting the picture.

Large ends of larger retractor should be used

Assistant should hold both retractors, pulling all the soft tissue laterally and forward; this makes it easier for the patient to bite together in occlusion and pulls the soft tissue away from the teeth.

The midlines if they are should be at the center of frame

The dental light should always be shown directly into the patients mouth, adequate depth of field is required, so it is important to focus on lateral incisor or mesial of canine to ensure that maximum number of teeth are in focus.

The center of the image is the contact point of the upper central incisors.

The reproduction ratio is about 1: 1.8

The edges of the photograph are in vestibular oris (20).

1.7.4. Positioning the Patient (16)

Both the patient and the clinician need to be positioned correctly in a standardized manner. If there is a difference in height between the patient and the clinician, one can stand on a platform and raise it to the appropriate level camera level in the middle of the face.

In extra-oral photography, it should be tried to focus on the patient's lower eyelid in order to allow the patient to fall into the depth of field from the tip of the nose to the ear.

Front view

Vertical view extending from the frame just above the head to the lower rim line around the larynx.

The photograph should be symmetrical, the line between the pupils should be parallel to the ground7

A focus screen with grid is very useful

The patient takes a natural head position and looks straight into the camera.

Camera position in the middle of the face and in portrait format.

There should be spaces on all sides of the photo.

The light should come diagonally from the front, leaving the patient's shadow out of sight of the camera. Three types of frontal photographs are usually taken.

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CHAPTER III

ULTRASONOGRAPHY AND ITS APPLICATION AREAS IN DENTISTRY

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It is a non-invasive and non-ionizing diagnostic method that has been used in medicine for many years and provides real-time images, resulting in the reflection of sound waves from tissues. The sound waves used in this method are high-frequency sound waves (2-20 MHz), and they are much higher than the sound waves that the human ear can hear. Sound waves are obtained in ultrasonographic (USG) examinations by means of the transducers in the probes. These high-frequency sound waves are produced by the piezo-electric effect (1-4). In other words, when an alternating current is applied, crystals such as quartz in ceramic discs used as transducers expand and contract and create sound with mechanical vibration, then the sound waves reflected back in the tissues cause changes in the crystal thickness and amplified electrical signals are produced. Simultaneous images are obtained on the monitor after the electrical signals are processed. While there were materials called lead zirconate-titanate in transducers in the past, it has recently been replaced by ceramic discs and the thickness of the discs is inversely proportional to the sound frequency they produce. As the thickness of the discs decreases, the frequency of the ultrasound increases, the wavelength of the sound becomes shorter, the sound beam becomes narrower, its resolution increases and its penetration decreases. While high-frequency sound waves create a high-resolution image, they cause less penetration of sound in soft tissue and only superficial tissues can be examined (2-4). Today, thanks to the Micromachined Ultrasonic Transducer (MUT) technology, which uses silicon-based material instead of piezoelectric material, studies are carried out on the production of cheaper and wide frequency range devices (5).

In USG, the energy source (ultrasound) and image receiver (probe) are outside of the body and on the same side. During its application, the acoustic gel is used to eliminate the air layer between the probe and the skin and to reduce reflection. Images are formed as spots whose brightness changes according to the intensity of the echoes returning from the tissue.

Images can be obtained in all sections including axial, coronal, and sagittal. Ultrasound waves directed to the body by USG undergo attenuation in the form of a combination of changes such as absorption, reflection, scattering, refraction, and diffusion. The acoustic resistance of the tissue (acoustic impedance) determines the interaction of sound with tissue. The acoustic resistance is determined by the density and elasticity of the tissue. Each tissue shows a characteristic internal echo feature in relation to this resistance. Sound waves passing through surfaces with different acoustic resistance are reflected. The amount of reflection is determined by the difference in acoustic resistance of the tissues. While the reflection between soft tissues is very low, the reflection between soft tissue and bone, and between soft tissue and air surfaces is very high. The greater the difference in acoustic resistance between tissues is, the greater reflection and scattering will be (2-5).

In the images, the high signal producing regions where echoes are dense are described as hyperechoic (white), regions with low signal producing echo are described as hypoechoic (black), tissues that do not produce signals such as cysts with no echo are defined as anechoic (ultra-black), and those with equal echogenicity are defined as isoechoic. The acoustic impedance difference in air-soft tissue and bone-soft tissue interfaces is very high, so air-containing structures such as lungs and bone tissue cannot be well visualized by ultrasonography. Soft tissue, on the other hand, is very well displayed and liquid and solid can be distinguished (2). The echoes obtained in USG are shown in 3 different modes as A (Amplitude), B (Brightness), and M (Motion), depending on their origin and amplitude differences. In the field of dentistry and medicine, B mode, which is the mode in which echoes are recorded as bright spots in proportion to their intensity, and two-dimensional cross-sectional images consisting of points of different brightness are obtained on the monitor (1).

The Doppler USG method, which is the basic method in evaluating the quality and quantification of blood flow, as defined by Johan Christian Doppler, an Austrian physicist, in 1842. This method is a type of imaging obtained on the basis of the physical principle called Doppler shift which suggests "A sound source with a fixed frequency is heard more as it approaches, and less as it moves away". In other words, objects moving towards the transducer reflect the sound at a higher frequency, while objects moving away from the transducer reflect the sound at a lower frequency. The basic principle when evaluating blood flow with this method is to determine the change in the frequency of the sound beam sent to the vein at a certain angle according to the direction and velocity of the current. It is applied in 3 different ways: Pulsed Doppler, Power Doppler

(PDUS), and Color Doppler (CDUS). In Pulsed Doppler USG, the delay time between the sending and return of the sound wave is evaluated and information about where the Doppler signal comes from is obtained. In PDUS, instead of the average frequency shift of the Doppler signal, the intensity of the Doppler signal is calculated. The coloration and brightness are independent of the velocity and direction of blood flow and depend on the motile blood volume. In CDUS, tissue morphology is shown in grayscale, and blood flowing into the vessel is shown in color mode simultaneously. An image is obtained by the signals received from erythrocytes, by coding the current moving away from the probe with blue and the current approaching the probe with red, depending on the direction of the current. Even small vessels can be monitored via CDUS due to the current in them. Vascularity of tumors and tissues can be easily diagnosed with PDUS and CDUS. (1-4,6).

With contrast ultrasonography (CEUS), intravenous contrast material is given as an acoustic signal enhancer, and after the mass and vascular lesions become visible, imaging can be achieved with USG. For this purpose, gas-filled microbubbles coated with phospholipid and albumin are used as contrast agents. Thanks to these microbubbles, the backscatter signal is amplified and harmonic echoes are produced. In recent studies, it has been reported that it can be used in the imaging of microvascular perfusion of salivary gland tumors (pleomorphic adenoma, Warthin tumor, etc.) in the maxillofacial region (7,8).

With the Doppler algorithm developed with the microvascular imaging technique, also called superb microvascular imaging (SMI), which has been developed in recent years, the thinnest flows can be monitored using USG without using contrast material. With this technique, tissue movement is suppressed better than conventional methods, and algorithms and filters are used to examine the real flow more clearly. It has also been stated that SMI is superior to conventional USG in evaluating the blood flow of the salivary gland in the maxillofacial region (9).

One can have an idea about the elasticity of the tissues through ultrasound elastography (UE), which is one of the current methods. The basic principle of this method is to obtain the hardness of the tissues by using different methods. The applied force can be created in two ways, namely the compression of the applicator (freehand technique) or the high-frequency application of the transducer (shear wave). Among the signal collection methods, strain elastography (SE), in which the strain values of the tissues and the velocities of the shear waves formed in the tissues are measured, is classified as acoustic radiation force impulse imaging (ARFI), shear wave elastography (SWE), and transient elastography (TE).

Elastography is used for the differentiation between benign and malignant tumors, for the evaluation of metastatic lymphadenopathies, and for the evaluation of calcifications in clinical applications (10).

Application Areas of Ultrasonography in the Head and Neck Region

1. Evaluation of swelling in the neck region

2. Evaluation of salivary glands and their pathologies (detection of opaque-nonopaque stones, cyst, tumor, etc.)

3. Evaluation of cervical lymph node metastases

4. Evaluation of tongue carcinomas

5. Evaluation of postoperative edema and hematomas

6. Evaluation of dental abscesses and cellulitis

7. Evaluation of the distraction zone in patients undergoing distraction osteogenesis

8. Interventions for diagnosis and treatment under the guidance of ultrasonography (aspiration biopsy, temporomandibular joint arthrocentesis, intramuscular injection, anesthesia applications, etc.)

9. Scanning the tissues of the tooth structure

10. Detecting carious lesions

11. Detection of dental fractures or cracks

12. Examination of soft tissue lesions (especially through Color Doppler imaging method, evaluating the relations of vascular structures and vascular properties evaluation of blood flow in the carotid, etc.)

13. Detection and evaluation of periapical lesions

14. Evaluation of maxillofacial fractures

15. Evaluation of periodontal bone defects

16. Measurement of gingival thickness and muscle thickness

17. Evaluation of temporomandibular diseases (evaluation of intramuscular spasm areas, temporomandibular interval evaluation, etc.)

18. Evaluation of sutural opening in rapid palatal expansion patients (1,11)

19. In the evaluation of oral mucosal lesions such as oral lichen planus and leukoplakia (Ultra-high frequency ultrasonography - use of higher frequencies, ranging from 30 to 100 MHz) (12)

USG, which is an excellent soft tissue examination method, has the advantages of being very cheap, portable, and, more importantly, non-ionizing and its application areas in the head and neck region are limited. Since sound waves are absorbed by bone, its use is limited to superficial structures. However, today, the areas of its use are expanding with the developing technology, and dentists should follow up-to-date developments about USG.

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CHAPTER IV

RESULTS OF MAJOR NEURONAL MICRORNA APPLICATIONS IN NEURODEGENERATION: CURRENT STATUS

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1-Neurodegeneration and Neurodegenerative Diseases

Neurodegeneration can be defined as the progressive loss of neurons, structurally or functionally. This can be caused by the effects of aging and genetic factors, as well as due to damage during delivery. Neurodegenerative diseases include a large group of diseases like Schizophrenia, Alzheimer Disease (AD), Parkinson‘s Disease (PD), Epilepsy (Amin et al., 2018). According to the World Health Organization (WHO), more than 6% of people in the world currently have various neurological diseases. For example, now more than 50 million people have epilepsy and there are at least 47.5 million dementia patients with 7.7 million new cases annually (Amin et al., 2018). The high prevalence rate of neurodegenerative disorders is linked to reduced life expectancy of people. Most of the disorders are sporadic while rate of genetic mutations are quite low (4-5% only) (Ribeiro et al., 2013). The pathogenesis of these disorders is not clear and effective treatment is not available yet. The identification and characterization of effective therapeutic agents is carried out by animal models for neurodegenerative disorders. Transgenic models have great potential of genetic variability with the aid of vast variety of genetic tools. The development of new genetic engineering techniques makes it possible to use large animal models for neurodegenerative disorders.

Neurodegenerative disorders (NDDs) are a group of chronic progressive neurological diseases based on primary neurodegeneration. The wide pathological characteristics of varied NDDs are neuronal degeneration, depletion, glial cell proliferation, and hypertrophy at specific locations in the nervous system (Dong et al., 2019). The neuroinflammatory mechanisms in the pathogenesis of NDDs have received increasingly attention, and it has become a research hotspot in related fields (Schain et al., 2017). The role of neuroinflammation in

the progression of different neurological diseases changes according to its role in the severity of the disease (Chen et al., 2016). Studies have confirmed that the occurrence and development of many NDDs are nearly related to neuroinflammation, among which activation of microglia is considered to be the key factor (Tang et al., 2016). Microglia are the localized macrophages of the central nervous system (CNS), accounting for about 10% of the total number of adult brain cells. Microglia plays the important role of immune defense and is responsible for protecting the brain from damage and irruption by pathogens. When neurons are damaged, microglia activates and undergoes morphological changes, releasing a series of inflammatory cytokines to alleviate neuronal damage.

Intensive studies on early diagnosis and treatment of NDDs have gained a different dimension with the discovery of the structure and functions of micro RNAs (miRNAs). Given the recent promising results from new miRNA-based medicines, the potential applications of miRNAs for clinical evaluations of the progression and treatment of neurodegenerative diseases have received much attention. Defining of the neuronal identity and connectivity relies on precise control of the expression of transcriptional regulators that gradually limit cell potential. miRNAs constitute a relatively new class of these transcriptional regulators, and their emerging features appear to hold a lot of promise for NDDs. In particular, diagnosis and treatment of NDDs may be much more effective based on neuronal patterning. It has been suggested that small non-coding RNAs produced by cytoplasmic RNaseIII Dicer, called miRNAs, may play an important role in neuronal patterning through their ability to inhibit mRNA translation (Bartel, 2018).

2-MicroRNAs: Overview of Its Production and Functions

MicroRNAs (miRNAs) have emerged as main regulators of glial cells. miRNAs are small non-coding molecules that regulate gene expression. Altered expression of miRNAs has been associated with several NDD pathological processes, among which regulation of the inflammatory response is key at present. Also, miRNAs are biological markers for diagnosis and potential targets for treating NDDs (Dong et al., 2019). They are involved in a series of biological processes such as cell proliferation and apoptosis, growth and development, metabolic activation, and DNA repair in vivo, which are closely related to the occurrence and development of various diseases. miRNAs regulate the target gene mRNA mainly by cutting off the RNA molecule of target genes, inhibiting target gene translation, and inhibiting combining

(Aalaei-Andabili et al., 2016). miRNAs affect cellular and physiological function in all multicellular organisms (Gaudet et al., 2018). They are produced by Dicer enzyme processing of about 70–90 single-stranded RNA precursors with a hairpin structure (Khandelwal et al., 2019). More than 5000 miRNAs exist in humans, and each miRNA binds an average of 200 RNAs. Specific immune-modulatory miRNAs can regulate a set of RNAs in a coordinated manner, suggesting that effective miRNA-based therapeutic manipulations for neuroinflammatory conditions may be revealed (Khandelwal et al., 2019).

miRNAs act to inhibit protein expression by slightly hybridizing to complementary sequences in the 3'UTR of functionally-related target RNA transcripts (Doxakis, 2013). They display a wide diversity of expression patterns and many of them are differentially expressed during development or disease (Wienholds et al., 2005). miRNAs perform substantiality to genetic programs conceivably by two opposing means: (a) miRNA and target mRNAs are either highly expressed in mutually exclusive tissues where the miRNA blocks translation of the unwanted mRNAs expressed from leaky promoters, (b) both the miRNA and target mRNAs are co-expressed in the same tissues where the miRNA acts as resistor to protein translation to optimal levels thus enabling customized expression (Hornstein&Shomron, 2006; Peterson et al., 2009). Currently, some 2,700 mature miRNAs have been identified in humans (miRbase 22.1). Transcription of miRNAs is usually mediated by RNA polymerase II and its associated transcription factors (Cai et al., 2004). As with production of protein-coding mRNAs, transcription of different miRNAs can be elicited by the same transcription factor. For instance, the canonical pro-inflammatory transcription factor NFκB increases expression of both pro-inflammatory (miR-155) and anti-inflammatory (miR-124, miR-146a) miRNAs (Doxaki et al., 2015; Ma et al., 2014).

Transcription of a miRNA gene produces miRNA primary transcripts (pri-miRNAs), which are several kilobases long and contain local stem-loop structures (Ha&Kim, 2014). Canonical miRNA biogenesis occurs in a multi-step process that involves the key processing proteins Drosha, DGCR8, and Dicer (Fig. 1) (Gaudet et al., 2018; Daugaard&Hansen, 2017). miRNAs also can be produced via alternative pathways that generate mature miRNAs independent of Drosha/DGCR8 and/or Dicer. For canonical miRNA biogenesis, the RNase type II proteins, Drosha, along with cofactor DGCR8, form a microprocessor complex that recognizes specific motifs in the pri-miRNA (Gregory et al., 2004; Kwon et al., 2016). Ultimately, the

microprocessor complex defines the mature miRNA sequence to be used by cleaving at the stem of the hairpin structure then releasing a small RNA hairpin called the pre-miRNA (Andrew et al., 2002). Next, the pre-miRNA is exported to the cytoplasm by the nuclear transport receptor Exportin-5. Exportin-5 binds cooperatively to the pre-miRNA and a cofactor, GTP bound Ran; once in the cytosol, GTP is hydrolyzed and the pre-miRNA cargo is released (Lund et al., 2004).

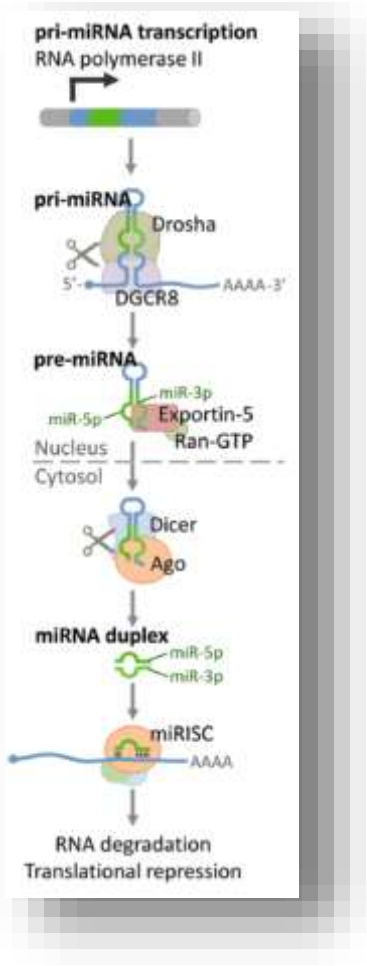

Figure 1. MicroRNA processing and function (Gaudet et al., 2018).

Most pri-miRNAs are transcribed by RNA polymerase II. The pri-miRNA is loaded into the microprocessor complex, which consists of the proteins Drosha and DGCR8. Drosha cleaves the pri-miRNA to create a small hairpin RNA, the 70- to 90-nucleotide pre-miRNA. The

pre-miRNA is bound by Exportin-5 (linked to Ran-GTP); then, the Exportin-5-miRNA complex is translocated to the cytosol. The pre-miRNA is then loaded into a protein complex including Dicer and Argonaute (Ago). Dicing the pre-miRNA results in a ~22 nucleotide long miRNA duplex. One of the strands (usually the more stable strand, which is often the -5p strand) is loaded into the Ago in the microRNA-induced silencing complex (miRISC). In animals, the miRNA seed sequence (5-7 nucleotides) binds with partial complementarity to sequences in the 3′ untranslated region of target mRNAs. The miRISC complex then directs these target mRNAs for degradation or translational repression (Daugaard&Hansen, 2017).

miRNAs that preferentially inhibit translation of many cellular anti-inflammatory proteins could drive a pro-inflammatory response. Key pro-inflammatory (miR-155, miR-27b, miR-326), anti-inflammatory (miR-124, miR-146a, miR-21, miR-223), and mixed immune-modulatory (let-7 family) miRNAs regulate neuroinflammation in various pathologies, including spinal cord injury, multiple sclerosis, ischemic stroke, and Alzheimer's disease (Gaudet et al., 2018). miRNAs represent a relatively newly emerging group whose therapeutic benefits in a variety of diseases await full exploration and exploitation.

3-Neuronal MicroRNAs

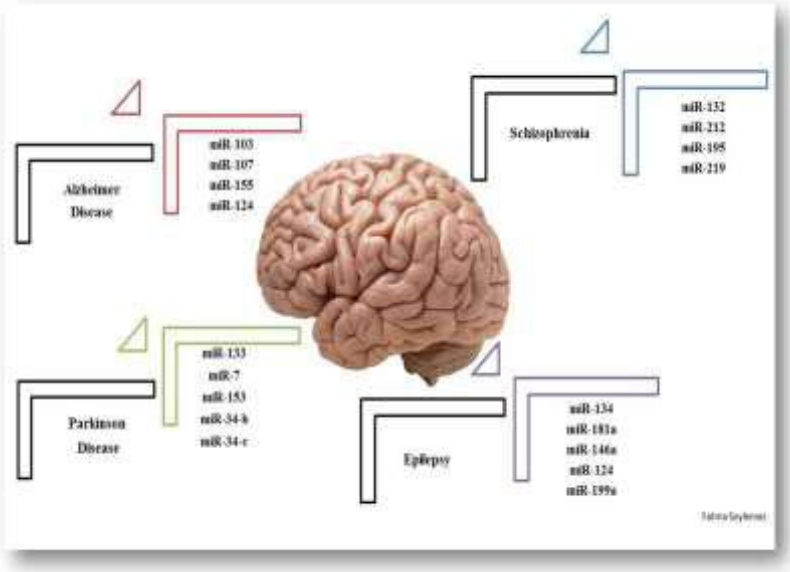

Figure 2. Major miRNAs related to neurodegenerative phenotypes.

To date, most miRNA data on the nervous system have focused on effects on development. More recently, miRNA functions related to neuronal plasticity in the mature nervous system have been discovered. Another issue that has emerged is the role of miRNAs in neurological diseases (Table 1). Here I discuss the functions of miRNAs specifically in these diseases and questions that future research may address.

Table 1. List of major miRNAs and their functions in various Neurodegenerative diseases.

miRNAs	Phenotype	Function	Reference
miR-132	Schizophrenia	Abnormalities in synaptic plasticity	27
miR-212	Schizophrenia	Abnormalities in synaptic plasticity	27
miR-219	Schizophrenia	Blocks NMDA receptors	28, 30
miR-195	Schizophrenia	Regulates Schizophrenia related genes	28, 29, 30
miR-103	Alzheimer Disease	Repress cofilin translation	36
miR-107	Alzheimer Disease	Repress cofilin translation	36
miR-155	Alzheimer Disease	Regulation of immune response	37, 38, 39
miR-124	Alzheimer Disease	Regulation of BACE1/Induce Tau pathology	41, 42
miR-133	Parkinson Disease	Regulation of NDRG1	44
miR-7	Parkinson Disease	Modify of α-synuclein expression	45, 46
miR-153	Parkinson Disease	Modify of α-synuclein expression	45, 46
miR-34b	Parkinson Disease	Decrease Park2 expression	47
miR-34c	Parkinson Disease	Decrease Park2 expression	47
miR-134	Epilepsy	Apoptosis control	54
miR-181a	Epilepsy	Induction of Apoptosis	55, 56
miR-146a	Epilepsy	Neuronal cell loss	58
miR-124	Epilepsy	CREB1 expression	60
miR-199a	Epilepsy	SIRT1 regulation	61, 62

In the NDDs, the substantial role of miRNA gene in their pathogenesis had been documented (Bilen et al., 2006). Pathogenic mechanisms in the development of psychiatric disorders and neurodegeneration are not always different and are suitable for therapeutic targeting by miRNAs. Altered miRNA profiles have been identified in diagnoses of diseases such as Schizophrenia, Alzheimer's disease, Parkinson and Epilepsy.

3.1.Schizophrenia

Schizophrenia is a chronic psychiatric disorder with a heterogeneous genetic and neurobiological background that influences early brain development, and is expressed as a combination of psychotic symptoms such as hallucinations, delusions and motivational and cognitive dysfunctions (Kahn et al., 2015). The combination of gene variants causes neurodegeneration associated with neurobehavioral syndromes caused by termination of neural networks. Hence, miRNAs have great potential for the intracellular gene silencing mechanism and regulation of target genes. During development, alterations occur in the neuronal network due to the disorder of miRNA that occurs in the mature brain. This is extremely important in the pathology of Schizophrenia and can be the regulatory factor in most of the human genes in the tissue-specific stages of development.

A group of miRNA altered in the brains of subjects diagnosed with schizophrenia, such as miR-132, miR-212, miR-219, and miR-195 deserves special attention. In the case of miR-132 and miR-212, their links to activity-dependent synaptic plasticity, and neuronal maturation, are of interest given the known defects in synaptic plasticity and connectivity observed in Schizophrenia (Mellios&Sur, 2012). miR-132 and miR-212 are involved in several abnormalities like synaptic plasticity in Schizophrenia and in neuronal cultures; the mature miR-132 stability is affected by NMDA inhibition (Beveridge&Cairns, 2012). Schizophrenia has been associated with NMDAR signaling dysfunction, and miR- 219 appears to be an integral component of the NMDAR signaling pathway. miR-219 responds rapidly to alterations in NMDAR signaling, which contributes to altered behavioral situations of schizophrenia patients (Dinan, 2010). Also, pharmacological blockade at NMDA receptors causes to down-regulated expression of miR-219.

Other important miRNAs that play a major role in Schizophrenia are miR-195, which regulate several of Schizophrenia-related genes such as brain-derived neurotrophic factor (BDNF). BDNF is a potential schizophrenia-susceptibility gene, and miR-195 is a novel regulator of

prefrontal BDNF expression in schizophrenia (Dinan, 2010). As very new information, researchers indicate that miR-195 is associated with cognitive impairment in female Schizophrenia patients, and it may be involved in the underlying mechanism of sex differences in cognitive impairment in Schizophrenia (Huang et al., 2020). It has been suggested that serum miRNAs such as miR-195 and miR-219 have a strong potential to reflect schizophrenia disease (Shi et al., 2012). The potential of different miRNA combinations to be used as biomarkers for the diagnosis of schizophrenia has begun to be investigated and promising results have been obtained (He et al., 2019).

3.2. Alzheimer Disease

Alzheimer's disease (AD) is a neurodegenerative disease with progressive impairment of behavioral and cognitive functions including memory, language, attention, reasoning, and judgment (Lane et al., 2019). The cause of AD is poorly understood. About 70% of the risk is believed to be inherited from a person's parents, with many genes usually involved. Other risk factors include head injuries, depression, and hypertension (Burns&Iliffe, 2009). The disease process is associated with plaques and neurofibrillary tangles in the brain. There is no cure for Alzheimer's disease, although there are treatments available that may improve some symptoms. AD brains have increased levels of cytoplasmic rod-shaped bundles of filaments composed of cofilin-actin in a complex (Bamburg&Bernstein, 2016). Cofilin complex affects the function of the cytoskeleton in abnormal conditions. Cofilin-actin formation mechanism although still unknown it has been determined that it plays a dynamic role in the pathogenesis of AD (Maloney&Bamburg, 2007). Studies have been conducted on miRNAs in this regard and it has been determined that miR-13 and miR-107 suppress the translation of cofilin (Yao et al., 2010). The reduction of miR-103 or miR-107 results in an increase in Cofilin protein levels in AD brains. In addition, miR-107 level was found to be decreased in postmortem AD brains.

Readhead and colleagues indicating that miR155 may be based in a point where several components of this multiplex of AD pathogenic mechanisms intersect (Readhead et al., 2020). It has been suggested that the inhibition of miR155 would represent a very effective therapeutic strategy. There are also studies suggesting that miR-155 may have therapeutic potential through regulation of T cells in AD (Song&Eun Lee, 2015). Immune response plays a crucial role in AD pathogenesis. The role of inflammatory miRNAs in AD and their ability to modulate glia responses were explored. Thus, regulation of diverse T-cell types

may reduce AD related severe pathologies. miR-155 controls characteristics such as survival, differentiation, proliferation, and activation of Th1, Th2, Th17, Treg, and CD8+ T-cells during inflammation. Guedes and colleagues suggested early miR-155 and c-Jun upregulation in the 3xTg AD mice, as well as in Aβ-activated microglia and astrocytes, thus contributing to the production of inflammatory mediators such as IL-6 and IFN-β. They reported that this effect was associated with a miR-155-dependent reduction in cytokine signaling 1 suppressor (Guedes et al., 2014).

miR-124 was first investigated in relation to Alzheimer's in 2007. Lukiw found that miR-124 expression was slightly down-regulated in AD hippocampus as compared to age-matched controls, but the difference was not significant at the $p < 0.05$ (Lukiw, 2007). miR-124 is a neuronal system-specific miRNA which play important roles in the nervous system. Therefore, its possible roles in AD pathology have been studied for a long time. It has demonstrated that miR-124 is a potent negative regulator of BACE1 in the cellular AD phenotype and might be involved in the pathogenesis of AD (An et al., 2017). They reported that miR-124 was an important modifier of development of AD which regulates the expression of BACE1 that contribute to pathogenesis of AD. Recently, researchers found that artificially replicating the abnormalities in miR-124/non-receptor–type protein phosphatase 1 (PTPN1) signaling induced tau pathology in the hippocampus of wild-type mice, including hyper phosphorylation at multiple sites, insolubility and soma dendritic aggregation, as well as learning/memory deficits (Hou et al., 2020).

3.2.1. Parkinson Disease

Parkinson's disease (PD) is characterized by its main motor symptoms bradykinesia, rigidity and tremor, but also has additional motor and non-motor characteristics. The onset of the disease is usually at an age of 65 to 70 years (Tysnes&Storstein, 2017). PD is the most common movement disorder and represents the second most common degenerative disease of the central nervous system. PD is characterized neuropathologically by the presence of α-synuclein-containing Lewy bodies in the substantia nigra of the brain. Loss of dopaminergic neurons in the substantia nigra leads to reduced facilitation of voluntary movements. The loss of dopaminergic neurons in the midbrain or the increase of protein and Lewy bodies causes PD. Also, it is known that miRNAs accompany the pathogenesis of PD. Over the last decade, many studies have been directed towards the identification of a specific miRNA signature in PD. Amongst the miRNAs analyzed, miR-133b

has been found specifically deficient in PD samples. Notably, pre-miR-133b was downregulated in PD samples more than 6 times (Kim et al., 2007). N-Myc down-regulated gene 1 (NDRG1) and junction plakoglobin (JUP) are two new transcriptional factors expressed in eleven genes that are identified by miRNA and the transcription factors associated with Parkinson's disease. It was determined that NDRG1 expression is regulated by miR-133. It was thought that transcriptional regulation was impaired in the absence of miR-133 and could lead to Parkinson's pathology. Therefore, mir-133 can be considered an important miRNA for PD.

miR-7 and miR-153 change the expression of α-synuclein in PD (Leggio et al., 2017). Doxakis found that miR-7, miR-153 and -SYN levels were higher specifically in cultured neurons, suggesting the two miRNAs as SYN expression modulators (Doxakis, 2010). Given the high levels of miR-7 and miR-153 expressed in the mouse midbrain, their deregulation may be important in PD onset. The decreased expression of miR-34b and miR-34c was shown in PD, and these changes were found in 17 regions of the brain, including the frontal cortex, cerebellum, and amygdala (Miñones-Moyano et al., 2011). The reduction of miR-34b or miR34c results in the decreased expression of Parkinson protein 2 (Park2) and Parkinson protein 7 (DJ1) and cell death. Performing in vitro studies, the depletion of miR-34b/c in differentiated SH-SY5Y neuroblastoma line resulted in cell death associated with impaired mitochondrial function and oxidative stress (Miñones-Moyano et al., 2011). Mitochondrial dysfunction is finally resulted by these kinds of cellular changes in PD.

3.2.2. Epilepsy

Epilepsy affecting 1 % of the world’s population and is a chronic neurological disorder that forms gratuitous seizures. It is caused by clusters of nerve cells in the brain which occasionally signal abnormally, causing seizures. Epilepsy is a symptom complex with multiple risk factors and a strong genetic predisposition rather than a condition with a single expression and cause (Thijs et al., 2019). The pathogenesis is thought to involve the expression of genes for controlling neural signaling, synaptic structure, cell death, and inflammation (Yihong, 2018). Anti-epileptic drugs are effective in less than half of patients, and specific antiepileptic drugs are not available (Walker, 2018). In addition, there is no evidence to show that drugs modulate the pathophysiology of seizure suppression or have other effects in research or clinical practice (Nair, 2016). As a key target,

miRNA has been shown to alter brain excitability and suppress seizures in epilepsy and other conditions (Meng et al., 2015).

miR-134, miR-181a, miR-146a, miR-124 and miR-199a play a role in the pathogenesis of epilepsy causing seizures. Evidence in vivo showed that miR-134 regulates spine volume in the hippocampus, validating the seizure-suppressive effect and suggesting a different triggering mechanism, including decreasing miR-134 reduced SE-like electrographic activity in the hippocampal neurons (Wang et al., 2014). The authors suggested that the decrease in the expression of has-miR-134 could be a potential non-invasive biomarker for use in the diagnosis of epilepsy (Spain et al., 2015). Experiments with the miR-181a antagomir showed that this particular miRNA led to the inhibition of caspase-3 expression and was up-regulated in the course of seizure induced neuronal apoptosis (Huang et al., 2015). Other research suggests that miR-181a may play a role in the impairment of cognitive function in epileptic rats by decreasing Bcl-2 protein levels and inducing apoptosis in the hippocampus. Ashhab observed a significant downregulation of miR-181a in the acute stage, an upregulation in the chronic stage and no change in the latent stage compared with the control group in a rat model (Ashhab, 2013). There is still a need for future development and discussion regarding the role of miR-181a in different stages of epilepsy.

Jun Wang et al. used a genome-wide circulating miRNA expression analysis and found that the target of miR-146a was down-regulated in patients compared with controls (Xiao et al., 2017). In cases of human TLE with hippocampal sclerosis, increased expression of miR-146a was observed mainly in regions where neuronal cell loss (Miller-Delaney et al., 2015). Cui found that the rs57095329 polymorphism in the promoter region of miR-146a is involved in the genetic susceptibility to seizure frequency, suggesting that miR-146a might be a potential biomarker for epilepsy evaluation (Cui et al., 2015). miR-124 was originally considered a key regulator in neuronal differentiation and the development of the nervous system. Its expression was suppressed in human patients with epilepsy and rats after drug induced-seizures. miR-124 alleviated seizure severity and prolonged onset latency, and a miR-124 inhibitor led to shortened onset latency in rat seizure models (Cui et al., 2015). miR-124 was also associated with the suppression of NMDAR. In addition, miR-124 injection resulted in decreased activity and expression of cAMP-response element-binding protein1 (CREB1), a key regulator in epileptogenesis models (Wang et al., 2016). These results revealed a previously unknown function of miR-124 in neuronal excitability and

provided new insight into the molecular mechanisms underlying epilepsy.

miR-199a regulates seizures and seizure damage by targeting the antiapoptotic protein silent information regulator 1 (SIRT1). Hippocampal expression levels of miR-199a, SIRT1, and p53 were quantified in a rat lithiumpilocarpine epilepsy model. Silencing of miR-199a expression in vivo was achieved by intracerebroventricular injection of antagomirs. The effects of targeting miR-199a and SIRT1 protein on seizure and epileptic damage post status epilepticus were assessed by electroencephalography (EEG) and immunohistochemistry, respectively (Wang et al., 2016). Furthermore, the seizure-suppressing effect of the antagomir was partly SIRT1 dependent. Silencing of miR-199a exerts a seizure-suppressing effect in rats, and SIRT1 is a direct target of miR-199a in the hippocampus. The effect of miR-199a on seizures and seizure damage is mediated by the down-regulation of SIRT1 (Jiang et al., 2016). Therefore, the miR-199a/SIRT1 pathway may represent a potential target for the prevention and treatment of epilepsy and epileptic damage.

4. Conclusions

Great progress has been made in the field of miRNA research, especially in the understanding of basic biology, function and disease involvement. From a neurodegeneration perspective, although the mechanical understanding and impact of miRNAs wait to be determined, researchers have found a significant number of miRNAs that have the potential to be targets in order to understand more about neurodegeneration and NDDs. Although most of the studies conducted to examine the efficacy of miRNAs in NDDs target the central nervous system, circulating miRNAs are also evaluated in terms of their potential to be biomarkers. It will also be important to understand the mechanisms of miRNA levels in NDD patients. miRNAs were previously considered as multiple targeting in in vitro and in vivo experiments, and although it is not yet clear in NDD patients, the focus is again on single or multiple miRNA targets. Focusing on multiple targeting functions of miRNAs could predict target and off target effects. For developing miRNA-based biomarkers for NDDs in humans, more powerful miRNA-profiling screens are needed to assess miRNA changes with greater confidence. There are many miRNAs that regulate numerous gene expressions. Not only gene expressions in the brain involved in NDD pathology, but also miRNA regulations in circulation have begun to be emphasized. Focusing on miRNAs in future NDD research will provide advanced knowledge about their

contribution to disease. Continued advances in studies on miRNA in the brain, circulation, and neurological disorders will lead to a better understanding, diagnosis, and treatment of neurodegenerative disorders. Therefore, it could provide new therapeutic targets for neurodegeneration and NDDs. Researchers who focus on miRNAs should great understand the causes, treatment, and diagnosis of NDDs. The exploration of these effects on the efficacy of miRNAs is worthwhile.

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CHAPTER V

SOME DRUGS USED IN THE COVID-19 OUTBREAK AND TREATMENT

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1. Introduction

Coronaviruses are common viruses found in the 21st century and are known to occur respiratory diseases in people after the 1960s (Perlman & Mcintosh, 2020). 229E and OC43 coronavirus types cause diseases in humans and they gained great importance with the SARS virus that emerged in China in 2002. Also, Middle East Respiratory Syndrome (MERS) virus, which appeared in 2012 in Saudi Arabia, has also caused respiratory diseases by infecting many people (Bartlett, 2004; Chan & Chan, 2013). Coronavirus Disease-19 (COVID-19) disease originated SARS-CoV-2 is like to the SARS-CoV virus, but it is a viral infection that occurs in Wuhan, China and causes severe acute respiratory distress syndrome (Shereen et al., 2020).

New cases of pneumonia, named COVID-19 by the World Health Organization (WHO) on February 11, 2020, were reported in December 2019 (Li et al., 2020). Epidemiological studies have shown that the virus may have emerged from a seafood market in Wuhan. In this market, there are especially fish, shellfish, hedgehogs, badgers, snakes, bats and various wild animals (Wu et al., 2020). Environmental samples taken from the market indicated that this could be the starting point of the virus. As a result of the researches, the virus was 95% similar to the coronavirus seen in bats and 75% similar to the SARS-CoV virus. Cases without contact with this market have also increased in number, suggesting that human-to-human transmission may ocur (Huang et al., 2020). It is stated that the virus spreads to people because close contact with the infected human, coughing, sneezing, and respiratory droplets.

These aerosols enter reaches the respiratory organ by inhalation through the nose or mouth (Riou & Althaus, 2020).

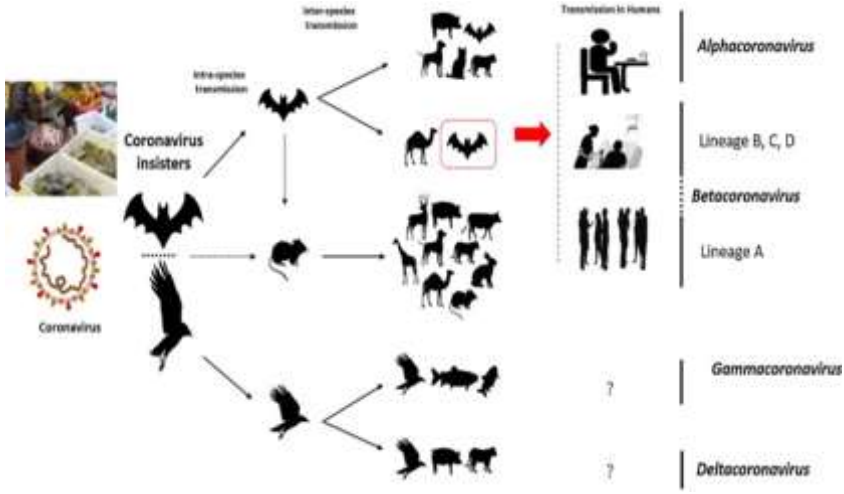

Figure 1. Transmission of coronaviruses. Only alpha and beta coronaviruses infect humans. Consuming the infected animal as food is the main cause of virus transmission to humans. Close contact with the infected person triggers transmission to humans. Black dotted arrows represent probability of bat contamination, dark black arrows represent definite contamination (Shereen et al., 2020).

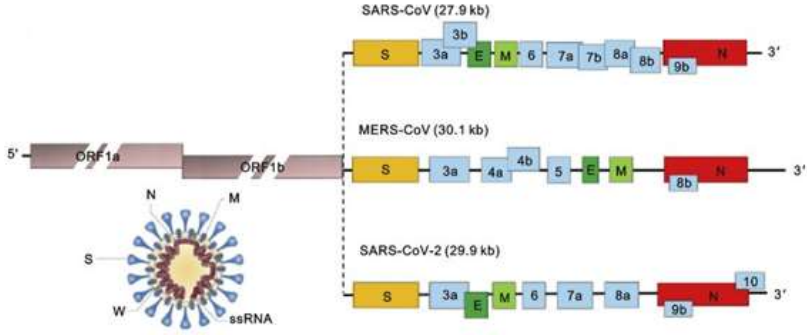

Figure 2. Genome sequences of SARS coronaviruses. 5'UTR (non-coding region), open reading frame (ORF1a/1b) encoding non-structural proteins (nsp), structural proteins (S, E, M, N), accessory proteins in the genome (3a, 6, 7a, 8a, 9b, 10) and 3'UTR zone (Li et al., 2020).

1.1 General features of coronaviruses

Coronaviruses are enveloped, single stranded positive RNA viruses which have the largest genome (26.4-31.7 kb) of RNA viruses. These viruses are divided into 4 genera as alpha, beta, gamma and delta coronaviruses and divided into three further subgroups as a, b, and c. In humans, alpha and beta coronaviruses usually cause infections in the upper respiratory tract. Apart from these infections, they can cause serious lower respiratory tract infections such as bronchiolitis and pneumonia (Lu et al., 2012). Coronaviruses also cause diseases in cats, dogs, rodents, cattle, pigs and birds (Bozkaya, 2006).

2. SARS-CoV-2

2.1 Structure of SARS-CoV-2

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is approximately 30,000 nucleotides long enveloped positive RNA virus. Its genome is similar to common coronaviruses, but contains 14 open reading frames (ORFs). Two-thirds of the 5 'terminal region in the genome where ORF1a / b is located encodes the polyproteins that make up the viral replicase transcriptase complex. These two polyproteins, pp1a and pp1b, are processed into 16 non-structural proteins (nsp1-nsp16) (Fehr & Perlman, 2015). Other ORFs in a third of the genome encode the four major structural proteins, spike glycoprotein (S), envelope (E), nucleocapsid (N), membrane (M) proteins, and several accessory proteins that are not involved in viral replication these proteins are encoded at the 3 'end of the viral genome (Masters, 2006).

S protein is a transmembrane protein consisting of 3 regions: the large external region, the transmembrane sequence, and the small internal region. S protein uses the N-terminal sequence to reach the endoplasmic reticulum and undergoes N-linked glycosylation. Trimers of the S protein form the spikes on the surface of the virüs (Beniac et al., 2006; Delmas & Laude, 1990). The trimeric S glycoprotein is a fusion protein and aids in binding to the host cell receptor (Bosch et al., 2003). Often times, the S protein of coronaviruses is cleaved by the host cell's furin-like proteins into two polypeptides called the S1 and S2 domains. The S1 region provides the receptor binding site of the S protein, while the S2 region forms the stem of the pointed molecule (de groot RJ et al., 1987).

M protein is the most abundant structural protein in the virion. The protein with three transmembrane domains forms the virion and allows the nucleocapsid to attach to the internal membranes. There is an N-terminal glycosylated ectodomain domain and a large C-terminal

endodomain domain extending into the viral particle (Nal et al., 2005). The presence of the E protein is in small amounts in the virion. This protein is found in the nucleus circles of infected cells and on the cell surface. E protein has N terminal ectodomain domain, C terminal endodomain domain, and shows ion channel activity. The E protein of the virus provides the ability to aggregate and spread (Nieto-Torres et al., 2014). The only protein found in the nucleocapsid is the N protein and this protein contains N-terminal and C-terminal domains (Chang et al., 2006). It binds the M protein with nsp3 in the N protein replicase complex. These protein structures allow the viral genome to bind to the replicase transcriptase complex and serve to keep the encapsulated genome in the form of viral particles (Hurst et al., 2013). Hemagglutinin-esterase (HE) as structural protein is only found in some coronaviruses. The protein acts like hemagglutinin, can bind to sialic acid and contains acetyl-esterase activity. These activities provide adhesion to cells of the virus and are thought to increase the spread of virus to the mucosa (Cornelissen et al., 1997; Klausegger et al., 1999).

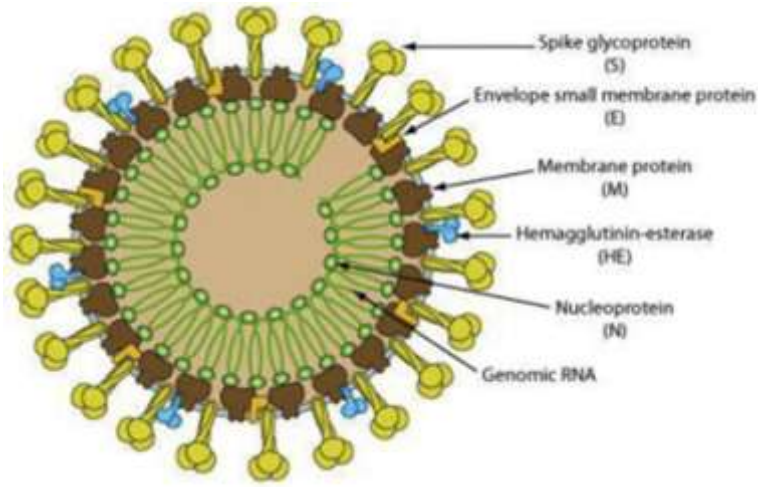

Figure 2.1. The structure of the coronavirus. S glycoprotein (S), envelope protein (E), membrane protein (M), hemagglutinin-esterase protein (HE), nucleoprotein (N), and genomic RNA (Mousavizadeh & Ghasemi, 2020).

2.2 Replication of SARS-CoV-2

Viral infection begins with binding of virus with receptor of the host cell, and this process is followed by viral envelope fusion and cell membrane. The primary target of virus is thought to be lung epithelial cells (Wan et al., 2020). The spikes of S glycoprotein on surfaces of

coronaviruses provide entry into host cell. The receptor binding site clings loosely to the virus, making it easier for the virus to bind to multiple hosts (Perlman & Netland, 2009; Raj et al., 2013). Proteases containing human airway trypsin-like protease (HAT), transmembrane protease serine 2 (TMPRSS2) and cathepsins are involved in entry of coronavirus into cells and changes in penetration (Bertram et al., 2011; Glowacka et al., 2011).

Angiotensin converting enzyme 2 (ACE2) is used as a receptor for coronaviruses (Raj et al., 2013). ACE2 is main receptor of SARS-CoV-2 and important for entry of virus to host cell in lung infection at the start. It is reported that ACE2 expresses in mouth, nose, lung epithelial cells, small intestinal enterocytes and proximal tubule (Xu et al., 2020). SARS-CoV-2 is similar to other coronavirus structures with its S protein, and expresses other membrane proteins such as RNA polymerase, 3-chymotrypsin-like protease, helicase, glycoprotein, papain-like protease and accessory proteins, polyproteins and nucleoproteins (Wu et al., 2020; Zhou et al., 2020). The lysine residue 31 on the human ACE2 receptor recognizes the 394-glutamine residue in the receptor binding site of SARS-CoV-2 (Wan et al., 2020).

When the S protein binds to the ACE2 receptor, viral infection begins. For virus to enter one cell, the S protein must be recognized by the TMPRSS2 protease enzyme, and the S protein separates into the S1 and S2 binding sites. After receptor binding, the S protein undergoes conformational changes, facilitating the viral envelope fusion with the cell membrane. It then releases the SARS-CoV-2 RNA to the host cell. Genomic RNA is split into small pieces by viral proteinases and converted into viral replicase polyproteins, polyprotein-1a (pp1a) and polyprotein-1b (pp1b). The polymerase enzyme transcribes both continuously and discontinuously, producing a series of subgenomic mRNAs and transformed into the relevant viral proteins in the final stage. Viral proteins are placed in endoplasmic reticulum, then they pass into endoplasmic reticulum (ER)-golgi space, and genomic RNA combines with viral proteins to form virions by budding in (ER)-golgi space, then transported through vesicles and removed from cell (Masters, 2006; Shereen et al., 2020).

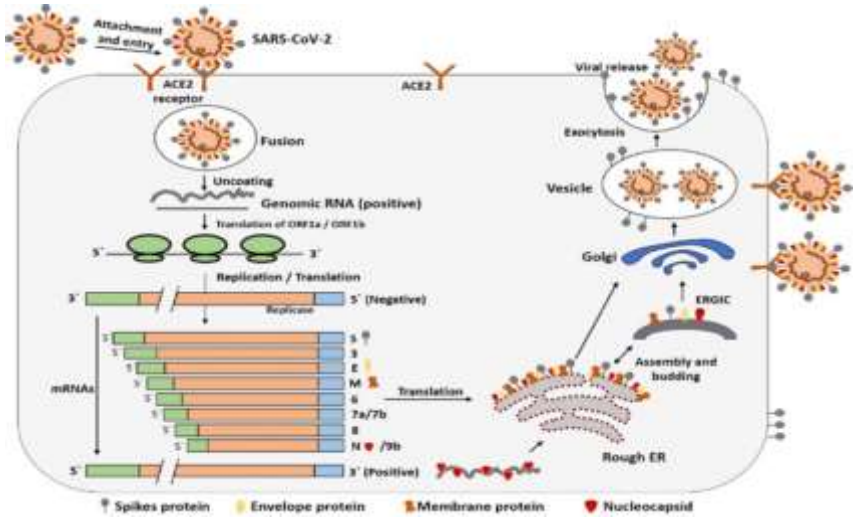

Figure 2.2. Life cycle of SARS-CoV-2 in host cell (Shereen et al., 2020).

2.3 Pathogenesis of SARS-CoV-2

SARS-CoV-2 is generally transmitted by respiratory drops and contact. Replication occurs in epithelial cells in the upper respiratory tract and in the gastrointestinal mucosa in the lower respiratory tract (Xiao et al., 2020). COVID-19 patients clinically show symptoms such as shortness of breath, cough, fever, fatigue, muscle pain, headache, nausea, diarrhea, and radiographically pneumonia. In case of progression of the disease, respiratory failure occurs with alveolar damage and death occurs (Wang et al., 2020). Patient individuals are exposed to development acute respiratory distress syndrome, which prevents sufficient oxygen from reaching the lungs and causes lung damage (Xu et al., 2020). In addition, the most important cause of death in SARS-CoV-2 is the occurrence of a strong inflammation during viral infection. After cellular damage and replication, large amounts of proinflammatory cytokines and chemokines are released (Fu et al., 2020). The severe occurrence of this release causes fatal disorders in organs and acute respiratory distress syndrome (Salvi & Patankar, 2020).

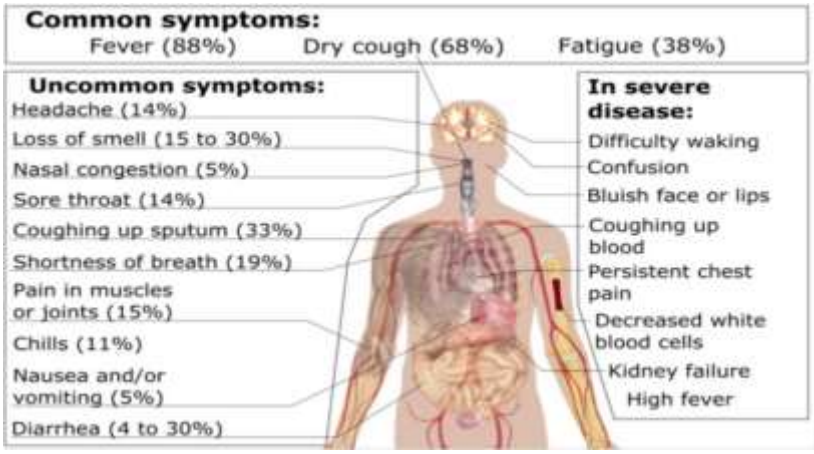

Figure 2.3. Common symptoms of COVID-19 infection (Salvi & Patankar, 2020)

2.4 Potential therapeutics and studies for SARS-CoV-2

The molecular mechanism must be well analyzed in order to use potential therapeutics in COVID-19. There are some suggestions in the studies (Glowacka et al., 2011; Holmes, 2003; Schrezenmeier & Dörner, 2020).

- SARS-CoV-2 S glycoprotein must be recognized by the host cell's TMPRSS2 protease enzyme, and the virus can enter the cell through ACE2 receptor. ACE2 and TMPRSS2 mediated cell entry can be blocked (Glowacka et al., 2011).
- Virus replication can be inhibited by targeting RNA-dependent RNA polymerase and SARS-CoV-2 main protease (Mpro) (Holmes, 2003).
- Antagonists can be used to suppress severe pro-inflammatory cytokine release (Conti et al., 2020).

According to the literature review, the most commonly used antiviral agents against SARS-CoV-2 until December 2020 are chloroquine, hydroxychloroquine, lopinavir/ritonavir, favipiravir and remdesivir.

2.4.1 Hydroxychloroquine and chloroquine

They are aminoquinoline compounds used for treatment of malaria and autoimmune diseases. It accumulates in the lysosomes of phagocytic cells and inhibits cellular mechanisms and pathways by causing changes in pH levels (Schrezenmeier & Dörner, 2020). Chloroquine and its analogs are weak bases and prevent viral fusion in

the cell by going to acid specific organelles like lysosomes or endosomes. In studies, these drugs prevent SARS-CoV-2 receptor attachment to ACE2 receptor and viral infection by affecting glycosylation of ACE2 receptor (Liu et al., 2020).

In an study, infecting Vero E6 cells with SARS-CoV-2 was revealed that chloroquine reduces viral replication by entering the lung tissues (Colson et al., 2020). Hydroxychloroquine is less permeable to blood retinal barrier and is excreted faster than retinal cells. There is less risk of retinal toxicity according to chloroquine (Singh et al., 2020). In a different in-vitro study, the dose-dependent antiviral effects of Chloroquine and hydroxychloroquine were examined using the Vero E6 cell line. Chloroquine has been observed to have a lower inhibitory effect even at the highest concentration than hydroxychloroquine (Yao et al., 2020). In a randomized clinical study of 62 COVID-19 infected patients with mild pneumonia, fever and cough were significantly reduced in group received hydroxychloroquine (Chen et al., 2020).

In some studies, it has been stated that both drugs can cause QT interval prolongation and sudden cardiac death depending on the dose (Chen et al., 2006). In a study conducted in the USA, it was stated that there are differences in QT intervals in adult COVID-19 patients received a combination of hydroxychloroquine/azithromycin. They showed that the QT interval became longer beyond the baseline and that these patients were included in the high-risk group for arrhythmia (Borba et al., 2020).

2.4.2 Lopinavir

Lopinavir is a protease inhibitor that treats HIV. Lopinavir is used with ritonavir to increase its half-life. Protease enzyme important for processing polyproteins in coronaviruses. It was reported that lopinavir was inhibited SARS-CoV virus. The lopinavir was also stated to show an antiviral effect for SARS-CoV-2 with a value of 50% in the Vero E6 cell line (Choy et al., 2020).

47 individuals infected by SARS-CoV-2 virus were assigned to the control and test group, depending on whether they were received the lopinavir or ritonavir. The control group patients took the adjuvant drug and the patients in the test group took lopinavir/ritonavir and adjuvant drugs, and the changes in fever measurement and blood biochemistry between these groups were compared. In patients on both sides, it was observed that body temperature decreased and it started to decrease regularly until the end of the treatment. The rates of alanine aminotransferase and aspartate aminotransferase, which were abnormal

levels in blood biochemistry, were observed at lower levels in test group according to control group. In addition, SARS-CoV-2 virus test results were observed to be negative (Ye et al., 2020).

The important point in the use of this drug is that the period from the onset of the side effect to the start of the drug is at most 13 days. It is thought that the treatment may be more effective in the early period (Şimşek & Ünal, 2020).

2.4.3 Favipiravir

It has been developed for bird flu and influenza virus resistant to neurominidase inhibitors. It is a drug that suppresses the activity of RNA polymerase enzyme in RNA viruses (Du & Chen, 2020). Favipiravir showed effective antiviral properties at EC50 concentration in Vero E6 cells line effected by SARS-CoV-2 (Wang et al., 2020). In a published paper, 80 patients infected by SARS-CoV-2 were separated as 35 patients who received oral favipiravir and additionally interferon-alpha with aerosol inhalation and 45 patients who received lopinavir/ritonavir with aerosol inhalation and additionally interferon-alpha (Cai et al., 2020).

It was noted that favipavir were significantly reduced fever and cough and that group treated favipiravir showed a shorter time to viral clearance compared to group treated lopinavir. In computed tomography (CT) imaging, the group treated with favipiravir with a rate of 91.43% showed a more significant improvement rate than group received lopinavir (Cai et al., 2020).

In a randomized open-label study, it was reported that early (1st day) and late (6th day) treatment with oral favipiravir showed rapid and significant viral clearance within 6 days in both patient groups (Doi et al., 2020). It has been concluded that COVID-19 will lose its effect in less than a week when favipiravir is used within the first few hours of symptoms (McCullough et al., 2020). However, taking oral antithrombotic has been recommended when there is a risk of respiratory distress or thromboembolism (Singhania et al., 2020).

2.4.4 Remdesivir

It is an antiviral drug that suppresses RNA polymerase enzyme that acts against many viruses such as Marburg, Ebola, MERS, SARS virus. By binding to the virus RNA, it terminates transcription early. Remdesivir showed strong virus inhibition at low concentrations in Vero E6 cell line infected by SARS-CoV-2 virus (Wang et al., 2020). In a previous study related to mice with MERS-CoV virus was reported virus titers in lungs and damage to lung tissue could be decreased by

remdesivir (Sheahan et al., 2020). Significant improvements were noted when remdesivir was administered intravenously to COVID-19 patients in the USA (Holshue et al., 2020). In another clinical study, using remdesivir for severe COVID-19 cases, 36 of 53 patients did not need oxygen support (Grein et al., 2020). In a placebo-controlled study with patients who recently had severe COVID-19, intravenous remdesivir and placebo administered groups were evaluated after reaching 28 days as a recovery time. As a result, it was reported that there was no change between these for mortality, recovery and viral clearance time when the groups were observed and remdesivir had no significant effect on patients undergoing severe COVID-19 (Wang et al., 2020).

2.4.5 Tocilizumab

In previous studies in SARS and MERS outbreaks, significant pro-inflammatory cytokine release TNF-α, IL-1β, IL-6, IL-8 has been reported to ocur (Lau et al., 2013; Li et al., 2013). Similar to these results, it has been recorded that the secretion of cytokines such as IL-2, IL-6, IL-10, IL-7, IFN-y, GM-CSF is excessive in COVID-19 and cytokine storm occurs (Huang et al., 2020).

SARS-CoV-2 adheres to alveolar epithelial cells and activates natural and acquired immunity, causing the emergence of many cytokines with IL-6. The increase of these pro-inflammatory cytokines allows excess fluid and blood cells to penetrate to the lung alveoli, causing impaired respiratory activity (Knudsen & Ochs, 2018; Leiva-Juárez et al., 2018). Histological examinations of biopsy samples taken by autopsy on a patient who died from COVID-19 determined fluid accumulation in the lungs, intense alveolar damage and inflammatory cells. Increased cytokine release causes pulmonary fibrosis and organ failure in patients (Xu et al., 2020).

In a study conducted, it was thought that IL-6 could play an important role in excessive cytokine release and interfering with IL-6 would be beneficial for COVID-19 and tocilizumab was used as the recombinant IL-6 receptor. Tocilizumab is also used in rheumatoid arthritis. IL-6 binds to interleukin 6 (IL-6) receptor and then binds to gp130, the cytokine receptor member in the cell membrane, showing its pro-inflammatory properties. Tocilizumab binds specifically to IL-6 receptors and prevents signal transmission by preventing IL-6 from binding to receptors. In this study, the results were evaluated by administering tocilizumab to 21 severe COVID-19 patients. Body temperature decreased to normal levels in the patients on the day after tocilizumab administration. Symptoms such as dry cough, nausea, chest pain, chest tightness improved in the following days. In the first 5 days,

15 out of 20 patients reduced their oxygen intake, and there was no need for oxygen intake in 1 patient. Lung lesions resolved on CT imaging in 19 patients. Low lymphocyte and increased C-reactive protein (CRP) levels reached normal levels within the first 5 days after tocilizumab. Before tocilizumab was given, IL-6 levels were quite high. IL-6 levels increased temporarily in serum after treatment because tocilizumab blocks IL-6 receptors. No adverse results were reported during the treatment phase. As a result, it was stated that tocilizumab could be an important therapeutic in COVID-19 disease by considering further studies (Xu et al., 2020).

2.4.6 Liposomal lactoferrin

In an study related with 75 patients with SARS-CoV-2 were supplemented with liposomal bovine lactoferrin nutritional syrup (32 mg lactoferrin (LF) and 12 mg vitamin C). The patients were isolated at home and treated remotely. Liposomal lactoferrin (LLF) was given orally in 4 or 6 doses for 10 days and an additional dose of zinc solution (LZ) was administered 2-3 times a day. In addition, nasal drops and mouth spray were given to patients with dry cough, headache, and nasal congestion. Aerosol LLF was given to patients with respiratory distress. Family individuals in contact with patients received half doses. Before taking liposomal lactoferrin (LLF) and additional zinc (LZ), patients experienced symptoms such as cough, difficulty in breathing, headache, muscle pain, fatigue, loss of taste and smell. After LLF + LZ intake, headache symptoms completely disappeared in patients 48 hours after treatment. Dry cough, muscle pain and fatigue percentages decreased significantly. No improvement in taste and odor was observed after 48 hours, but it was reported that the loss of taste and smell improved in later times. After 10 days, the improvements increased significantly. Similar results were obtained in family members treated with half the doses. By the same treatment has shown a protective character effect for COVID-19 infection in healthy individuals (Serrano et al., 2020). Lactoferrin is a glycoprotein found in milk, tears, saliva, nasal and bronchial fluids, semen, gastrointestinal fluids, vaginal fluid, urinary mucosal fluids (García-Montoya et al., 2012).

Lactoferrin has antiviral activity against DNA and RNA viruses such as Hepatitis C, Zika virüs, Herpes Simplex virus, Cytomegalovirus, Rotavirus and Human Papilloma virus (HPV) (Berlutti et al., 2011; Carvalho et al., 2017; Wakabayashi et al., 2004; Wrapp et al., 2020; Yen et al., 2011). Viruses use common molecules found in the cell membrane, such as heparan sulfate proteoglycans (HSPG), to increase spread into cells. Common molecules allow the

virus to communicate with its host. Lactoferrin, on the other hand, can bind to HSPGs, preventing the virus from coming into contact with host cells (Lang et al., 2011). Endogenous lactoferrin increases during inflammation and infection process. Lactoferrin shows antibacterial and antiviral effects, regulates the immune system, ensures the maturation of immune cells (Martorell et al., 2017).

The antiviral role of lactoferrin has been linked to the receptors viruses use to enter cells (Dix & Wright, 2018). Lactoferrin is a specific ACE2 blocker and blocks the fusion of virus S glycoprotein with ACE2 receptor in host cell (Liu et al., 2020; Wrapp et al., 2020).

Lactoferrin normalizes increased or decreased parameters in the over-release of proinflammatory cytokines and significantly reduces cytokine storm by protecting the lungs. This suggests that lactoferrin has anti-inflammatory properties (Siqueiros-Cendón et al., 2014). In addition, low zinc levels have been observed in patients infected by COVID-19, which may exacarbate the infection. Zinc exhibits strong antiviral effects and reduces both cell influx and cell fusion, so it is supported for COVID-19 infection (Ishida, 2018; Shankar & Prasad, 1998). As a result, the effects of lactoferrin on COVID-19 were found to be curative.

2.4.7 DHODH inhibitors (S312-S416)

Dihydroorotate dehydrogenase (DHODH) inhibitors are candidate therapeutics for COVID-19. DHODH enzyme provides nucleotide sources in the cell by converting dihydroorotate to orotate in pyrimidine synthesis. Nucleotide biosynthesis is very important for viral replication in virus-infected cells. Viruses need cellular pyrimidine synthesis to reproduce. Inhibition of the DHODH enzyme can restrict virus replication. DHODH inhibitors are used to limit inflammation in amelioration for rheumatoid arthritis disease (Xu & Jiang, 2020; Xiong et al., 2020). In a study, A549 lung cells with the DHODH enzyme removed were re-infected with the Influenza virus, and virus formation was observed to decrease 1000-fold in the DHODH enzyme deleted group. They stated that the presence of the DHODH enzyme is not necessary for cell proliferation, but for the replication of the virus. It is claimed that a dose-dependent inhibition was observed for brequinar and S312 and S416 as inhibitors. S312 and S416 inhibited viral replication by 90%. In addition, DHODH inhibitors have been reported to show low toxicity in the Vero E6 cell line sensitive to the SARS-CoV-2 virus. Considering the results of this study, it is revealed that DHODH inhibitors may be beneficial for COVID-19 (Xiong et al., 2020).

2.4.8 Emodin

In a study, emodin, produced from *Polygonum and Rheum* species, has been shown to block S protein and ACE2 activity in SARS coronavirus depending on the dose. Emodin, an anthraquinone derivative, is one of the main components of Rheum rhabarbarum. Emodin has anti-bacterial, anti-inflammatory and anti-cancer effects. It has been stated that emodin, an anthraquinone compound, is a virucidal agent and it has been stated that this compound can exhibit a natural therapeutic effect for COVID-19, considering the interaction of S protein and ACE2 (McKee et al., 2020).

2.4.9 Oleanolic acid, ursolic acid and carvacrol

In an in-silico study, it has been shown by molecular modeling method that oleanolic acid, ursolic acid and carvacrol have an inhibitory effect by acting on the main protease (Mpro) of the SARS-CoV-2 virus. In molecular modeling, these chemicals have been reported to bind strongly to main protease in SARS-CoV-2 virus. It is thought that oleanolic acid, ursolic acid and carvacrol may act as inhibitory agent for SARS-CoV-2 replication and regulation management of main protease (Kumar et al., 2020).

Main protease in SARS-CoV-2 virus (Mpro or 3CLpro) has an significant activity during replication (Wu et al., 2020). Triterpenoids are compounds common in nature. Ursolic acid and oleanolic acid, which are among these compounds, have antibacterial anti-cancer, antiviral and antioxidant activities (Jesus et al., 2015).

In in-vitro studies, it has been stated that oleanolic acid and its analogs show antiviral effects against HIV, influenza and hepatitis viruses (Khwaza et al., 2018). Carvacrol is a natural monoterpenoid phenol derivative and has antiviral and antimicrobial effects (Gilling et al., 2014). It is an important component of Labiatae family plants and is a molecule used for therapeutic purposes (Hyldgaard et al., 2012). In this modeling study, considering the predicted effects of oleanolic acid, carvacrol and ursolic acid and its strong binding with the main protease, it was revealed as an important inhibitor against COVID-19 infection (Kumar et al., 2020).

3. Conclusion

In the literature reviews, SARS-CoV-2 virus as structural and candidate therapeutics that can be used for cure of COVID-19 disease were evaluated. Accordingly, studies aimed at suppressing RNA polymerase enzyme activity showed positive results by inhibiting virus replication, but more comprehensive studies were needed. In addition,

the drugs used to block ACE2-dependent cell entry in studies inhibited the attachment the S protein of virus with ACE2 and prevented viral infection, but combination therapies with hydroxychloroquine should be considered to have various side effects in some patients. In addition, tocilizumab and liposomal lactoferrin, which is used to decrease cytokine storm, one of the most important death reasons in COVID-19 infection, draws attention to correct the course of the disease as a result of significant improvements.

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CHAPTER VI

PRIMARY MICROGLIA ISOLATION FROM NEONATAL MOUSE BRAIN TISSUE*

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1. Introduction

In the central nervous system, there are different cell types, neuron and glia, in terms of structure and function. The main difference between these two types of cells is that neurons have the ability to generate and transmit electrical impulses, while glia cells undertake different functions such as support for neuronal cells, myelination, and defense. Cells commonly referred to as glia cells are in fact microglia with macrophage characteristics that have different functions, astrocytes responsible for the blood-brain barrier, oligodendrocyte cells that form the myelin sheath for neurons in the CNS, thereby increasing the electrical conduction velocity, and ependymal cells surrounding the CNS cavity (Allen & Barres, 2005; Hamilton, Hillard, Spector, & Watkins, 2007; Ni & Aschner, 2010).

The neuroimmune system plays a role in the development, normal functioning, aging and injury to the central nervous system. Microglia, first discovered a century ago, are the main neuroimmune cells of the CNS and these cells have three basic functions;

1-Protective function: They are responsible for the constant perception of changes in their environment.

2- Cleaning function: It removes cellular residues or harmful structures by phagocytosis for neuronal health.

3- Defense function: Protects the CNS against harmful structures that have passed the blood brain barrier (Hickman, Izzy, Sen, Morsett, & El Khoury, 2018).

Microglia make up about 12% of the CNS cell population. Microglia, which are a part of the immune system, continuously scan the CNS to

* This work was produced from author's PhD thesis

investigate factors such as inflammation in the environment and can gather at the damaged local area by forming an enormous network (Figure 1) (Ransohoff & Perry, 2009). Thus, they make a cytotoxic intervention by secreting neurotropic factors against damage. Microglia cells are responsible for protecting the central nervous system from inflammation. These cells constitute the first line of defense and they are the main immune cells for the brain. When there is an invasion of pathogens or cellular debris, microglia cells are rapidly activated and eliminate negative structures (Gutmann & Kettenmann, 2019). Meanwhile, they secrete pro-inflammatory cytokines to mediate the inflammatory reaction. The presence of active microglia cells surrounding the lesions in various neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis has proven the importance of these cells in neurodegeneration (Xu, He, & Bai, 2016). However, overactive microglia cells may accelerate the process of some neurodegenerative diseases (183). Active microglia can release cytotoxic factors, cytokines, and chemokines. The increase in chemokine leads more microglia cells to migrate to the area of inflammation. Increased cytokine release can further damage neurons. Some researchers consider increased inflammation mediated by microglia as a possible mechanism for the occurrence of neurodegenerative diseases or damage (Magni et al., 2012; McGeer, Itagaki, Boyes, & McGeer, 1988). In addition, monitoring the activation of microglia cells in the brain is thought to be a marker for diseases such as AD and PH that show clinical symptoms in the last stages of the disease. Thus, observing overactive microglia cells in neurodegenerative diseases and inhibiting their activation appropriately is seen as an alternative way to reduce neurodegenerative damage (Xu et al., 2016) (Yıldızhan & Nazıroğlu, 2019).

Due to the heterogeneous cellular structure of the brain, it has been reported that it is very difficult to obtain pure Microglia cells at the desired level in in vivo studies (Tamashiro, Dalgard, & Byrnes, 2012). Today, the search for a better technique continues in order to produce pure and healthy microglia in a sufficient quantity for the study on the neuroprotective and neurotoxic functions of microglia cells against damage to the CNS.

The primary microglia cell isolation method, which we will explain in detail in this section, was obtained by modifying existing protocols (Gordon et al., 2011; Kato et al., 2008; Lian, Roy, & Zheng, 2016). It was clearly photographed and described so that future researchers can benefit from it (Figure 2).

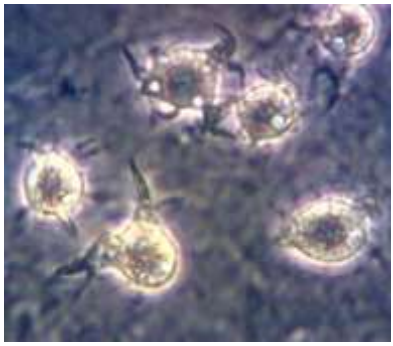

Figure 1. Microglia cells

2. Obtaining Microglia Cells

2.1. Equipment and Materials for Isolation

For the isolation of pure microglia cells from newborn P1-P3 (1-3 days old) C57BL/6J race mouse pups;

- Laminar Flow
- Incubator (5% CO_2, 37 °C)
- Ice machine
- Automatic pipettes and sterile pipette tips (1 ml, 20-200 µl, 0.5-10 µl)
- Rechargeable automatic pipette and steripipette tips (10 ml and 5 ml)
- Dissecting microscope
- Scissors, spatulas, and forceps suitable for dissection
- Centrifuge
- Inverted microscope
- Shaking water bath (37 °C)
- Ethanol (70%)
- Dulbecco's modified Eagle's medium (DMEM)
- Deoxyribonuclease I (DNase I)
- Fetal bovine serum (FBS) inactive
- Antibiotic (penicillin/streptomycin)
- Trypsin-EDTA (2.5%)
- 20% glucose solution
- Bovine insulin
- Phosphate buffered saline (PBS)
- Poly-L-lysine
- Different sizes of petri dishes
- Flask (25 cm^2 and 75 cm^2)
- Falcon (15 ml and 50 ml)
- Syringe filter (100 µm pore size)

2.2. Isolation Procedure

Currently accepted microglia studies are based on the isolation of pure cells from neonatal offspring or *in vivo* studies, taking sections from the brain tissue of animals at the end of the experiment and examining microglia cells detected with specific markers (Farber & Kettenmann, 2006). For this reason, we designed our isolation method by considering the difficulties and criticisms about microglia cell studies in the literature review (Wolf, Boddeke, & Kettenmann, 2017). The method of isolation of microglia cells was obtained by modifying the existing protocols (Gordon et al., 2011; Kato et al., 2008; Lian et al., 2016). We made sure that the new method was more understandable and applicable, and we tried to explain it with photographs as clearly as possible so that future researchers can benefit from it (Figure 2-4).

Stages of the Study;

- In line with the prepared plan, care should be taken to ensure that the offspring production is obtained within a certain schedule; the amount and proportions to be explained throughout the method are for 6-8 mice pups. The medium content used during the study was prepared in DMEM with the final concentrations of 10% FBS (inactive), 1% antibiotic (penicillin/streptomycin), 5% (20%) glucose solution, and 0.2% bovine insulin (Gordon et al., 2011; Kato et al., 2008; Lian et al., 2016).

- All materials and media were sterilized before cell isolation. Sterilized materials and chemicals were taken into laminar flow and should not be removed until the end of the study.

- In the initial stage of the study, the ice taken from the ice machine was taken into a deep bowl and a sterile gelatin was laid on it.

- When the animals were brought to the laboratory, they were placed directly on gelatin and kept until anesthesia occurred (Figure 2a). Anesthetized offspring were taken into sterile laminar flow by dipping and removing one by one in 70% ethyl alcohol placed in 50 ml falcon for sterilization (Figure 2b).

- They were sacrificed one by one using sterile forceps and scissors. The heads of the animals were placed in a container containing 10 ml of 70% ethanol (Figure 2c). After being washed here (in 70% alcohol), they were placed in one of the 4 plastic petri dishes containing 1xPBS (petri dishes on ice cubes).

- During the stages of brain tissue removal, they were taken into the other clean petri dish each time (Figure 2d). All parts except the brain were removed, the meninges were removed precisely under the microscope

(Figure 2e), the hippocampus was removed by separating the brain into two hemispheres (Figure 2f).

- Sterilization should be ensured at every stage.

- Only the brain tissue was taken to the petri dish filled with the previously prepared medium, these steps were repeated for all the mice pups. The next steps were carried out very quickly, paying attention to sterilization.

- All brain parts were placed in a 15 ml flask (for 6-8 animals) (Figure 2).

- The brain tissue was pipetted for mechanical disruption with the help of glass pipettes. Trypsin (2.5% Trypsin-EDTA and 1% final concentration) was added onto the completely creamy tissue.

- It was kept in the shaking water bath for 8 minutes at 37 °C. Deoxyribonuclease I (DNase I) was added to 1mg/ml on the tissue, then it was completed to 10 ml with Donor Horse Serum (DHS) and quickly centrifuged (300 g, 5 min).

- The supernatant remaining on the upper part of the falcon tube was thrown away and 10 ml of fresh medium was added on it and re-pipetting was performed with the help of a glass pipette.

- In order to reduce the risk of contamination, the cells were filtered into a new sterile 50 ml falcon tube with a filter that has a pore diameter of 100 μm.

- In the last stage, the cell name and date were written on each of the 75 cm^2 (250 ml) flasks. 10 ml of fresh medium was added to the cells in the falcon tube and the implantation was performed according to the number of brains (one 75 cm^2 flask per 4 brains).

- It was left in the cell culture medium at 37 °C for 10-15 days (Figure 2h). In this period of 10-15 days, the medium was checked every other day and changed according to need.

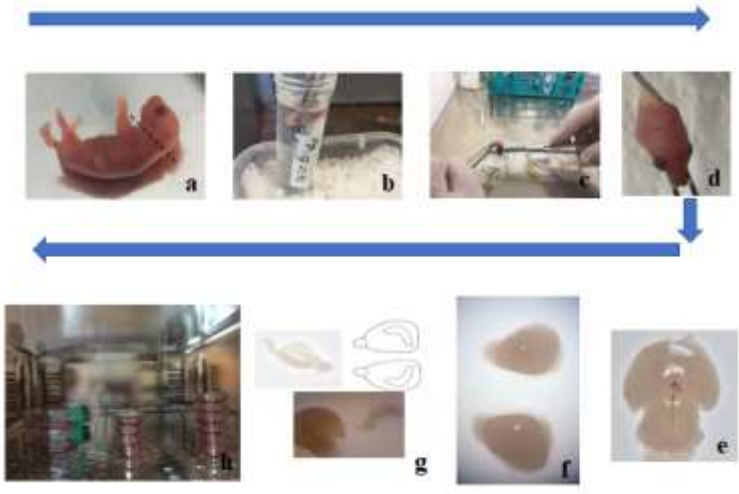

Figure 2. Taking brain tissue from neonatal mice and adapting it to *in vitro* conditions.

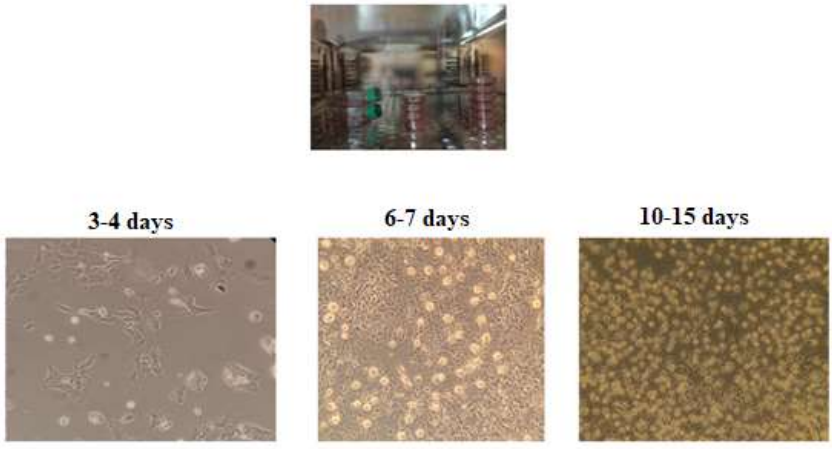

Figure 3. The brain cells reaching confluency in culture medium and the appearance of microglia cells as if budding on other cells at the top.

- Approximately 10-15th day of the isolation of microglia cells, it was observed that the microglia cells completely filled the upper surface of the flask in the flasks examined under the microscope (Figure 3).

- After this step, the mouth of the 75 cm^2 flask was wrapped with parafilm and placed on a shaker in the incubator at 37 °C and 180 rpm for 1 hour (NB-203XXL, N-BIOTEK Inc., South Korea). As stated in the literature, microglia with low adsorption properties started to float in the medium.

- Without touching the bottom of the flask, the medium was carefully drawn with the help of an automatic pipette and transferred into a 15 ml sterile falcon tube. This was done with 10 ml sterile glass pipettes. 15 ml tubes were centrifuged at 1000 rpm for 6 minutes at room temperature and the supernatant on top was discarded.

- A sufficient amount of fresh medium was placed on the microglia cells in the falcon tube and taken into containers (flasks or petri dishes) suitable for the technique to be carried out (Figure 4).

- Approximately 1-3 x 10^6 cells were obtained from 6-8 mice pups. Errors in the process will cause a decrease in the number of cells to be obtained.

- If the study is to be done with confocal microscopy or a similar technique, petri dishes should be coated with Poly-lysine beforehand so that microglia cells with low adhesion adhere to the ground.

- For poly-lysine coating (Sigma-Aldrich, 6282-5GM) under laminar flow, 50 ml of sterile water is added to 5 mg of poly-lysine. 1 ml/25 cm^2 of the prepared solution is added to the containers with the help of a pipette and it is spread over the entire surface by shaking slightly. After 5 minutes, the poly-lysine in the petri dishes are withdrawn. The petri dishes are thoroughly washed with distilled sterile water. It is left to dry for 2 hours in laminar flow, the microglia cells are cultivated in these petri dishes and left in the incubator during the working process.

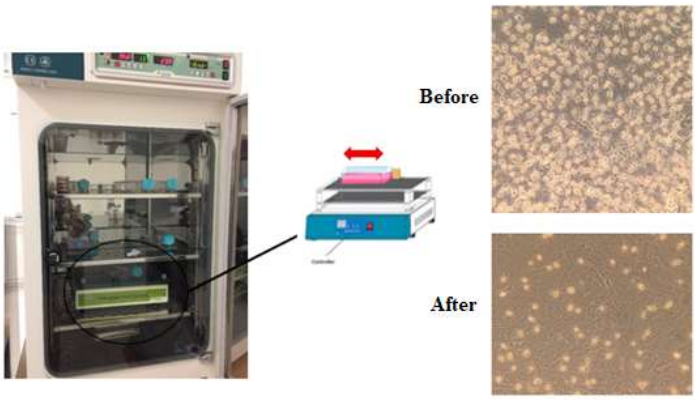

Figure 4. Separation of microglia cells from the brain cells with a shaker in the incubator (90 minutes at 180 rpm).

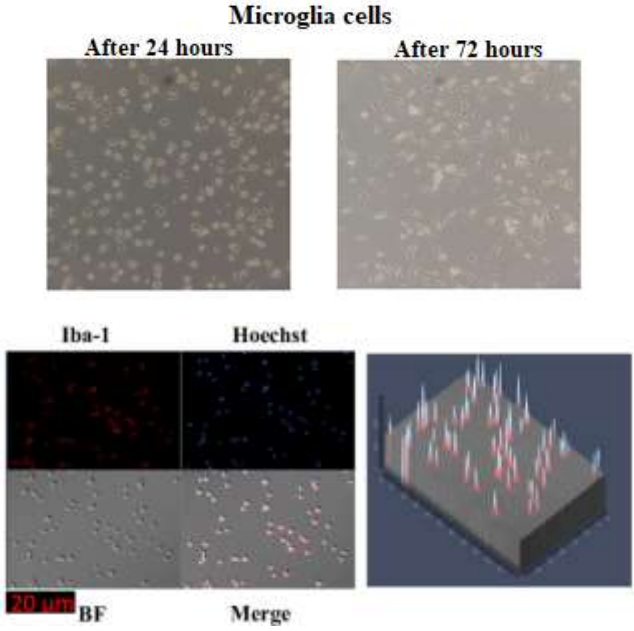

Figure 5. Images of pure microglia cells after 24-72 hours under an inverted microscope. In addition, Iba-1 microglia marker was used with laser confocal microscope (Zeiss LSM 800) to make sure that the obtained cells are pure microglia cells. (Bright field (BF): Bright field view, Merge:

Merging multiple images taken simultaneously using a laser confocal microscope).

3. Discussion & Conclusion

Microglia located in the CNS are susceptible to brain damage and disease, their morphology and phenotype are activated in response to pathophysiological brain movements. Morphologically activated microglia, like other tissue macrophages, appear in different phenotypes depending on the nature of tissue damage. The microglial response to damage suggests the acceptability of these cells as diagnostic markers of disease onset or progression, and that they may also contribute to the diagnosis/treatment of neurodegenerative diseases (Ransohoff & Perry, 2009). The persistence of activated microglia after acute injury and in chronic disease indicates tissue damage and degeneration of these cells have an innate immune memory. The microglial phenotype is also modified by systemic infection or inflammation. Evidence from some preclinical models suggests that systemic manipulations can improve disease progression, but data from other models suggest that systemic inflammation exacerbates disease progression (de Haas, Boddeke, & Biber, 2008). Systemic inflammation is associated with decreased function in patients with chronic neurodegenerative disease, both acute and long-term (Hanisch & Kettenmann, 2007). Today, the roles of microglial cells in indirect or direct neurodegenerative diseases such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis, Neuropathic pain, Traumatic brain injury, neuro-AIDS, Stroke, and Schizophrenia are being investigated.

With the new isolation protocol we have created, it will be easier to investigate the roles of microglia cells in experimental neurodegenerative disease models in vitro. To summarize the important points for obtaining pure microglia cells in a sufficient amount with our experience we have learned from previous studies (Akyuva, Naziroglu, & Yildizhan, 2021; Yildizhan & Naziroglu, 2020)with this easy and applicable protocol, sterilization should be ensured at all stages, the brain of the mice pups should not be damaged while the brain is taken, and all parts except the brain (cerebellum, hippocampus, etc.) should be carefully separated. Chemicals and medium should be fresh. In the mixed brain cell culture prepared in the first stage, the microglia cells should be shaken to allow them to float in the medium, and maximum care should be taken not to touch the other cells adhered to the bottom of the flask while the microglia cells floating in the medium are taken. Attention should be paid to the amount of time and chemical substances explained in the protocol.

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CHAPTER VII

FALLING RISK ASSESSMENT OF THE HOSPITALIZED PATİENTS IN PALLIATIVE CARE SERVICE

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1. Introduction

The World Health Organization (WHO) defines fall as an event which results in a person coming to rest inadvertently on the ground or floor or other lower level (1). Falls are a major cause of morbidity and mortality, in addition to present health problems. Although they are a significant issue in patients aged above 65 years, they are deemed important only in the presence of a comorbidity in patients aged below 65 years (2). Fall rates rise as high as 34% among patients above 65 years of age (3,4). Falls make up as high as 70% of multi-trauma patients presenting to the emergency units (2). Among multiple-trauma patients aged above 65 years, this rate is around 35% (1) and approximately 25% of these patients have at least one comorbidity (2). Falls account for 44% of all home accidents presenting to the emergency units among all age groups (5).

Often, adult patients are hospitalized in the palliative care units and majority of these patients have comorbidities affecting the quality of life. In addition to the present diseases, morbidities associated with falls further deteriorate the quality of life and bring about higher costs. Family medicine specialists following the biopsychosocial model are important factors in such cases because they put special emphasis on preventive medicine. Determining potential risks of falling for the patients and educating them on these risks while recommending mobility aids for those in need of one, should protect them from future traumas and reduce the health costs.

In this study, the risks of falling were evaluated and their relation with comorbidities were investigated in patients admitted to the Family Medicine Palliative Care Unit, Tepecik Teaching and Research Hospital.

2. Material and method

This non-randomized and cross-sectional study, designed to determine the risks of falling in patients admitted to the Family Medicine Palliative Care Unit, Tepecik Teaching and Research Hospital, was performed between November 2014 and May 2015.

2.1 Study Sample

The patients that were admitted between the above mentioned dates constituted the study sample. Since some of the inpatients were immobile and some were unconscious, the Tinetti test could be applied only for 70 patients. Between these dates, 116 patients were admitted and 60.34% were eligible according to the study criteria.

2.2 Statistical Analysis

Study data were obtained from face-to-face interviews including questionnaires and the Tinetti test results. Collected data were analyzed with the SPSS (Statistical Package for the Social Sciences) program, using descriptive statistics and chi-square test. $P<0.05$ was recognized as statistically significant.

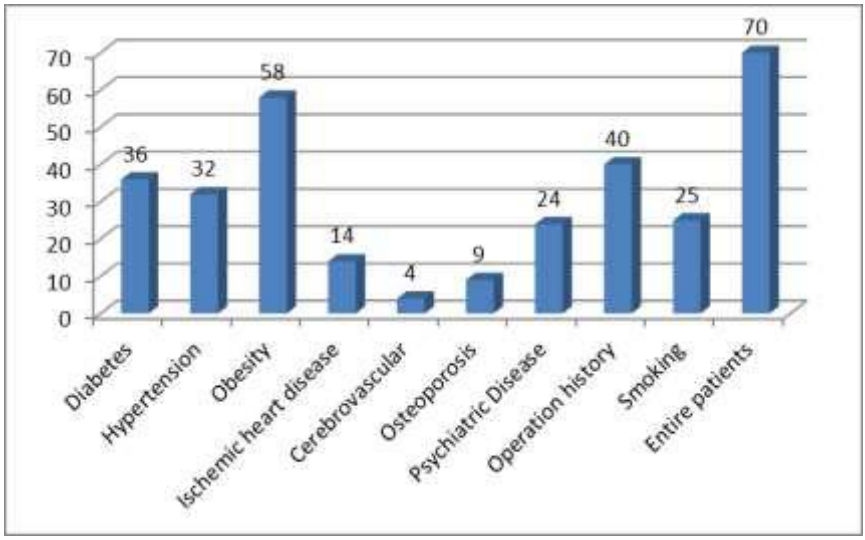
2.3 Study Method

Study was performed by applying two forms, one questionnaire including items focusing on the sociodemographic characteristics and one form including the Tinetti test. Sociodemographic characteristics included the following information on each patient: age, gender, marital status, body mass index, educational level, chronic diseases, profession, use of mobility aids, and medication. The participants were also asked if they lived alone and if they had any children. The balance and gait sections of the Tinetti test were performed and recorded as two forms. This test has been developed by Mary Tinetti, working at the Yale university (6). The Tinetti test is recognized as the gold standard assessing balance and gait disorders in the elderly. The maximum total score is 28 points; maximum balance score is 16 points and maximum gait score is 12 points. Scores ≥ 24 are deemed low risk group for falls, 19-23 are deemed moderate risk group, while scores ≤ 18 are deemed as high-risk group (7). In high-risk results, the risk has been observed to rise as high as 5-fold (8). The specificity and sensitivity of the Tinetti test have been shown to be 85% and 93%, respectively (9).

3. Results

The mean age among the study population was 56.95±14.28 (min: 21, max: 87). As 75.7% (n=53) were <65 years of age, 24.3% (n=17) were ≥65 years of age. 71.4% (n=50) of the patients were female and 28.6% (n=20) were male. The mean age among women was 58.62 ± 12.23 years, whereas it was 52.80 ± 18.20 years among men.

The primary diagnosis of the study patients was excess weight (obesity-morbid obesity) in 74.3% (n=52), diabetes mellitus in 7.1% (n=5), malignity in 4.2% (n=3), osteomyelitis in 2.9% (n=2), and other diseases in the remaining 11.5%. Other diseases included asthma, iron deficiency anemia, cervical disc disease, peripheral vascular disease, ischemic heart diseases, and gonarthrosis. The history of the patients are shown below in graphic 1.



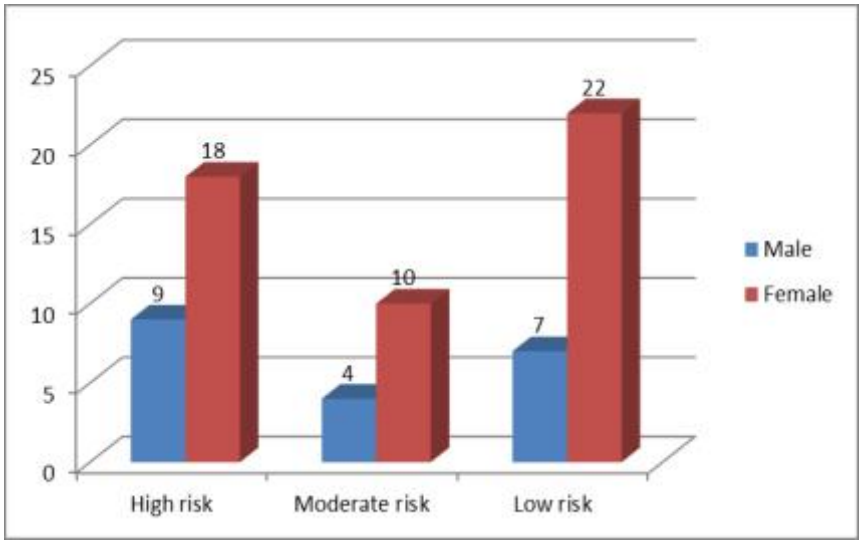
Graphic 1: The history of the study population

Based on the body mass index, 82.8% (n=58) of the study population were assessed as obese, while 8.6% (n=6) were overweight, and 8.6% (n=6) were normal. The mean body mass index value was 48.19±16.34 for all the patients. 74.3% (n=52) of the patients were not using any mobility aid, while 25.7% were using at least one mobility aid. 83.3% (n=15) of the patients with mobility aid were obese and morbid obese. As 38.8% (n=7) of the patients aged ≥65 years were using a mobility aid, this rate was 23.1% (n=12) among patients aged <65 years

Table.1 Tinetti scores relative to various parameters

	Number of patients (%)	Mean age	Tinetti score	p
Male	20	52.80±18.20	19.05±7.74	P=0,445
Female	50	58.62±12.23	19.36±8.27	P=0,445
≥65 years	19	73.47±7.07	17±7.80	P=0,030
18-64 years	51	50.80±11.02	20.11±8.08	P=0,648
Women aged 18-54 years	14	43.64±8.99	24.21±4.64	P=0,004
Women aged >55 years	36	64.44±7.35	17.47±8.64	P=0,006
History of diabetes	36	60.22±10.36	18.42±8.30	P=0,577
History of hypertension	47	59.48±11.46	19.10±8.30	P=0,663
Obese patients ≥65 years	16	71.31±5.28	17±7.80	P=0,010
Obese patients between 18-64 years	42	50.16±11.51	20.90±7.45	P=0,037
Using mobility aid	18	63.05 ±11.08	12.70±6.14	P=0,000
İnability to self-care	27	57.92±16.01	12.92±7.72	P=0,000
Living Alone	13	61 ±15.84	19.23 ±8.32	P=0,648
Low educational level	54	59,98 ±13,64	17,68±7,93	P=0,003
Divorced	9	72,66 ±9,87	13,77±9,13	P=0,030
History of diabetes and surgery	21	63,19±7,04	15,57±8,62	P=0,037
Smoking	25	51,16±15,66	20,76±7,46	P=0,805
use of antidepressant or hypnosedative	20	56.90±12,96	20,15±7,41	P=0,703
Total patients	70	56.95±14.28	19.27±8.07	

According to the Tinetti test results, 38.6% (n=27) of the patients were evaluated as at high risk for falls (score ≤15), while 20.0% (n=14) and 41.4% (n=29) were evaluated as at moderate risk (score=19-23) and at low risk for falls (score >24), respectively. In the high-risk group, 66.6% (n=18) were female and 33.4% (n=9) were male; in the moderate-risk group, 71.4% (n=10) were female and 28.6% (n=4) were male; in the low-risk group, 75.8% (n=22) were female and 24.2% (n=7) were male. The mean Tinetti score was 19.27 ± 8.07 (SD). The mean Tinetti score was 17 ± 7.80 (SD) in patients ≥65 years of age and 20.11 ± 8.08 (SD) in patients <65 years of age. The risk distribution of the study population relative to gender difference is shown below (Graphic 2).



Graphic 2. Tinetti test results of the study population

Based on the Tinetti test results, married patients were categorized as follows: 30.4% (n=14) at high risk, 21.7% (n=10) at moderate risk, and 47.8% (n=22) at low risk for falls. The same categorization for the singles was as follows: 46.7% (n=7) at high risk, 13.3% (n=2) at moderate risk, and 40% (n=6) at low risk for falls. On the other hand, among divorced patients, 66.7% (n=6) were found to be at high risk, 22.2% (n=2) at moderate risk, and 11.1% (n=1) at low risk for falls. The Tinetti test results showed a statistically significant difference relative to marital status; the divorced patients with a Tinetti result of 13.77±9.13 were at higher risk for falls ($p<0.05$).

The groups did not exhibit a significant difference relative to diabetes alone, however, those with a history of diabetes showed lower Tinetti scores (18.42±8.30). Moreover, patients with a history of diabetes combined with a history of surgery were observed to display significantly higher risk for falls (mean Tinetti score, 15.57±8.62) as compared to other groups ($p<0.05$). It is seen that history of diabetes is a major risk factor for falls and that it significantly increases the risk in the presence of comorbidities and surgical interventions.

According to the body mass index; 34.4% (n=20) of the obese patients were at high risk, 22.4% (n=13) were at moderate risk, and 44.2% (n=25) were at low risk for falls. The risk for falling was higher in patients ≥65 years of age ($p<0.05$). Similarly, the risk for falling was lower in obese and young patients than in other patients ($p<0.05$).

The patients with inability to self-care (n=27) displayed a mean Tinetti score of 12.92±7.72, showing a significant correlation with risk of falls. The patients with limited self-care abilities demonstrated significantly higher risk of falls (p=0.000).

4. Discussion

In general, the mean Tinetti score was 19.27±8.07; it was 17±7.80 in patients ≥65 years of age and 20.11±8.08 in patients <65 years of age. In total, 58.6% of the patients were at moderate and high risk for falls, while among patients ≥65 years of age, 84.1% were at moderate and high risk for falls.

A study conducted in the inner regions of Turkey evaluated 66 (23 male and 43 female) cases. The mean age was 68.45±6.2 and the mean Tinetti total score was 24.5±5.8 (10). Another study conducted in the province of Nigde assessed 90 patients above 65 years of age. The mean age was 71.11±6.62 and the mean Tinetti score was 24.58±3.62 (11). A study performed in the province of Denizli evaluated 80 patients with end-stage renal failure receiving hemodialysis. The mean age of the patients (35 male and 45 female) was 40.02 ± 8.93, while the mean Tinetti score was 24.10 ± 5.62 (SD) (12). In the present study, similar to other studies, presence of more than one chronic diseases in patients needing palliative care was observed to significantly affect the walking ability and other functions in addition to increasing the risk for falling and showing a negative influence on the Tinetti score.

Ratio of using mobility aid was 25.7% (n=18). Among patients ≥65 years of age, 38.8% (n=7) were using a mobility aid. According to the Tinetti rest results, 77.7% (n=14) of the patients using a mobility aid were at high risk and 22.3% (n=4) were at moderate risk for falls. The mean Tinetti score of patients using a mobility aid was 12.70 ± 6.14 (SD). The risk for falling was significantly higher in patients using a mobility aid (p=0.000). A study in Brasil evaluated 61 patients and found 47.9% at high risk and 10.4% at moderate risk for falls. The mean Tinetti score was 15.23±11.03 (SD) (13). The risk for falling was similar in patients using a mobility aid in the palliative care unit and in those with a history of stroke. Nonetheless, only 8% of the patients using a mobility aid had a history of stroke. The highest risk for falling was demonstrated by the patients using mobility aid. The risk for falling in these patients appears to be higher than the risk occurring after stroke.

Onat et al. evaluated a total of 164 patients, 83 elderly, and 81 non-elderly. In this study, the rate of using mobility aid was 48.2% in elderly patients and a significant correlation was found between age and use of

mobility aid (14). 38.8% of the patients aged ≥65 years were using a mobility aid. The absence of a correlation between age and use of mobility aid supports the view that chronic diseases requiring palliative care affect walking performance regardless of the age.

In the literature, low levels of education, advanced age, and female gender are mentioned among risk factors for falls. In the past, higher risk for falls in women has been associated with higher prevalence of age-related osteoporosis and musculoskeletal diseases. Low educational levels, low socioeconomic status, and poor environmental conditions have been claimed to elevate the risk of chronic diseases, indirectly leading to increased rates for falls (15). In the present study, the results obtained in the palliative care unit were consistent with those in the literature.

A study in Australia evaluated 5681 patients aged ≥65 years. In this study, the risk for falling was 31% higher in elderly and obese patients. Moreover, there was no significant difference between individuals with normal weight and elderly obese patients with regard to fall-related injuries (16). In the US, annual fall rate among people ≥65 years of age is higher than 33%. One study evaluated 31602 cases aged ≥65 years between 1998 and 2006, investigating the relationship between falls and obesity. The fall rates were significantly higher in obese patients. Class I and II obesity cases exhibited considerably higher fall-related injury risks, while morbid obesity (class III) cases displayed lower fall-related injury risks. The reason behind lower fall-related injury risk in morbid obese patients was explained by the reduction of the trauma effect by obesity (17,18). Higher fall rates in the elderly and obese patients relative to those in the literature supports that obesity is a significant risk factor for falls at advanced ages. Decreases in bone density and musculoskeletal mass over age, as well as higher arthrosis rates at advanced ages are believed to be the contributing factors making obesity a major risk factor for falls.

5. Conclusion

The above mentioned statistically significant factors (age, gender, obesity, mobility aid, educational level, marital status, history of diabetes and surgery) should always be borne in mind as risk factors for falling. In addition to the personnel in the palliative care unit, the patients and their relatives should also be educated in this matter to raise awareness and take due precautions.

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CHAPTER VIII

MANAGEMENT OF VERTICAL ROOT FRACTURES: NOVEL APPROACHES

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1.Introduction

Vertical root fractures (VRFs) are the fractures that extend through the longitudinal axis of the dental root mesiodistally or buccolingually (Tamse et al., 1999) and they comprise 2 to 5% of crown and root fractures (Gher et al., 1987). These types of fractures are reported to be the third most frequent reason of tooth loss after tooth decays and periodontal diseases (Liao et al., 2017; Takeuchi et al., 2009). Due to the classification of Leubke, VRFs can be defined as complete or incomplete according to the fragments whether separated (Dwan et al., 2014). These fractures may influence the root or expand towards periodontal attachment in cervical region (García-Guerrero et al., 2018; Liao et al., 2017).

The root which is narrower mesiodistally is more deteriorated than buccolingually for VRFs. Thus, the most prone to VRF teeth are maxillary and mandibular premolars, mandibular incisors, mesiobuccal roots of maxillary molars and mesial roots of mandibular molars (Tamse et al., 1999). Besides, VRF stated to be presented more frequently in males because of their stronger chewing forces and less elastic supporting bone (Chan et al., 1999). VRF is challenging in case of diagnosis, treatment and prognosis of the affected teeth (Unver et al., 2011).

2.Etiology

Various causes and factors may constitute the etiology of VRFs. Kallel et al., 2018). In a retrospective study, it was shown that 94% of root fractured teeth had been previously endodontically treated (Takeuchi et al., 2009). Prevalence of VRFs in root canal treated teeth is stated to be 2% to 20% (Chang et al., 2016). In endodontically treated teeth, some of the possible iatrogenic reasons of VRF are below.

- Long term usage of calcium hydroxide as intracanal medicament. Calcium hydroxide causes the changing of the organic matrix and might increase fracture risk of the root (Selden, 1996).
- Excessive removel of tooth structures during different stages of endodontic treatment (Kallel et al., 2018), and post space preparation (Morfis, 1990).
- Tapered and rigid posts cause increased stress and VRF (Cooney et al., 1986). Another possible reason of the root fractures is corrossion products of posts (Saw & Messer, 1995).
- Increased forces during lateral compaction technique have been reported to be the etiological factor for 48% to 84% of VRF (Lertchirakarn et al., 2003; Tamse, 1988). Besides, warm gutta-percha obturation techniques such as Thermafil and Obtura cause coronal strain and thermal expansion in root dentin (Lustig et al., 2000).
- Round root canal preparations of the oval canals reduce the dentin thickness and increases the risk of VRF (Masudi et al., 2012).

Clinicians are recommended to take preventive precautions to avoid occurring the VRF during and after the endodontic treatment. Moreover, warning the patients to avoid chewing hard foods or items until receiving the permanent coronal restoration is essential (Adorno et al., 2011; Mullally & Ahmed, 2000; Tang et al., 2010).

VRFs rarely might occur in non-endodontically treated teeth that is also called spontaneous VRF (Yeh, 1997). Spontaneous VRF cases have been seen most common in eastern Asia, especially in China, according to the literature (Chan et al., 1998; Chan et al., 1999). In vital teeth, the cracked tooth syndrome may be the major reason for VRFs (AAE, 2008).

3.Diagnosis

Diagnosis of the VRF is clinically difficult particularly in the early stage, because of the nonspecific symptoms that may also mimic an insufficient endodontic treatment or a periodontal disease (River & Walton, 2010). Undetermined VRF cases cause widespread bone loss,

improper treatment, and poor prognosis of the teeth (Zhang et al., 2019). Hence, accumulative symptoms and signs should be taken into consideration with the radiographic findings to achieve the certain diagnosis (Dua & Dua, 2015).

3.1. Clinical diagnosis

In most of the VRF cases, a mild pain might be the only sign (Meister et al., 1980) and it may occur as a result of lateral or occlusal forces (AAE, 2008). In case of a longitudinal fracture, narrow and deep probing defects might be seen on facial or lingual direction (Nicopoulou-Karayianni et al., 1997). Slight tooth mobility may be observed in some of the cases, while a sinus tract is often presented in many VRF cases (Khasnis et al., 2014). The origin of the sinus tract is suggested to determine by using gutta percha (Kallel et al., 2018). Moreover, VRF might be found out during root canal obturation stage by signs such as popping or cracking sound and a sharp pain (Pitts & Natkin, 1983).

There are some adjunct methods to define the fracture lines such as transillumination with a fiberoptic light, dyes staining, bite test (Barkhordar et al., 1988) and surgical revelation (Meister et al., 1980). In a case series study, Walton carried out flap reflection to a identify VRF in suspected teeth, and he indicated that the only reliable way to reach the proof of VRF is to visualize the fracture line directly by surgical exploration (Walton, 2017).

3.2. Radiological diagnosis

Various radiographic signs are possible to be seen in VRF cases (Kallel et al., 2018). Some of these signs may be vertical bone loss (Khasnis et al., 2014), expanding diffuse periodontal ligament (Meister et al., 1980), separated root fragments (Tamse et al., 1998), extrusion of filling or cement, unexplained bone loss in bifurcation area in molar teeth (Kallel et al., 2018), and bony fenestrations and dehiscence's (Tamse et al., 1999; Tamse et al., 2006). Radiographic halos (halo-like radiolucencies) that may be present through the whole root length are important signs of VRF (Kallel et al., 2018; Khasnis et al., 2014). Besides, oblique fractures usually seem as ‘step-like bone defects’ that may imitate endodontic lesions (Kallel et al., 2018).

3.2.1. Periapical radiography

Intraoral periapical radiography is firstly used and most common visualization technique to evaluate VRFs (Bechara et al., 2013; Junqueira et al., 2013). Periapical radiographs have been used for a long time at

several vertical and horizontal positions to detect the VRF due to their high resolution. However, they have limitations such as overlapping the structures because of two-dimensional imaging (Tsesis et al., 2008).

Radiographic diagnosis depends on several parameters including the angulation, density, contrast, and interpretation of the radiographic findings by the physician (Khasnis et al., 2014). Rud and Omnell reported that only 35.7% of the VRF cases may be detected by periapical radiographs, hence X-ray beam should be 4° paralel to or at an angulation to both sides of the fracture plane to visualize the fractures. (Rud & Omnell 1970). In another words, X-ray beam should get through just about directly to the fracture line to be seen on periapical radiography (Kallel et al., 2018). The visualization and detection of VRF is limited particularly when the fracture line is placed mesiodistally (Hassan et al., 2009; Kambungton et al., 2012; Jakobson et al., 2014). The major reason for this limitation other than the beam direction is the overlap of adjacent tissues in conventional radiography (Khedmat et al., 2012; Kondylidou-Sidirae et al., 2013; Youssefzadeh et al., 1999; Tsesis et al., 2008).

3.2.2. Novel approaches to detect VRFs and Cone Beam Computed Tomography

To increase the accuracy of the radiographic diagnosis, a digital subtraction radiography (DSR) technique that may determine slight changes is recommended (Kapralos et al., 2020; Mikrogeorgis et al., 2004). In this technique, there is no need a spescific equipment except the software. (Kapralos et al., 2020). DSR is based on comparing two sequential radiographs by substracting the unrevised anatomical distractions. The results of an ex-vivo study reported that DSR had a greater accuracy in VRFs of single root canal filled teeth compared with conventional digital periapical radiographs (Kapralos et al., 2020).

Recently, cone beam computed tomography (CBCT) has been commonly used for detection of VRF in in-vitro and in-vivo studies (Edlund et al., 2011; Kajan al., 2012; Makeeva et al., 2016; Wang et al. 2011). Researchers stated that CBCT has superior accuracy compared to periapical radiographs when used to diagnose VRFs in nonendodontically treated teeth (Corbella et al., 2014; Talwar et al., 2016). Moreover, CBCT has the ability of imaging the structures three dimensionally, therefore handicaps seen in intraoral radiographs such as superimposition is eliminated in this imaging method (Hassan et al., 2009; Jakobson et al., 2014).

Although CBCT provides greater imaging of the fracture line through axial, sagittal and coronal planes, radiopaque materials such as gutta-

percha and posts may be the source of artifacts and distortion because of beam hardening (Hekmatian et al., 2018; Walton, 2017). An in vitro study evaluated the validity of identification of VRFs in case root canals with or without gutta-percha. The researchers found that gutta-percha decreased the accuracy diagnosis of VRFs and suggested to image the root canals after removing filling materials to enhance the visualization (Hekmatian et al., 2018).

CBCT is indicated in detection of the suspicious root fractured cases which is difficult to image and diagnose with intraoral conventional radiographs. The European radiation protection report (No.172) recommended to use CBCT with 0.2 mm or smaller voxel sizes and limited volume in these cases where conventional intraoral radiographs are insufficient. (SEDENTEXCT, 2012; Patel et al., 2014 ESE). Consistently, Gulibire et al. suggested to use a specific mode of CBCT (increased time of exposure, smaller size of voxel) subtle cases that the teeth are endodontically treated, to enhance accuracy of diagnose based on the findings of an in vivo study (Gulibire et al., 2020). Guo et al. stated that fracture width and voxel size of CBCT have been shown to affect the accuracy of determining the VRFs of non-endodontically treated teeth (Guo et al., 2019). They found that impact of the voxel size was dependent on the type of the CBCT unit used. (Guo et al., 2019).

4.Treatment

In most of VRF cases, demolition of periodontal tissues and loss of alveolar bone may be occurred because of bacteria and irritants' entrance due to gingival sulcus is involved (Moule et al., 1999). Thus, treatment of the teeth with VRF is challenging and it depends on the location, extent, duration of the fracture as well as the tooth type (Prithviraj et al., 2014). Resection of fractured root-hemisection or root amputation might be a proper treatment option in multi-rooted teeth (Prithviraj et al., 2014). Hence, five- and ten-year retention rates of the root resected teeth reported to be 94 and 68 percent respectively, in the literature (Buhler, 1988; Langer et al., 1981).

The prognosis of single rooted teeth with VRF is suspicious, therefore extraction is usually the treatment choice (Prithviraj et al., 2014). A conservative treatment option is extracting and intentional replanting the teeth after reuniting the fractured fragments by an adhesive material. (Unver et al., 2011; Nizam et al.,2016). For this purpose, several materials and techniques have been used such as MTA (Floratos & Kratchman, 2012), Biodentine (Hadrossek & Dammaschke, 2014), adhesive resin cement (Yıldız et al., 2019), and 4 META/MMA/TBB (Unver et al., 2011).

Hadrossek and Dammaschke first used Biodentine to fill the fracture fragments in an incomplete VRF case of a maxillary central incisor before replantation (Hadrossek & Dammaschke, 2014). They noticed no pathological sign after a 2 year follow up. In a recent case report, researchers also demonstrated a successful treatment of using Biodentine with a bonding agent, fiber post and a dual-cure resin cement in complete VRF of a maxillary left central incisor. Interestingly in that case, treatment was completed without tooth extraction and replantation. The researchers suggested to fill the root canals with Biodentine for healing and joining the vertically fractured segments (Baranwal et al., 2020).

4-Methacryloxyethyl trimellitate anhyride/methacrylate-tri-n-butyl borane resin cement (4-META/MMA-TBB) has been used to bond the fractured fragments because of its superior tensile strength and tolerance to the water and blood (Miles et al., 1994; Hayashi et al., 2002; Tagami et al. 1990, Tao et al. 1991). An in vitro study reported that Super-Bond C&B was found to be higher resistant than self-adhesive dual-cured resin cement when used to attach the vertical root fractured fragments (Yıldız et al., 2019). Moreover, case reports and in vivo studies showed that successful clinical outcomes were achieved when single rooted teeth with VRFs were replanted after reuniting the fragments by 4-META/MMA-TBB cement (Super Bond C & B, Sunmedica Co., Moriyama, Shiga, Japan). (Unver et al., 2011; Nizam et al.,2016). Nizam et al. stated that periodontal parameters reduced after 6 months, and mobility was in normal limits after 12 months of replantation (Nizam et al.,2016).

Replantation with a 180-degree rotation is suggested to attach the residual healthy periodontal membrane to the periodontal connective tissue in socket wall (Kallel et al., 2018). Nevertheless, anatomical variations and root curvatures might limit the indication of rotational replantation. (Hayashi et al., 2004).

5. Conclusion

Diagnosis and treatment of VRFs are substantially challenging and a great majority of them are seen in teeth with history of endodontic treatment. For this reason, considering the predisposing factors, it is extremely important to avoid procedures that may cause vertical fractures during and after root canal treatment. CBCT and surgical exploration might be beneficial for the accurate detection of VRFs in suspicious teeth. While molar teeth with VRF are usually treated by hemisection or root amputation, single rooted teeth are mostly considered to be extracted. However, a treatment option including atraumatic extraction, attaching the fragments by a proper adhesive cement and replanting has a successful

outcome for single rooted teeth with VRF according to the current literature.

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CHAPTER IX

ALTERNARIA SP AND *CLADOSPORIUM* SP.: WHERE DO SEASONAL CHANGES PLACE ALLERGEN FUNGUS SPORES IN OUR CITY LIFE?

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1. Introduction

The air: the most essential element we need in order to survive. Besides its vital importance, it is also an important carrier. It carries a wide range of objects, such as pollens, seeds, infectious bacteria, viruses, and industrial production-based pollutant particles. All of the mentioned particles can either have positive or negative effects on the human life. In fact, the coexistence of both pollutants and allergen-effective organic particles (pollens, spores, etc.) can create even more unfavorable conditions (cross-reaction). Some of these airborne particles stand out in terms of their allergic effects and fungal spores are at the top of them. When inhaled in by allergen-sensitive individuals, it causes critical conditions such as allergic rhinitis and asthma (Bush and Portnoy, 2001-Dutkiewicz 1997). Clinical studies in children and adolescents with asthma revealed that 10% of patients developed allergic symptoms from fungal spores (Andri et al 1986). Therefore, determining fungal spores' seasonal airborne concentrations is convenient for allergen-sensitive individuals as well as allergists who treat these diseases.

Especially Alternaria sp. spore count reaches high numbers during late summer (Hyde and Willams 1946). Being one of the most significant mold fungi, *Cladosporium* sp. is a prevalent allergen in both the home and the atmosphere.

Fungal spores are known to induce numerous diseases such as chronic asthma, allergies, conjunctivitis, and hypersensitivity pneumonitis. According to a study conducted by Tilak (1991); Alternaria sp. and Cladosporium sp. are responsible for %2 - %30 of the total respiratory allergies caused by fungal spores (Pepeljnjak and Segvic 2003). The risk of death in patients is doubled when the incidence of mold spores is high

(Targonski et. al. 1995). The first local study in our country was conducted by Özkaragöz et. al. in 1967; followed by Ince and Pehlivan, 1991, Bıçakçı et. al., 1999, Tatlıdil et. al., 2001, Şakıyan and Inceoğlu, 2003, and Çelenk et. al. In 2007. Ataygül et. al. did atmospheric spores in 2007.

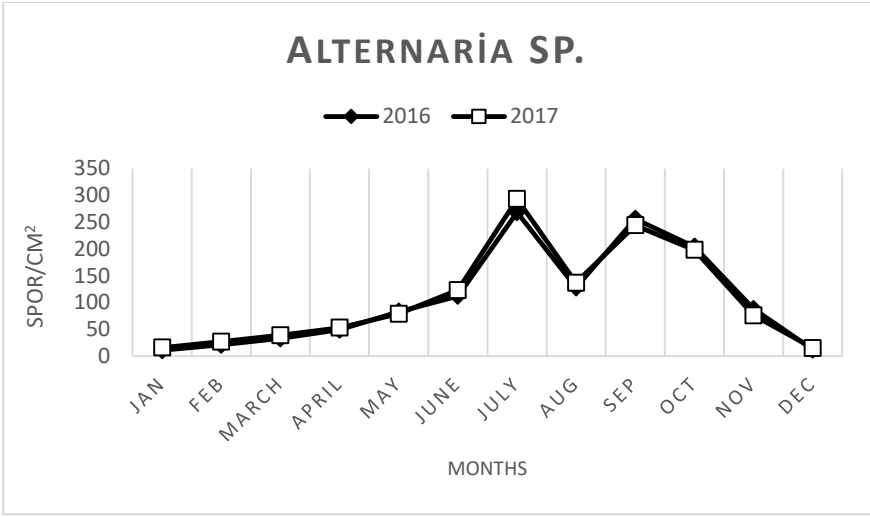
In this study, the amount of allergenic Alternaria and Cladosporium spores in Bursa were evaluated by meteorological parameters. The number of spores in the atmosphere and temporal distribution difference caused by the climate change were determined with the help of previous years' studies. Therefore, conducting these studies in a regular fashion is highly important and beneficial for the patients suffering from fungal spore allergy and their allergy specialists.

2. Material and method

According to the data of 2019, Bursa is the 4th largest city in Turkey with a population of 3.05612 million people with 40° latitude and 28° - 30° longitude. Even though the Mediterranean climate is prevalent, Black Sea climate transition areas are also present. The temperature ranges between +42.6°C and –25.7°C. Approximately, Bursa takes 113 days of rain throughout the year with 456.2 mm – 1217.4 mm precipitation. Mountainous Uludağ region is usually covered by snow in summer with forests up to a thousand meters. 92% of Bursa is suitable for cultivation, 43% is covered by forests, 44% by fields such as olive groves and 5% by meadows and pastures.

Durham sampler along with the gravimetric method was used to collect atmospheric fungus spores for this study (Durham O.C. 1946). The Durham sampler is positioned on the upper floor of a downtown building which is approximately above 15 meters above ground level and open to the air currents. Each week, basic fuchsin and glycerin gelatin slides were replaced and examined (Charpin J. and Surinyach R. 1974). Weekly spore count was observed on the preparations covered with a coverslip via an objective with 100x magnification under the light microscope. Monthly numerical changes of Alternaria sp. and Cladosporium sp. spore count is shown in Figure-1. Meteorological parameters are shown in Figure-2.

Figure-1 *Alternaria* sp. and *Cladosporium* sp. monthly distribution of sports in the atmosphere of Bursa.



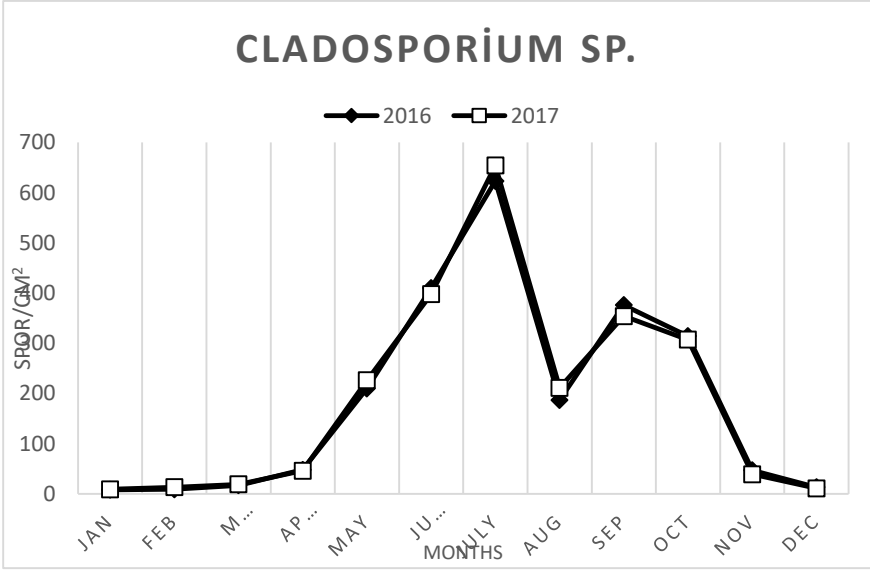
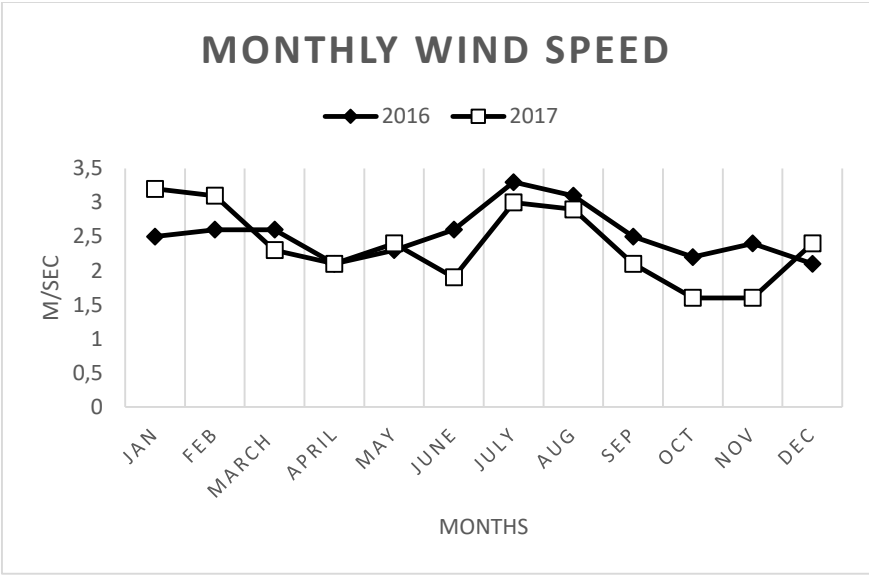
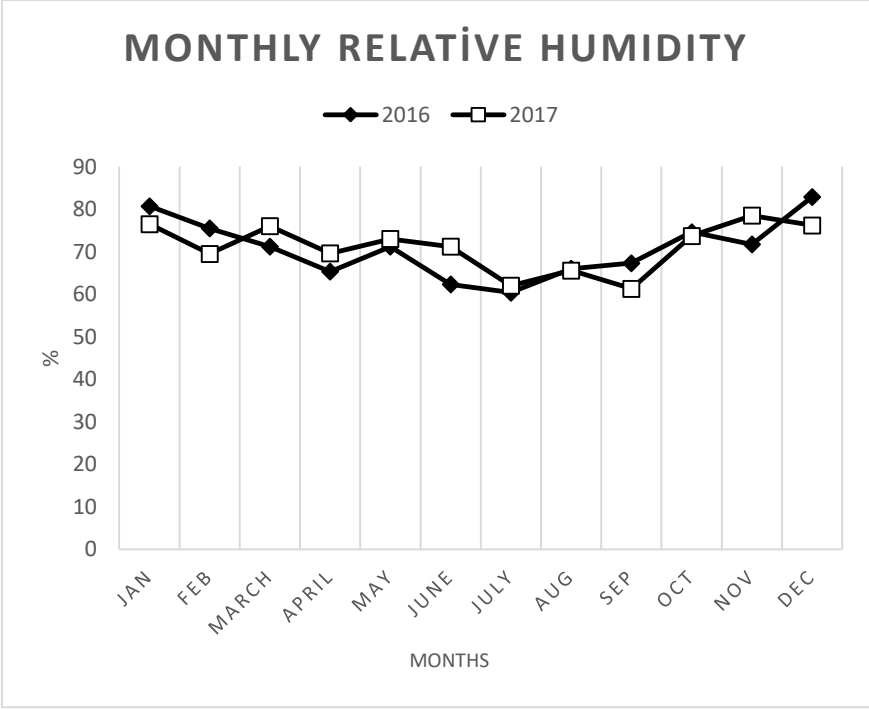
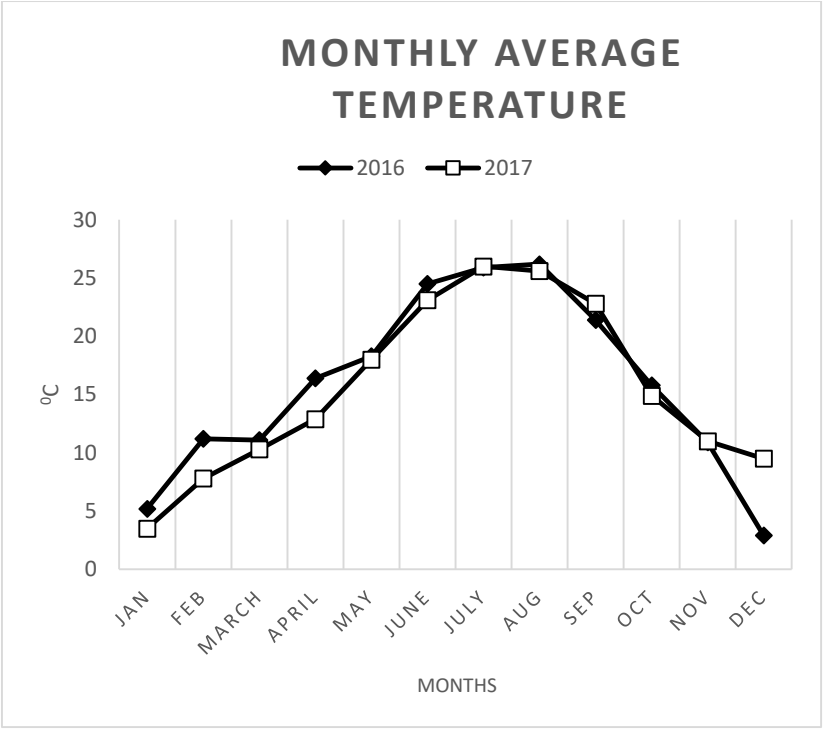
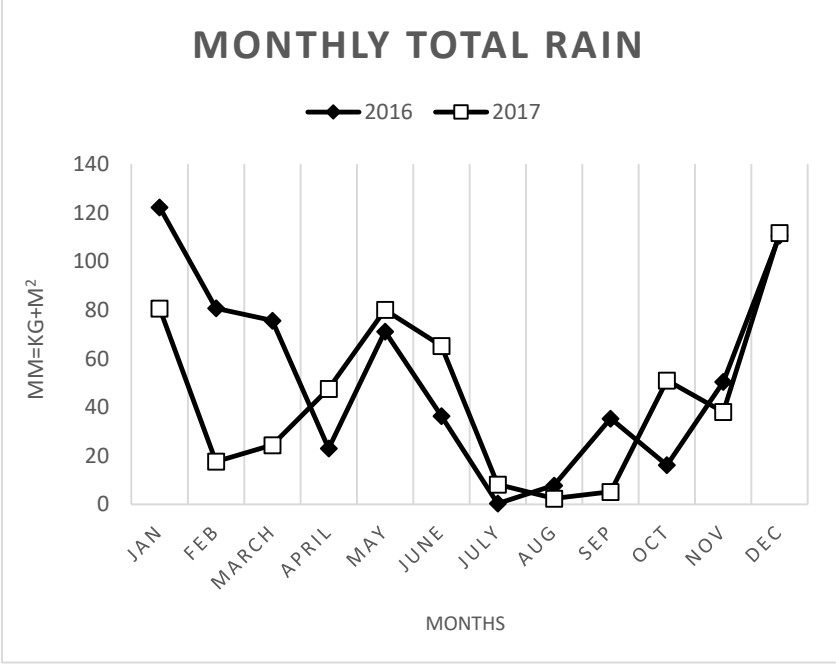


Fıgure-2 Bursa Province Monthly Average Meteorological Data






MONTHLY AVERAGE TEMPERATURE
2016
2017
30
25
20
15
10
5
0
^{0}C
JAN
FEB
MARCH
APRIL
MAY
JUNE
JULY
AUG
SEP
OCT
NOV
DEC
MONTHS


MONTHLY TOTAL RAIN
2016
2017
140
120
100
80
60
40
20
0
MM=KG+M^2
JAN
FEB
MARCH
APRIL
MAY
JUNE
JULY
AUG
SEP
OCT
NOV
DEC
MONTHS

3. Results

A total of 7114 spore/cm^2 were counted over the span of two years. Of these, 2565 spore/cm^2 belong to *Alternaria* sp. and 4549 spore/cm^2 belong to *Cladosporium* sp. The spores of both taxa were detected every month in the Bursa atmosphere.

Minimum spore concentration was observed during the months of January, February, and March for both years. The low spore concentration is due to both low temperature and precipitation. The number of spores increase along with the air temperature during April, May, and June.

The *Alternaria* sp. and *Cladosporium* sp. spore count increases significantly in July and both taxa reach their maximum spore level in the atmosphere. Even though the temperature is at its peak; the spore count is even higher due to the contributing factors of low rainfall and low average relative humidity.

Despite the high air temperature and low precipitation, the number of spores in the Bursa atmosphere decline significantly in August for both taxa. Comparatively low relative humidity and the decrease in wind speed can be the reason for this decline (Figure-2).

The spore concentration in September is observed higher than August. Lower monthly average temperature of September can be the reason for this increase. In the following months of October, November and December, the number of spores in the atmosphere decrease gradually. Decreasing temperatures and increasing precipitation are seen as the reasons for this decline.

4. Conclusion

Our study has shown that meteorological factors such as temperature, relative humidity, wind speed and precipitation effect *Alternaria* sp. and *Cladosporium* sp. Thus, the number of spores in the Bursa atmosphere change with the seasons. Both *Alternaria* sp. and *Cladosporium* sp. spores were encountered in the Bursa atmosphere in 2016 and 2017 while this study was ongoing. July was the month with highest concentration of spores for both years. After July, only month with higher spore concentration compared to its previous one was September (Figure-1). We know that the climatic conditions and vegetation of each geographical region differ. For example, Eskişehir, a nearby city of Bursa, reach its highest spore count in May (Erkara et. al.2007).

Bursa is a region with intense industrial and agricultural activities. While industrial activities decrease air quality, agricultural activities increase the number of saprophytic *Alternaria* sp. and *Cladosporium* sp.

Fungi found on plants and soil. In our study area, the agricultural harvest starts in June and continues until the end of September. Landecker (1996) pointed out that the number of spores in the atmosphere is linked to a variety of professions. Agriculture is counted as such because it causes the spores in the air to act as pathogens on plants instead. Elevated level of spores was detected in Bursa atmosphere once the period of crop cutting and bailing started. Grains, fruits, and vegetables stored after the harvest further supports the growth of the stored fungi (Landecker 1996).

Cladosporium sp. Spores were more abundant than the spores of *Alternaria* sp. in the Bursa atmosphere. Similarly, Davies (1965), Hamilton (1959), Vittal and Krishnamoorthi (1981) have also observed greater *Cladosporium* sp. spores compared to any other fungus species. Spore levels were at minimum during the first 3 months of the year. In earlier studies, it was shown that lower temperatures cause a decline in spore numbers in the atmosphere (Halwagy 1989; Hjelmros 1993). In April, a rise in rainfall and temperature increases the concentration of spores in the atmosphere. Enough precipitation combined with elevated temperatures are observed to optimize the conditions for *Alternaria* sp. and *Cladosporium* sp. sporulation. After precipitation, a significant increase in spore concentration is observed (Kramer et. Al. 1959). Higher temperatures were recorded in June compared to May. Which resulted in higher *Alternaria* sp. and *Cladosporium* sp. spore concentrations. Once the relative humidity rises above 45% and wind is stong, concentration of *Alternaria* sp. spores increase (Hjelmros 1993). Moreover, wind speed had the greatest influence on the spore concentration. Especially when the other climatic factors were optimal.

In conclusion, the highest level of *Alternaria* sp. and *Cladosporium* sp. spores in Bursa atmosphere were observed in July for both years. The highest *Alternaria* sp. and *Cladosporium* sp. spore concentration in Kemalpaşa province of Bursa was in July (Bicakci et. al. 2001). In Burdur (Tatlidil et. al. 2001) and Ankara (Sakiyan and Inceoglu 2003) the spore concentration at its highest was during July. Ataygul (2007) found high concentrations of *Alternaria* sp. and *Cladosporium* sp. spores in Bursa atmosphere. The *Alternaria* sp. spore concentration peaked during July and the *Cladosporium* sp. Spore concentration peaked during Juna in Bursa atmosphere(Ataygul et. al. 2007). We found a positive correlation between mean temperature and the spore concentration in the atmosphere (Figure-1, Figure-2), as have Corden and Willington (2001) in Derby, UK. Moreover, we agree with Mitakakis et. al. (1997)'s study in Australia which found a slight negative correlation between daily precipitation and the spore concentration in the atmosphere. Gregory and Hist (1957) found the highest *Alternaria* sp. spore concentration in August, in warm dry

spells after a period of wet weather. Therefore, seasonal changes, urbanization, agricultural and industrial activities in the geographic region affect the monthly concentration of spores in the atmosphere. Along with the global warming, a sudden heavy rain followed by a warm air during the summer months changes the number of spores greatly. Determining local allergenic airborne fungal spores' monthly concentrations and progresses will benefit patients who are sensitive to these allergens and suffer from asthma or rhinitis. Patients should take some measurements during the dense allergen spore periods because one of the leading treatments for spore-sensitive cases is avoiding these allergens. It is recommended that, fungal spore sensitive allergen individuals and people with allergic diseases to take a shower after coming back to their homes, change their clothes and clean their leaves in their gardens while having a mask on.

Examining the atmospheric spore distributions and regularly monitoring them and sharing them with allergy specialists will facilitate the treatment process of allergy patients.

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CHAPTER X

DIAGNOSTIC CONTRIBUTION OF THE ^{68}Ga-DOTATATE PET/CT IMAGING IN PATIENTS WITH NEUROENDOCRINE TUMOR PRESENTING WITH LIVER METASTASES

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1. Introduction:

Primary focus cannot be detected by routine imaging in 12-22% of neuroendocrine tumor (NET) patients (1-4). According to the SEER Database, the NET rate with an unknown primary focus is 0.84/100.000 in the USA, and they constitute <5% of patients with metastatic cancer of unknown primary focus (1,5). In NET patients,detection of the primary focus and complete determination of metastatic lesions are important in terms of appropriate treatment planning and surgical treatment with complete excision or debulking method for the primary focus.

According to the NCCN Guideline, thorax computerised tomography (CT) and Abdominal CT/magnetic resonance imaging (MRI) should be used for routine imaging in patients with NET of unknown primary origin. If the primary focus could not be detected by these studies,

somatostatin receptor scintigraphy, ^{68}Ga-PET/CT imaging, upper GIS endoscopy and colonoscopy are recommended (6).

Somatostatin receptor scintigraphy (In-111-Octreotide) has been used in molecular imaging for many years, and the effectiveness of this modality in primary focus detection was limited (4,7). PET/CT imaging with ^{68}Ga-DOTA-Octreotide analogues such as ^{68}Ga-DOTA TATE, ^{68}Ga-DOTATOC, and ^{68}Ga-DOTANOC have much higher affinities for somatostatin receptors than In-111-Octreotide for staging and metastases detection in NET patients (8,9,10).

The aim of our study was to investigate the diagnostic contrubitionof the ^{68}Ga-DOTA TATE PET/CT imaging in detecting primary focus and the extent of metastasis in patients with NET presenting with liver metastases.

2. Material and Methods:

2.1. Patients:

15 patients with a diagnosis of NET presenting with liver metastases and who had ^{68}Ga-DOTATATE PET/CT imaging between July 2017 and December 2020 for primary focus research were included in the study. ^{68}Ga-DOTATATE PET/CT images were retrospectively re-evaluated. Medical data of the patients and histopatological findings of existing lesions were obtained from the hospital information processing system.

All patients signed written informed consent forms for the purpose of reviewing and publishing their results. Ethics committee approval was obtained with decision number 449, dated 15.12.2020 for this clinical study which was designed retrospectively

2.2. ^{68}Ga-DOTATATE PET/CT imaging protocol and Imaging analysis:

A fully automated Scintomics GRP synthesis module with Scintomics Control Center and GRP-Interface software was used for the radiolabeling of ^{68}Ga-DOTATATE. The ^{68}Ge/^{68}Ga generator was purchased from iThemba LABS, South Africa. DOTATATE was purchased from Scintomics GRP, Germany via a local distributore. Whole-body PET-CT scans were performed using LSO-based full ring PET scanner (Siemens Biograph 6, Chicago, IL, USA). 2 MBq/kg ^{68}Ga-DOTATATE was injected intravenously. After 1 hour, whole-body CT scans were obtained from the base of the skull to the upper thigh with a slice collimation of 5 mm and a slice interval of 3.4 mm. The emission data were acquired for 2,5 min per bed (6-7 beds), which were later attenuation corrected with the digital CT data. Image reconstruction used ordered subsets expectation maximization algorithm of 2 iteration and 8 subsets. Image analysis was carried out on the Esoft multimodality computer platform (Siemens Medical Solutions, Erlangen, Germany). Images were

visually interpreted by consensus between two experienced nuclear physicians. The foci of uptake were identified as representing a tumor if the accumulation of ^{68}Ga-DOTATATE was increased relative to comparable normal contralateral or surrounding tissues.

2.3. Statistical analysis:

During the evaluation of the study data, descriptive statistical methods such as mean, median, frequency, ratio, minimum, and maximum value were used.

3. Results:

11 of the patients included in the study were female and 4 were male. The mean age was 57.8 (39-79). According to the biopsy results from liver metastases, 3 patients were Grade 1, 9 patients were Grade 2, and 3 patients were Grade 3 NET The mean Ki 67 index was 9.06 (1-30).

With ^{68}Ga-DOTATATE PET/CT imaging, the primary focus could be detected in 10 (66.6 %) of 15 patients. In 7 (46.6 %) of 12 patients, metastatic lesions were detected outside the liver. The lesion identified as the primary focus was in the pancreas in 3 patients, the lung in 1 patient, the rectum in 1 patient, the ovary in 1 patient, the ileum in 1 patient and the abdominal-pelvic retroperitoneum in 3 patients. Extrahepatic metastases detected by ^{68}Ga-DOTATATE PET/CT were bone in 3 patients, mediastinal lymph nodes in 3 patients, pelvic lymph nodes in 2 patients, supraclavicular lymph nodes in 1 patient, abdominal lymph nodes in 2 patient, and peritonitis carcinomatosis in 1 patient.

In 3 patients, bone and mediastinal lymph node metastases could not be detected by routine imaging and were detected by ^{68}Ga-DOTATATE PET/CT. In 1 patient, in addition to abdominal lymph node metastases, metastatic lymph node in the supraclavicular region was detected with ^{68}Ga-DOTATATE PET/CT.

4. Discussion:

In NET patients with unknown primary, detection of the primary tumor and accurate disease staging are crucial in terms of determining what operative approach will be taken and planning appropriate medical treatments.

In a study by Prasad et al., they found that ^{68}Ga-DOTANOC PET/CT identified the primary site in 35 out of 59 patients (%59) with the most commonly identified site being pancreas, followed by small intestine (11). In a similar study by Pruthi et al. ^{68}Ga-DOTANOC PET/CT identified the primary location in 40 out of 68 patients (%59) and most common primary location was small intestine (12). In our study, detection rate of primary focus was higher compared to these studies. We were able to identify the primary focus in 10 out of 15 (66.6 %) patients presented with hepatic metastases. The primary focus was in the pancreas in 3 patients, the lung in 1 patient, the rectum in 1 patient, the ovary in 1 patient, the

ileum in 1 patient and the abdominal-pelvic retroperitoneum in 3 patients. The reason for the higher detection rate of our study could be the differences in the diagnostic tests that patients in our study population previously underwent.

In the present study, ^{68}Ga-DOTATATE PET/CT detected extrahepatic metastases in 7 out of 15 patients. Lymph node metastases were detected in 6 patients and skeletal metastases were detected in 3 patients on ^{68}Ga-DOTATATE PET/CT imaging. In 3 patients, bone and mediastinal lymph node metastases could not be detected by routine imaging and were detected by ^{68}Ga-DOTATATE PET/CT imaging. In a study by Naswa et al., lymph node metastases were detected in 3 out of 20 patients on ^{68}Ga-DOTANOC PET/CT imaging and one of these correlated on contrast enhanced CT. Skeletal metastases were detected in 6 out of 20 patients, with 3 of them showing CECT correlation (13).

Our study has some limitations. The retrospective study design may have introduced selection bias in our data. Another limitation was that histopathologic verification was not available for all patients.

5. Conclusion:

^{68}Ga-DOTATATE PET/CT imaging is an effective imaging modality for primary focus detection and detection of extrahepatic metastatic lesions in patients with NET presenting with liver metastases.The treatment management of NET patients presenting with liver metastases of unknown primary origin will be more accurate with ^{68}Ga-DOTATATE PET/CT imaging.

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TABLE 1: Patient Characteristics / ^{68}Ga-DOTATATE PET/CT findings

Age	**Ki 67**	**Primary Lesion**	**Extrahepatic Metastases**
58	30	Lung	Mediastinal LN, Bone
46	18	Pancreas	
72	1	Rectum	Pelvic LN
59	3	Negative	
49	7	Negative	
63	8	İlium	Supraclavicular and Abdominal LN
79	3	Abdominal retroperitoneum	Bone, Mediastinal LN
49	2	Negative	Pelvic LN
64	4	Pancreas	
67	30	Pelvic retroperitoneum	
58	8	Negative	
39	5	Ovary	Peritonitis carcinomatosa
61	1	Abdominal retroperitoneum	
65	6	Negative	
39	10	Pancreas	Abdominal and Mediastinal LN, Bone

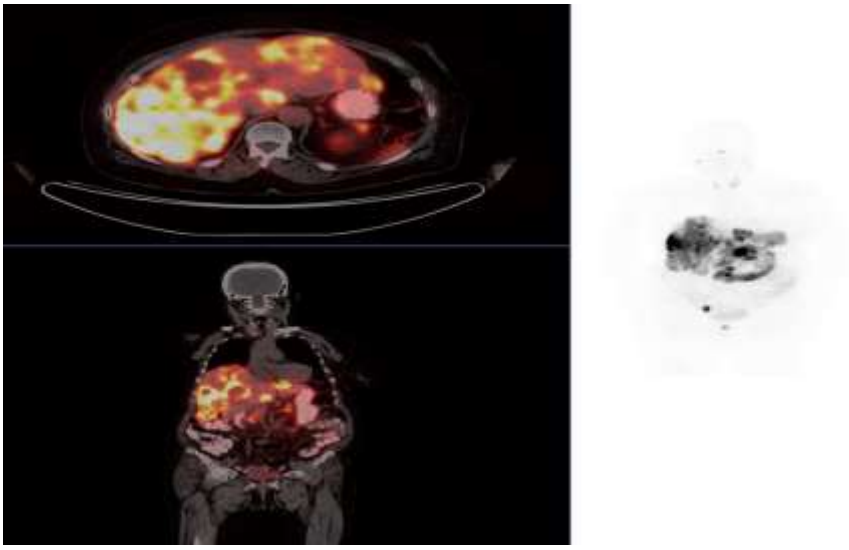

Figure 1:A 72-year-old female patient was diagnosed as Grade 1 WDNET metastases with Ki 67 1% as a result of biopsy performed on the detection of metastatic lesions in the liver in routine imaging. Primary focus could not be detected with routine imaging. ^{68}Ga-DOTA TATE PET/CT imaging revealed that multiple metastatic lesions showing intense somatostatin receptor expression in the liver, and a lesion showing dense somatostatin receptor expression, which could be compatible with the primary focus, in the anterior wall of the middle part of the rectum, and a focus compatible with the pelvic metastatic lymph node was detected.

CHAPTER XI

OBESITY AND PANCREAS CANCER

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Obesity is a important health problem. It was epidemic in the last fifty years. Obesity is a multifactorial disease. Increases diabetes mellitus, cardiovascular diseases, hypertension, hyperlipidemia and some type of cancers. Especially breast, endothelium, gall bladder, pancreas, oesophagus, heart, colon, rectum, kidneys and liver. Obesity means abnormal fat in the body. It is the second most preventable cause of death after smoking. For obesity treatment included type of terapies like diet, exercise, medical and surgical. Nearly 10% weight loss can improve quality of life. It has been shown that for every increase of 5 kg/m2 in Body mass index (BMI), increase in mortality 30 % and increase in cancer-related deaths nearly 10% (1-8). Obesity causes more than 700 billion dollars health expenses every year. BMI is calculated by dividing weight in kilograms by the square of height in meters. BMI is a standard screening tool for Obesity, Obesity can be classified according to their BMI:

Table 1. Obesity Classification

Underweight	less than 18.5 kg / m2
Normal range	18.5 - 24.9 kg / m2
Overweight	25 -29.9 kg / m2
Obese	
Class I Obese	30 - 34.9 kg / m2
Class II Obese	35 - 39.9 kg / m2
Class III Obese (Morbid Obese)	> 40 kg / m2

If the waist-to-hip ratio is more than 1: 1 in men and 0: 8 in women, it is considered as obesity. Skin fold, bioelectrical impedance analysis, computed tomography (CT), magnetic resonance imaging (MRI), Dural energy radiographic absorptiometry (DEXA) and air densitometry are further investigations for the diagnosis of obesity. The cheapest, easiest and simplest is BMI (9-13). It is associated with obesity, chronic inflammation and oncogenesis. Recently, these mechanisms have been investigated at the cellular level. (14, 15). Pancreatic cancer (PC) is a deadly disease. Nearly 95% of PC originates from exocrine cells. Because of its deep retroperitoneal location and no early symptoms, the diagnosis can only be made at a later stage. PC risk factors are elderly age, male gender, obesity, smoking, chronic pancreatitis, diabetes mellitus and gene mutations. (16, 17).

High calorie and fatty diets cause obesity. And then insulin resistance and leptin levels increase. As a result, pancreatic fat develops. Pancreatic fat causes the activation of inflammatory cytokines, especially IL6. Thus, it prepares the ground for PC development. (18). Leptin activates Notch signaling and receptors thereby leading to PC activation. It includes adipose tissue, preadipocytes, endothelial cells, immune cells, fibroblasts, and stem cells. These are many inflammatory cytokines. It secretes TNF-α, TGF-β, IL-6 and leptin etc. These factors activate the development of cancer (19). Obesity is a risk factor for many types of cancer such as pancreatic cancer, hematologic cancers, prostate and breast cancer and also type 2 diabetes mellitus (T2DM), cardiovascular diseases (20). Last studies have revealed that obesity and PC associated. BMI greater than 35 is one of the risk factor in both gender (20, 21). Obesity is associated with pancreatic and other types of cancers (22–24). The current theory is that in obesity, the size and number of adipocytes increase due to triglyceride

(TG) excess. This causes devascularization, hypoxia and ultimately macrophage activation. Thus, adipose tissue cytokines are released. Adipoectin, leptin, tumor necrosis factor alpha (TNF-α), interleukins and monocyte chemotaxin proteins cause local inflammation. Results from studies have shown that increased levels of adipocytokines alter the gut microbiota and play a role in PC development due to inflammation (23, 25). High AdipoQ levels were associated with PC in one study.

At the cellular level, studying 16 tumor tissues, the authors observed positive or strong positive expression of AdipoR1 in 87.5% of cases, while positive or strong positive expression of AdipoR2 was observed in> 97% of cases. In contrast to this publication, Kadri et al. no correlation was observed between adiponectin levels and PC. (26).

Leptin is a 16kDa glycoprotein secreted by adipocytes (> 95%). It is specific to adipose tissue. It is a hormone that regulates appetite and weight gain. (27). Leptin provides energy management under normal conditions. It causes a feeling of satiety by gaining weight by regulating energy consumption (28). Leptin is known as the satiety hormone. Serum leptin levels increase in obesity due to central leptin receptor resistance. Leptin has a mitogenic effect on cancer cells through mitogen activated protein kinase (MAPK) mediated pathways. However, in some types of pancreatic cancer, it also has cancer inhibiting properties. Leptin role is unclear at present (20, 29,31). High levels of adiponectin are inversely related to the incidence of pancreatic cancer (31). High expression of plasminogen activator inhibitor-2 (PAI-2) is an indicator of well survival in patients with PC (32). VEGF is an adipocyte derived polypeptide that is involved in cancer growth that is overexpressed in PC. Its expression indicates poor survival (33, 34).

Leptin mediates communication between cells by binding to the long receptor (OBR1) and short receptor (OBRs) in PC (35). Last evidences suggest that diet, environmental factors and microbial components can contribute to the development of cancer in liver and pancreas, a high-fat diet can alter the gut microbiome and start an inflammatory cascade (36). Gram-negative bacteria secrete lipopolysaccharide (LPS). LPS binds to toll-like receptors (TLRs) and CD 14 co-receptors found in monocytes, macrophages, and neutrophils and provides low-level inflammation (37, 38). The altered gut microbiota may cause a decrease in gut binding proteins (ZO-1 and occludin) that allow LPS to enter the circulation (39). The binding of LPSs to CD14 and TLR receptors on the immune cell surface causes PC cell proliferation (40, 41). T2DM is an important risk factor in PC. Studies have shown that T2DM patients have a 1.8-fold increase in the risk of developing PC (42). The literature suggests that

insulin resistance and diabetes are the result of PC, not the cause (in 50-80% of cases). Clinical studies have shown that from 0.85% to 7% of diabetic patients are first diagnosed with PC (43, 44). In PC, the increase in free fatty acids synthesized from adipose tissue causes lipotoxicity in β cells. This results in PC-associated diabetes mellitus (PCDM) (42, 45, 46). Performed operation to tumor tissue than increased survival of PC patients was associated with higher insulin sensitivity (47).

Insulin resistance is common in patients with type 2 DM and hyperinsulinemia is a result of this. Insulin has two types of receptors that transmit signals: Insulin receptor (IR) -A and IR-B. IR-A has higher affinity for IGF-1 and IGF2; IR-B plays a major role in maintaining glucose homeostasis. Insulin achieves its effects on cell growth and proliferation by binding to IR-B. In addition, insulin increases hepatic expression of the insulin-like growth factor-1 receptor (IGF-1R) and increases the level of free active IGF-1 in the blood by increasing hepatic clearance of IGF binding protein (IGFBP). Cell growth is stimulated as a result of IGF-1 binding to IGF-1R. In fact, insulin acts by binding to the IGF-1 receptor. Its affinity for IR-A is 1000 times higher than IGF-1 receptor. IR-A and IGF-1 are frequently expressed in tumor cells (48). In experimental models, reduction of IR-A and IGF-1 receptors has been shown to inhibit tumoral growth (49,50). After the activation of the insulin / IGF cycle, 2 important signaling pathways are stimulated. First, it is the main signaling pathway involved in tumor cell growth and proliferation. This pathway is the phosphoinositol 2 kinase (PI3K) / protein kinase B (Akt) / Mammalian target of rapamycin (mTOR), the fokhead box O and the Ras / MAPK pathway. The second is the activation of the β-catenin signaling pathway, which is responsible for resistance to chemotherapeutics (51). Insulin has direct effects. In addition, adipose tissue origin cytokines [resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6)], and some vascular factors which are thought to contribute to carcinogenesis in obese and type 2 DM individuals (52). The "Warburg effect" was defined by Warburg in 1956. It showed an increased glucose metabolism (anaerobic glycolysis) in tumoral cells (53). Thus, hyperglycemia acts as a "fuel pump" for tumor cells, stimulating faster proliferation. In some experimental studies, hyperglycemia was observed to increase tumor growth and proliferation, and insulin therapy reversed this situation (54). On the other hand, in experimental studies in which type 1 DM and associated hyperglycemia were created with pancreatic β-cell damage, it was found that the tumor growth was decreased (55). This suggests that hyperglycemia contributes to carcinogenesis when combined with insulin, not alone. In a meta-analysis including only prospective studies, it was reported that every 0.56 mmol / L increase in blood glucose

level increased the frequency of pancreatic cancer by 14% (56). It has been reported in many studies that DM is an independent risk factor for pancreatic cancer, but all of these studies are retrospective. In a recent meta-analysis, DM was associated with an increased risk of pancreatic cancer [relative risk (RR) 1.97] (57). While the risk is highest (RR 6.69) in early DM (first year after diagnosis), the risk decreases in the long term (10 years after the diagnosis of DM) (RR 1.36). In subgroup analyzes of meta-analyzes, it has been stated that the relationship between DM and pancreatic cancer is independent of body mass index (BMI) and insulin resistance (and hence metabolic syndrome) (58,59). However, in a recent meta-analysis, a relationship was found between BMI and pancreatic cancer risk (60). Overweight and obesity in adolescence increase the risk of PC in later periods and lead to a worse survival (61). Obesity was shown to cause poor prognosis pancreatic cancer in a multicenter cohort study in Sweden (62). Animal experiments have shown a strong association between pancreatic cancer and obesity. The development of PanIN in a pancreatic cancer mouse model overexpressing active KrasG12D was studied using selectively pancreatic Cre drivers as early as two weeks (63). PanIN-1a lesions developed in only 10% of the mice fed normal food in three months. Feed these mice a high-fat, high-calorie diet and when they became obese, they developed PanINs in 45% of the ductal cells. This made us think that obesity accelerates the progression of pancreatic cancer (64).

Hertzer et al. showed that conditional Kras G12D mice fed a fatty, calorie diet showed strong inflammation in peripancreatic adipose tissue with increased proinflammatory cytokines. As a result that adipose tissue inflammation caused by obesity is important in the development of pancreatic cancer (65). Several possible mechanisms bariatric surgery may reduce the cancer risk. It can reduce secretion of inflammatory cytokines and reduce tissue inflammation (66,67). Schneck et al. 33-week old diet-induced obese C57BI/6 J mice was performed sleeve gastrectomy. Decreased activated T cells ratio and increased anti inflammatory regulatory T cells in epididymal adipose tissue 3 weeks after surgery(66). Clinical and experimental data showed clearly that bariatric surgery can lead to decreased tissue inflammation and secretion, improve microbiota and decreased insulin resistance (66–70).

As a result, we believe that it plays an important role in the development of obesity and PC. More detailed prospective studies are needed on this topic. It is important that the studies are multi-centered and conducted with

large participation. In the future, these publications will provide new information about obesity and cancer association.

Table 1: Obesity and pancreas cancer

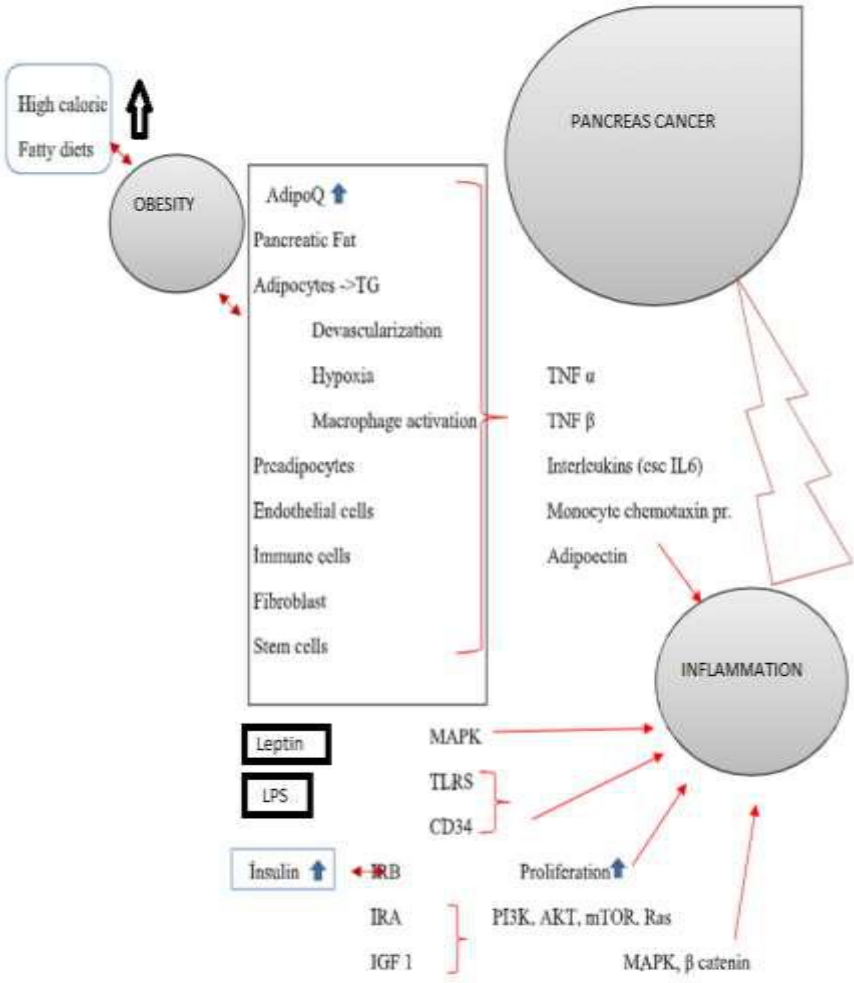

Table 1: Obesity and pancreas cancer

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CHAPTER XII

THE FRACTAL ANALYSIS AND THE APPLICATION IN DENTISTRY

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Fractal means broken parts that are not related to each other and it contains irregular geometric shapes, describes the objects in nature as curves, points and surfaces [1]. It is a way to analyse complex structures that cannot be expressed by integral dimension [2]. Fractal analysis can describe fractal structure complexity and consequently numerical value obtained as fractal dimension (FD) obtained [3].

In fractal geometry, each part of the shape viewed from different scales is the whole body and it is measured degree of irregularity of these objects.

There are no simple shapes such as triangle, square and circle as in classical Euclidean geometry; but in fractal geometry there are irregular shapes in many aspects.

Fractal analysis described by B.B Mendelbrot in 1975, was used in different fields such as physics, chemistry, medical sciences , dentistry, fluid mechanics and in engineering to evaluate topographic data [4].

Fractals are sometimes similar and sometimes have different shapes. The irregular shapes repeat on smaller scales (Figure 1).

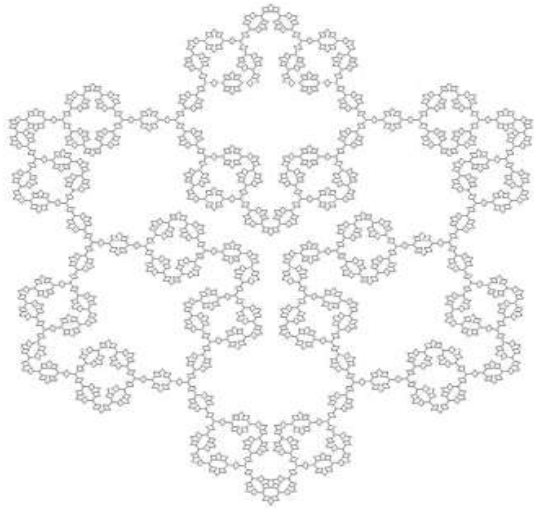


Figure 1. An example of fractal image (Resim yazara aittir)

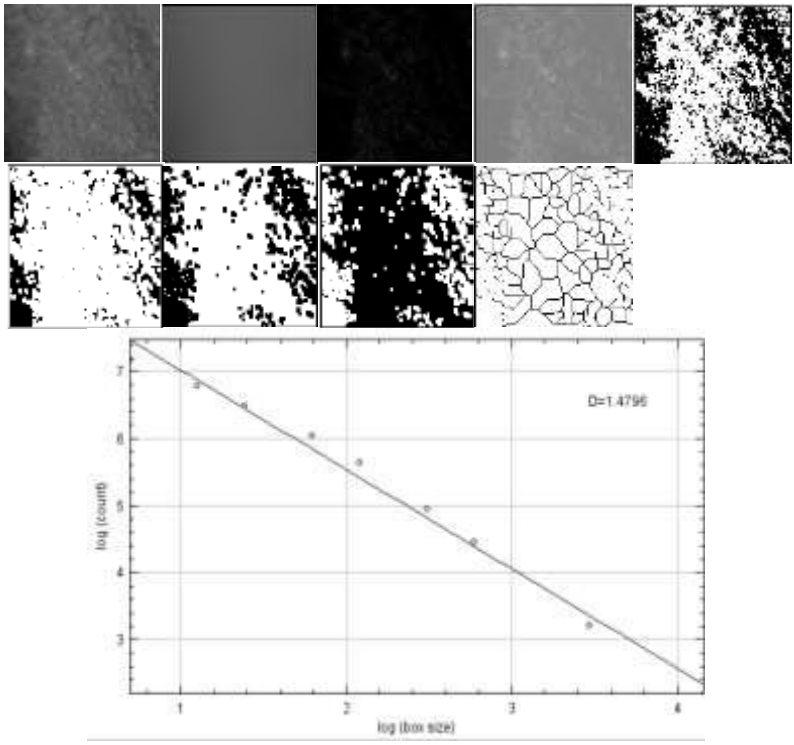
This can take forever. It can be found in nature as well as in the snow-flake example. This type of natural fractals in random in probability, therefore scalable fractals cannot be distorted statistically because they are irregular (they do not look the same when related around them).

Pulmonary branching and trabecular bone can be classified as biological fractals and their structure is considered as anisotropic. Recently trabecular analysis is widely used due to its accessibility, projection geometry and its ability to provide objective data about the trabecular internal structure and not being affected by variables such radiodensity [5-7].

Complexity of medical images allows us to use fractal geometry rather than Euclidean geometry. In FD analysis on the thyroid ultrasound images, dimensions of lesions, nodules and cysts in the region are determined by using fractal concepts. It will be helpful for the physician in early diagnosis and the treatment [8].

While the higher fractal dimension value shows more complex structure, the lower value indicates simple internal structure. In fractal analysis, ROI (region of interest) is taken as a unit. Steps of measuring FD with box counting method are presented in Figure 2. In FD analysis, box counting method is commonly used to calculate the complexity of structure [2, 3, 6].

Figure 2. Top row left to right; cropped and duplicated ROI, adding Gaussian blur filter, subtracted blurred image from the original image, addition of a gray value of 128, thresholding to 128. Middle row from left to right; erosion, dilatation, inversion, skeletonization. Bottom row shows the FD value of the image. (Resim yazara aittir)



In fractal measurement , image texture analysis was used to detect potential abnormalities and severity of existing disorders [9]. In medical field, fractals were widely used, especially in the evaluation of bone architecture.

Bone's mechanical properties are related with different parameters as density of trabecular bone and cortical thickness. Due to higher metabolic activity than the cortical bone, trabecular bone is a better choice for evaluating the changes on the bone [6].

Majumdar et al mentioned that the most effective method was fractal analysis to evaluate bone quality on 2D radiograps [10].

Many researchers also found that [1, 5, 7] complex bone architecture had a higher fractal dimension and density.

Rothe L.E et al investigated the effect of relapse on trabecular and cortical bone after orthodontic treatment by using fractal analysis and stated that there were no statistically significant differences in trabecular structure but thinner mandibular cortices were at an increased risk for dental relapse [11].

Arsan B and Köse TE stated that the patient with TMD exhibited a decreased fractal dimension of mandibular condyle when more severe degenerative change was present [12]. Gümüşsoy et al who also assessed FD values of patients with degenerative joint disease found that increased severity of the degenerative changes causes lower FD values [13]. In another study that investigated the condyle with FD, Cosgunarslan et al found lower FD values for total edentulous patients than the dentate subjects [14].

Trabecular bone healing in orthodontic surgery, implant and endodontics was assessed by fractal analysis. [15-17].

Bachtler et al investigated the FD values of patients with MRONJ and healthy subjects at CBCT images. They found lower values at MRONJ group, an concluded that FD is a good descriptor for MRONJ [18].

FD was used to assess the effects of different systemic diseases on the jaw bones. Bayrak et al. [19] investigated FD values and mandibular indexes at dental panoramic radiography of thalassemia patients. They found that thalassemia patients have lower mandibular cortical width and FD values. In another study by Neves et al, FD values of women with celiac disease and of healthy subjects at panoramic radiography were investigated. They found no significant difference between two groups [20]. Kurşun-Çakmak et al assessed FD values at panoramic radiographs of patients with diabetes type 1 and type 2, and also the healthy subjects. They found no difference among the three groups for FD values [21]. FD was also investigated in the osteoporosis field. Mostafa et al reported no difference in FD values obtained from CBCT images between control and the osteoporotic groups [22]. Alam et al also investigated the postmenopausal osteoporosis patients' FD values obtained from panoramic radiographs and they also reported no significant difference between the osteoporotic and healthy subjects' FD values [23]. Uğur Aydın et al. investigated periapical lesion healing in healthy and type 2 diabetes mellitus patients. They found more significant increases in FD values of healthy groups after endodontic treatment one year later and they concluded diabetes mellitus had a negative effect on FD increase [24].

Demirbaş et al investigated the FD values of two different groups as healthy and patients with sickle cell anemia. They found that patients with sickle cell anemia who were younger than 20 years old, had lower FD values than the control group [25].

In orthodontics, Cesur E and Bayrak S investigated the effects of functional appliances by using fractal analysis and they found significant changes in trabeculation of condyle and mandibular corpus in the treatment group compared to the control group [26].

The fractal analysis was used not only in radiographic images also in photographic images. Cano-Fernandez et al. investigated crenulation of molars in different hominids as humans, chimpanzee, gorilla and orangutan using fractal analysis at photographs of the upper and lower molars and found more complex occlusal morphology in orangutans [27].

The fractal analysis also can be used in the dysplastic lesions. Iqbal et al photographed 121 patients' oral leukoplakia lesions before and after toluidine staining. They concluded that fractal analysis is an effective way to assess complexity of the leukoplakia regions and it may be used to early detect malignant transformation [28].

In conclusion, FD is a useful method that used in different areas of the dentistry field.

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CHAPTER XIII

PROBIOTIC AND PREBIOTIC FOOD CONSUMPTION FREQUENCY AND BODY MASS INDEX

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1. Introduction

The human gut microbiota contains a crowded and heterogeneous microbial ecosystem of at least 10 trillion to 100 trillion microbial cells belonging to more than 2,000 species, weighing a total of 1.5 kilograms. While older studies have shown that there are at least 800 types of bacteria on average in our digestive tract, recent data suggest that the actual figure may actually be as much as 40,000 (1). There is a symbiotic relationship between the human (host) that hosts this variety of bacteria and the intestinal microbiota (guest). While human provides the nutrients necessary for its survival and proliferation to the microbiota that it hosts, guest bacteria in the microbiota take on multiple important tasks of improving human health, such as protecting the host from enteropathogenic organisms, regulate energy metabolism, extracting indigestible dietary components, modulating the immune system and synthesizing essential vitamins (2). The composition of the microbiota is influenced by many endogenous and exogenous factors such as genetics, physiology of the home host (age, diseases, stress conditions) and environmental factors (lifestyle, drug use, pesticides, contaminants, diet) (3). Since the human gut microbiota depends on the metabolism of dietary contents, diet is one of the most researched and decisive factors for understanding the microbiota and its effects on health. Among these dietary components, consumption of prebiotics is known to positively affect the composition of gut microbiota and metabolic functions at the level of the small intestine and colon (4). Probiotics are defined by the FAO and WHO as "living microorganisms that, when administered in sufficient quantities, can benefit the health of the host" (5). Some studies of probiotic Lactobacillus and Bifidobacterium that have been done on animals and humans have been reported to have beneficial health-beneficial effects on many health issues associated with metabolic syndrome such as weight loss, reduced visceral fat, improved glucose tolerance (6). However, some studies have reported that the application of probiotics alone may not show

the beneficial effects on health as it alone may not be able to change the gut microbiota, and it has been noted that prebiotics taken together with probiotics may be more beneficial in improving microbiota composition (6). Today, prebiotics are defined as "an indigestible food component that can improve the health of the host by selectively stimulating the growth and/or activity of one or more bacteria in the colon" (7). Consumption of prebiotic-rich diets increases microbial diversity by affecting both microbial metabolic activity and theformation of fermentative end products such as short-chain fatty acids (SCFAs), branched fatty acids, organic acids, peptides, ammonia, amines, phenolic compounds and gases (8-9). One of the strategies used in the control and treatment of metabolic diseases is the prebiotic or probiotic consumption of beneficial bacteria using a single type of gut microbiota. For example, the bacterium Akkermansia muciniphila degrades mucin and can be used as a prebiotic to reduce obesity and diabetes risks. This bacterium is more found in the gut microbiota of healthy individuals than those who are obese or diabetic (10).

Body Mass Index (BMI) is a method used to define obesity when it is not practically possible to directly measure the amount of body fat. BMI is achieved by dividing the body weight in kilograms by the square of the body height in meters and its unit is kg/m2. BMI between 25-29.9 kg/m2 is defined overweight, BMI $\geq$ 30 kg/m2 is obese, BMI $\geq$ 40 kg/m2 is morbidly obese, BMI $\geq$ 50 kg/m2 is defined as super obese (11). BMI benefits in population-based studies because it is used and accepted to identify health problems by creating specific categories according to body mass, but it is an insufficient scale to inform us about the body fat percentage of the individual. Skinfold caliper, which is a method used to determine fat ratio by measuring skin fold thickness, was not preferred in this study because it feels uncomfortable in some individuals.

There have been many studies on human and animal subjects about the effect of probiotics and prebiotics on body weight and diseases, but in the vast majority of these studies probiotics and prebiotics are given as food supplements or preparates. The main purpose of this study and the contribution it is intended to make to the literature is to investigate whether foods known to have a probiotic and prebiotic effect have a significant relationship between consumption frequency in daily life and body mass indexes of individuals, and as a result bring a new perspective to the dietary approaches used in the treatment of obesity and metabolic diseases related to obesity.

2. Materials and Method

2.1. Place and Time of the Research

This study was carried out between June 5, 2020 and July 15, 2020 to members of a private gym in Istanbul and to 225 females and 77 males, a total of 302 individuals aging 18-65 that are taking diet and nutrition counselling in the aforementioned gym. Patients with diagnosed cancer, Type 2 Diabetes and complications with one or more diseases of the gastrointestinal tract, individuals that were vegan, vegetarian-fed in the last 1 year, individuals that followed an elimination diet, pregnant women or nursing mothers, lactose and/or gluten intolerant individuals were left out of the study.

2.2. Type and Pattern of the Research

This study is a cross-sectional type of study. Before the study, Ethics Committee permission was obtained from the Ethics Committee of the Healthy Sciences Institute of Yeditepe University on the date of 03.06.2019. A survey consisting of 2 (two) sections was conducted, one including sociodemographic characteristics (age, educational status, working conditions, etc.), general health questions (cancer, Type 2 Diabetes, gastrointestinal tract diseases) in which the criteria for inclusion in the study were questioned, and diet, pregnancy/lactation period, alcohol consumption frequency on a weekly basis and two including a Food Consumption Frequency survey. For the Probiotic and Prebiotic Food Consumption Frequency, The consumption frequency survey (FFQ) prepared by the National Health and Nutrition Examination Survey (NHANNES) was adapted to the study and applied by the researcher to the participants with a one-to-one interview technique. Length and body weight were determined from anthropometric measurements of the participants and body mass index (BMI) was calculated.

2.3. Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis of the data found in the study. Descriptive statistical methods (average, standard deviation, median, frequency, ratio, minimum, maximum) were used when analysing the study data. Pearson Ki-Square test and Fisher-Freeman-Halton Exact test were used to compare qualitative data. The significance was evaluated at $p<0.05$.

3. Results

Table 1. Distribution of Demographic Characteristics of Individuals by Gender (n=302)

	Total (n=302)	Gender	
		Female (n=225; 74.5%)	Male (n=77; 25.5%)
Weight (kg)			
Min-Max (Median)	42-115 (65)	42-110 (60)	61-115 (80)
Avg±St	66,86±14,59	61,96±11,70	81,17±12,69
Height (cm)			
Min-Max (Median)	150-194 (167)	150-180 (164)	167-194 (178)
Avg±St	168,09±8,63	164,28±5,69	
BMI (kg/m^2)			
Min-Max (Median)	15,5-39 (23)	15,5-39 (22)	19,2-36,3 (25,2)
Avg±St	23,56±4,28	22,98±4,32	25,25±3,70
Underweight	26 (8,6)	26 (11,6)	0 (0)
Normal weight	174 (57,6)	138 (61,3)	36 (46,8)
Overweight	80 (26,5)	46 (20,4)	34 (44,2)
Obese	22 (7,3)	15 (6,7)	7 (9,1)
Marital status			
Single	198 (65,6)	146 (64,9)	52 (67,5)
Married	104 (34,44)	79 (35,1)	25 (32,5)
Educational status			
High school and less	42 (13,9)	32 (14,2)	10 (13,0)
University and higher	260 (86,1)	193 (85,8)	67 (87,0)
Weekly alcohol consumption			
Does not consume alcohol	165 (54,6)	145 (64,4)	20 (26,0)
1-5 units per week	112 (37,1)	67 (29,8)	45 (58,4)
5-10 units per week	18 (6,0)	10 (4,4)	8 (10,4)
10-15 units per week	7 (2,3)	3 (1,3)	4 (5,2)

The distribution of demographic characteristics of individuals by gender is given in Table 4.1. 77 males (25.5%) and 225 females (74.5%), a total of 302 volunteers between the ages of 18 and 65 participated in the study. BMI measurements of participants vary between 15.6 and 39 kg/m^2 and the average for women is22.98±4.32 kg/m^2 and for men it is25.25±3.70 kg/m$^{2;}$8.6% (n=26) are underweight, 57.6% (n=174) are normal weight, 26.5%(n=80) are overweight and 7.3% (n=22) are obese. When their marital status is examined; It was determined that 65.6% (n=198) were single and 34.4% (n=104) were married. When the educational status of the participants was examined, it was found that 13.9% (n=42) were high school and less, and 86.1% (n=260) were university and higher. When the weekly alcohol consumption frequency of the participants was examined, 54.6% (n=165) did not consume alcohol,

37.1% (n=112) consumed 1-5 units of alcohol per week, 6.0% (n=18) consumed 5-10 units of alcohol per week and 2.3% (n=7) consumed 10-15 units of alcohol per week. The consumption frequencies of foods that are known to have probiotic and prebiotic effects are given in Table 2.

Table 2. Distribution of Probiotic and Prebiotic Food Consumption Frequencies of Individuals

Nutrients	I don't consume		2-3 times a month or less		3-4 times a week		Once a day		2-3 times a day	
	n	%	n	%	n	%	n	%	n	%
Oat based food	130	43,0	114	37,7	47	15,6	9	3,0	2	0,7
Cereal bar	148	49,0	126	41,7	21	7,0	5	1,7	2	0,7
Breakfast cereal	153	50,7	92	30,5	48	15,9	7	2,3	2	0,7
Whole grain bread derivatives	48	15,9	97	32,1	79	26,2	46	15,2	32	10,6
Assorted nuts	12	4,0	111	36,8	119	39,4	43	14,2	17	5,6
Banana	18	6,0	183	60,6	85	28,1	16	5,3	0	0,0
Pineapple	133	44,0	153	50,7	14	4,6	2	0,7	0	0,0
Red plum	143	47,4	138	45,7	21	7,0	0	0,0	0	0,0
Fresh-squeezed fruit juice	83	27,5	161	53,3	48	15,9	9	3,0	1	0,3
Dried fruit	65	21,5	136	45,0	76	25,2	16	5,3	9	3,0
Raw green leafy vegetables	21	7,0	101	33,4	125	41,4	45	14,9	10	3,3
Onion	11	3,6	39	12,9	111	36,8	101	33,4	40	13,2
Pea	54	17,9	178	58,9	61	20,2	8	2,6	1	0,3
Tomato	7	2,3	30	9,9	91	30,1	105	34,8	69	22,8
Fresh-squeezed vegetable juice	228	75,5	49	16,2	20	6,6	4	1,3	1	0,3
Pickled cabbage or cucumber	57	18,9	146	48,3	76	25,2	19	6,3	4	1,3
Cow's milk	27	8,9	72	23,8	116	38,4	63	20,9	24	7,9
Soy milk	276	91,4	19	6,3	7	2,3	0	0,0	0	0,0
Soy containing products	278	92,1	20	6,6	4	1,3	0	0,0	0	0,0
Yogurt	3	1,7	34	11,3	114	37,7	99	32,8	50	16,6
Probiotic yogurt	212	70,2	58	19,2	17	5,6	11	3,6	4	1,3
Instant drinks with probiotic effect	261	86,4	28	9,3	8	2,6	3	1,0	2	0,7

Nutrients	I don't consume		2-3 times a month or less		3-4 times a week		Once a day		2-3 times a day	
	n	%	n	%	n	%	n	%	n	%
Types of cheeses	9	3,0	21	7,0	80	26,5	146	48,3	46	15,2
Breakfast olives	24	7,9	31	10,3	91	30,1	121	40,1	35	11,6
Kefir	181	59,9	71	23,5	27	8,9	19	6,3	4	1,3
Ice cream	27	8,9	157	52,0	84	27,8	28	9,3	6	2,0
Boza	208	68,9	67	22,2	19	6,3	6	2,0		0,7
Red meat	5	1,7	123	40,7	152	50,3	18	6,0	4	1,3
Types of white meats	9	3,0	129	42,7	150	49,7	14	4,6	0	0,0
Fermented sucuk (Fermented meat product)	66	21,9	171	56,6	64	21,2	1	0,3	0	0,0
Dried meat and meat products	191	63,2	82	27,2	27	8,9	1	0,3	1	0,3
Coffee	11	3,6	28	0,3	74	24,5	96	31,8	93	30,8
Beer	144	47,7	120	39,7	34	11,3	3	1,0	1	0,3
Red wine	179	59,3	106	35,1	14	4,6	2	0,7	1	0,3

Table 3. Evaluation of Probiotic and Prebiotic Food Consumption Frequency of Individuals According to their BMI Levels

Nutrients	Answers	Individuals' BMI Levels				p
		Underweight (n=26)	Normal weight (n=174)	Overweight (n=88)	Obese (n=22)	
		n (%)	n (%)	n (%)	n (%)	
Oat based food	Does not consume	12 (46,2)	66 (37,9)	40 (50)	12 (54,5)	[a]0.530
	Consumes occasionally	10 (38,5)	71 (40,8)	27 (33,8)	6 (27,3)	
	Consumes	4 (15,4)	37 (21,3)	13 (16,3)	4 (18,2)	
Cereal bar	Does not consume	10 (38,5)	82 (47,1)	43 (53,8)	13 (59,1)	[a]0.389
	Consumes occasionally	15 (57,7)	73 (42)	29 (36,3)	9 (40,9)	
	Consumes	1 (3,8)	19 (10,9)	8 (10)	0 (0)	
Breakfast cereal	Does not consume	7 (26,9)	89 (51,1)	47 (58,8)	10 (45,5)	[a]0.015*
	Consumes occasionally	15 (57,7)	47 (27)	20 (25)	10 (45,5)	
	Consumes	4 (15,4)	38 (21,8)	13 (16,3)	2 (9,1)	
Whole grain bread derivatives	Does not consume	6 (23,1)	27 (15,5)	11 (13,8)	4 (18,2)	[a]0.389
	Consumes occasionally	8 (30,8)	57 (32,8)	21 (26,3)	11 (50)	
	Consumes	12 (46,2)	90 (51,7)	48 (60)	7 (31,8)	
Assorted nuts	Does not consume	1 (3,8)	9 (5,2)	1 (1,3)	1 (4,5)	[a]0.644
	Consumes occasionally	8 (30,8)	61 (35,1)	32 (40)	10 (45,5)	
	Consumes	17 (65,4)	104 (59,8)	47 (58,8)	11 (50)	
Banana	Does not consume	3 (11,5)	6 (3,4)	6 (7,5)	3 (13,6)	[a]0.195
	Consumes occasionally	14 (53,8)	105 (60,3)	52 (65)	12 (54,5)	
	Consumes	9 (34,6)	63 (36,2	22 (27,5)	7 (31,8)	
Pineapple	Does not consume	10 (38,5)	75 (43,1)	39 (48,8)	9 (40,9)	[a]0.169
	Consumes occasionally	14 (53,8)	86 (49,4)	41 (51,3)	12 (54,5)	
	Consumes	2 (7,7)	13 (7,5)	0 (0)	1 (4,5)	
Red plum	Does not consume	13 (50)	82 (47,1)	39 (48,8)	9 (40,9)	[a]0.713
	Consumes occasionally	13 (50)	79 (45,4)	36 (45)	10 (45,5)	
	Consumes	0 (0)	13 (7,5)	5 (6,3)	3 (13,6)	
Fresh-squeezed fruit juice	Does not consume	2 (7,7)	51 (29,3)	24 (30)	6 (27,3)	[a]0.331
	Consumes occasionally	17 (65,4)	94 (54)	39 (48,8)	11 (50)	
	Consumes	7 (26,9)	29 (16,7)	17 (21,3)	5 (22,7)	
Dried fruit	Does not consume	12 (46,2)	33 (19)	33 (19)	5 (22,7)	[a]0.057
	Consumes occasionally	6 (23,1)	84 (48,3)	84 (48,3)	11 (50)	
	Consumes	8 (30,8)	57 (32,8)	57 (32,8)	6 (27,3)	

Nutrients	Answers	Individuals' BMI Levels				p
		Underweight (n=26)	Normal weight (n=174)	Overweight (n=88)	Obese (n=22)	
		n (%)	n (%)	n (%)	n (%)	
Soy containing products	Does not consume	26 (100)	26 (100)	73 (91,3)	21 (95,5)	[a]0.298
	Consumes occasionally	0 (0)	0 (0)	7 (8,8)	0 (0)	
	Consumes	0 (0)	0 (0)	0 (0)	1 (4,5)	
Yogurt	Does not consume	0 (0)	3 (1,7)	2 (2,5)	0 (0)	[a]0.979
	Consumes occasionally	3 (11,5)	20 (11,5)	8 (10)	3 (13,6)	
	Consumes	23 (88,5)	151 (86,8)	70 (87,5)	19 (86,4)	
Probiotic yogurt	Does not consume	19 (73,1)	118 (67,8)	60 (75)	15 (68,2)	[a]0.944
	Consumes occasionally	5 (19,2)	36 (20,7)	13 (16,3)	4 (18,2)	
	Consumes	2 (7,7)	20 (11,5)	7 (8,8)	3 (13,6)	
Instant drinks with probiotic effect	Does not consume	23 (88,5)	149 (85,6)	70 (87,5)	19 (86,4)	[a]0.752
	Consumes occasionally	2 (7,7)	15 (8,6)	9 (11,3)	2 (9,1)	
	Consumes	1 (3,8)	10 (5,7)	1 (1,3)	1 (4,5)	
Types of cheeses	Does not consume	2 (7,7)	5 (2,9)	2 (2,5)	0 (0)	[a]0.853
	Consumes occasionally	Numbering	10 (5,7)	6 (7,5)	1 (4,5)	
	Consumes		159 (91,4)	72 (90)	21 (95,5)	
Breakfast olives	Does not consume	2 (7,7)	18 (10,3)	4 (5)	0 (0)	[a]0.369
	Consumes occasionally	5 (19,2)	17 (9,8)	7 (8,8)	2 (9,1)	
	Consumes	19 (73,1)	139 (79,9)	69 (86,3)	20 (90,9)	
Kefir	Does not consume	13 (50)	103 (59,2)	48 (60)	17 (77,3)	[a]0.593
	Consumes occasionally	7 (26,9)	42 (24,1)	20 (25)	2 (9,1)	
	Consumes	6 (23,1)	29 (16,7)	12 (15)	3 (13,6)	
Ice cream	Does not consume	2 (7,7)	14 (8)	10 (12,5)	1 (4,5)	[a]0.529
	Consumes occasionally	16 (61,5)	94 (54)	38 (47,5)	9 (40,9)	
	Consumes	8 (30,8)	66 (37,9)	32 (40)	12 (54,5)	
Bora	Does not consume	23 (88,5)	121 (69,5)	49 (61,3)	15 (68,2)	[a]0.188
	Consumes occasionally	3 (11,5)	35 (20,1)	23 (28,8)	6 (27,3)	
	Consumes	0 (0)	18 (10,3)	8 (10)	1 (4,5)	
Red meat	Does not consume	0 (0)	3 (1,7)	2 (2,5)	0 (0)	[a]0.811
	Consumes occasionally	12 (46,2)	68 (39,1)	31 (38,8)	12 (54,5)	
	Consumes	14 (53,8)	103 (59,2)	47 (58,8)	10 (45,5)	

Nutrients	Answers	Individuals' BMI Levels				P
		Underweight (n=26) n (%)	Normal weight (n=174) n (%)	Overweight (n=80) n (%)	Obese (n=22) n (%)	
Types of white meats	Does not consume	1 (3,8)	4 (2,3)	3 (3,8)	1 (4,5)	[3]0.915
	Consumes occasionally	11 (42,3)	77 (44,3)	32 (40)	9 (40,9)	
	Consumes	14 (53,8)	93 (53,4)	45 (56,3)	12 (54,5)	
Fermented sucuk (Fermented meat product)	Does not consume	7 (26,9)	42 (24,1)	17 (21,3)	0 (0)	[3]0.141
	Consumes occasionally	12 (46,2)	93 (53,4)	50 (62,5)	16 (72,7)	
	Consumes	7 (26,9)	39 (22,4)	13 (16,3)	6 (27,3)	
Dried meat and meat products	Does not consume	21 (80,8)	106 (60,9)	49 (61,3)	15 (68,2)	[3]0.533
	Consumes occasionally	3 (11,5)	49 (28,2)	24 (30)	6 (27,3)	
	Consumes	2 (7,7)	19 (10,9)	7 (8,8)	1 (4,5)	
Coffee	Does not consume	0 (0)	10 (5,7)	1 (1,3)	0 (0)	[3]0.638
	Consumes occasionally	3 (11,5)	15 (8,6)	8 (10)	2 (9,1)	
	Consumes	23 (88,5)	149 (85,6)	71 (88,8)	20 (90,9)	
Beer	Does not consume	12 (46,2)	85 (48,9)	33 (41,3)	14 (63,6)	[3]0.330
	Consumes occasionally	9 (34,6)	66 (37,9)	37 (46,3)	8 (36,4)	
	Consumes	5 (19,2)	23 (13,2)	10 (12,5)	0 (0)	
Red wine	Does not consume	12 (46,2)	100 (57,5)	47 (58,8)	20 (90,9)	[3]0.015*
	Consumes occasionally	14 (53,8)	64 (36,8)	26 (32,5)	2 (9,1)	
	Consumes	0 (0)	10 (5,7)	7 (8,8)	0 (0)	

[3]Pearson's Chi-Square Test, [3]Fisher Freeman Halton Exact Test, *p<0.05

In Table 3, the consumption frequencies of probiotic and prebiotic foods included in the questionnaire were evaluated. No statistically significant difference was shown between individuals BMI levels and the consumption of Oat-based foods, cereal bar, whole grain bread derivatives, types of nuts, banana, pineapple, red plum, fresh-squeezed fruit juice, dried fruit, raw green leafy vegetables, onion, peas, fresh-squeezed vegetable juice, cow's milk, soy milk, soy-containing products, yogurt, probiotic yogurt, instant drinks with probiotic effect, cheese varieties, breakfast olives, kefir, ice cream, boza, red meat, white meat varieties, fermented sucuk(fermented meat product), dried meat and meat products, coffee, beer ($p>0.05$). A statistically significant difference was found between the BMI levels of individuals and their breakfast cereal consumption rates ($p = 0.015$; $p <0.05$). As a result of the paired comparisons; it was found meaningful that these ratios were higher; underweight individuals consumed breakfast cereal occasionally (57.7%), while normal weight and overweight individuals did not consume it (51.1% and 58.8%, respectively.) There was no significant difference between the breakfast cereal consumption rates of the other groups. A statistically significant difference was found between the consumption rates of pickled cabbage or cucumber according to the BMI levels of the individuals ($p=0.045$; $p<0.05$). As a result of the paired comparisons; The rates of obese individuals consuming pickled cabbage or cucumbers (50%) were found to be higher than the consumption rates of underweight and overweight individuals (19.2% and 26.3%, respectively). The rates of not consuming pickled cabbage or cucumbers (34.6% and 25%, respectively) of underweight and overweight individuals were also found higher than normal weight individuals (14.4%). There was no significant difference between the consumption rates of pickled cabbage or cucumber among the other groups. A statistically significant difference was found between the consumption of red wine according to the BMI levels of the individuals ($p=0.015$; $p<0.05$). As a result of the paired comparisons; The rates of red

wine consumption of underweight, normal weight and overweight individuals (53.8%, 36.8% and 32.5%, respectively) were higher than obese individuals (9.1%). There was no significant difference between the red wine consumption rates of the other groups.

Table 4. Evaluation of Probiotic and Prebiotic Food Consumption Frequency of Individuals According to Their Gender

Nutrients	Answers	Gender		
		Woman (n=225)	Male (n=77)	
		n (%)	n (%)	p
Oat based food	Does not consume	97 (43.1)	33 (42,9)	*0.911
	Consumes occasionally	86 (38,2)	28 (36,4)	
	Consumes	42 (18.7)	16 (20.8)	
Cereal bar	Does not consume	105 (46,7)	43 (55.8)	*0,324
	Consumes occasionally	97 (43.1)	29 (37,7)	
	Consumes	23 (10.2)	5 (6,5)	
Breakfast cereal	Does not consume	110 (48,9)	43 (55.8)	*0,174
	Consumes occasionally	75 (33,3)	17 (22,1)	
	Consumes	40 (17.8)	17 (22,1)	
Whole grain bread derivatives	Does not consume	30 (13,3)	30 (13,3)	*0.088
	Consumes occasionally	72 (32)	72 (32)	
	Consumes	123 (54.7)	123 (54.7)	
Assorted nuts	Does not consume	11 (4,9)	1 (1.3)	*0.274
	Consumes occasionally	79 (35,1)	32 (41.6)	
	Consumes	135 (60)	44 (57,1)	
Banana	Does not consume	14 (6,2)	4 (5,2)	*0.073
	Consumes occasionally	128 (56,9)	55 (71,4)	
	Consumes	83 (36,9)	18 (23,4)	
Pineapple	Does not consume	94 (41.8)	39 (50.6)	*0.375
	Consumes occasionally	118 (52,4)	35 (45,5)	
	Consumes	13 (5.8)	3 (3,9)	
Red plum	s not consume	102 (45.3)	41 (53,2)	*0.312
	Consumes occasionally	105 (46,7)	33 (42,9)	
	Consumes	18 (8)	3 (3,9)	
Fresh-squeezed fruit juice	Does not consume	63 (28)	20 (26)	*0.942
	Consumes occasionally	119 (52,9)	42 (54.5)	
	Consumes	43 (19,1)	15 (19,5)	
Dried fruit	Does not consume	48 (21.3)	17 (22,1)	*0.035*
	Consumes occasionally	93 (41,3)	43 (55.8)	
	Consumes	84 (37,3)	17 (22,1)	
Raw green leafy vegetables	Does not consume	11 (4,9)	10 (13)	*0.002*
	Consumes occasionally	68 (30,2)	33 (42,9)	*
	Consumes	146 (64,9)	34 (44,2)	
Onion	Does not consume	9 (4)	2 (2,6)	*0.054
	Consumes occasionally	23 (10.2)	16 (20.8)	
	Consumes	193 (85.8)	59 (76.6)	
Pea	Does not consume	46 (20.4)	8 (10,4)	*0.124
	Consumes occasionally	127 (56,4)	51 (66,2)	
	Consumes	52 (23.1)	18 (23,4)	
Tomato	Does not consume	4 (1,8)	3 (3,9)	*0.451
	Consumes occasionally	24 (10.7)	6 (7,8)	
	Consumes	197 (87.6)	68 (88,3)	
Fresh-squeezed vegetable juice	Does not consume	172 (76,4)	56 (72.7)	*0.667
	Consumes occasionally	34 (15,1)	15 (19,5)	
	Consumes	19 (8,4)	6 (7,8)	
Pickled cabbage or cucumber	Does not consume	40 (17.8)	17 (22,1)	*0.034*
	Consumes occasionally	102 (45.3)	44 (57,1)	
	Consumes	83 (36,9)	16 (20.8)	

Nutrients	Answers	Gender		
		Woman (n=225) n (%)	Male (n=77) n (%)	p
Cow's milk	Does not consume	20 (8,9)	7 (9,1)	*0.701
	Consumes occasionally	51 (22.7)	21 (27,3)	
	Consumes	154 (68,4)	49 (63.6)	
Soy milk	Does not consume	203 (90,2)	73 (94.8)	$0.333
	Consumes occasionally	15 (6,7)	4 (5,2)	
	Consumes	7 (3,1)	0 (0)	
Soy containing products	Does not consume	209 (92,9)	69 (89.6)	$0.175
	Consumes occasionally	12 (5.3)	8 (10,4)	
	Consumes	4 (1,8)	0 (0)	
Yogurt	Does not consume	4 (1,8)	1 (1.3)	$0.073
	Consumes occasionally	20 (8,9)	14 (18,2)	
	Consumes	201 (89,3)	62 (80,5	
Probiotic yogurt	Does not consume	151 (67,1)	61 (79.2)	*0,132
	Consumes occasionally	48 (21.3)	10 (13)	
	Consumes	26 (11.6)	6 (7,8)	
Instant drinks with probiotic effect	Does not consume	193 (85.8)	68 (88,3)	*0.309
	Consumes occasionally	20 (8,9)	8 (10,4)	
	Consumes	12 (5.3)	1 (1.3)	
Types of cheeses	Does not consume	8 (3,6)	1 (1.3)	*0.436
	Consumes occasionally	14 (6,2)	7 (9,1)	
	Consumes	203 (90,2)	69 (89.6)	
Breakfast olives	Does not consume	18 (8)	6 (7,8)	*0,998
	Consumes occasionally	23 (10.2)	8 (10,4)	
	Consumes	184 (81,8)	63 (81,8)	
Kefir	Does not consume	124 (55,1)	57 (74)	*0.013*
	Consumes occasionally	60 (26.7)	11 (14.3)	
	Consumes	41 (18,2)	9 (11.7)	
Ice cream	Does not consume	18 (8)	9 (11.7)	*0.217
	Consumes occasionally	113 (50,2)	44 (57,1)	
	Consumes	94 (41.8)	24 (31,2)	
Boza	Does not consume	150 (66,7)	58 (75,3)	*0.352
	Consumes occasionally	54 (24)	13 (16.9)	
	Consumes	21 (9,3)	6 (7,8)	
Red meat	Does not consume	5 (2,2)	0 (0)	$0.308
	Consumes occasionally	95 (42,2)	28 (36,4)	
	Consumes	125 (55.6)	49 (63.6)	
Types of white meats	Does not consume	7 (3,1)	2 (2,6)	*0.025*
	Consumes occasionally	106 (47,1)	23 (29.9)	
	Consumes	112 (49,8)	52 (67,5)	
Fermented sucuk (Fermented meat product)	Does not consume	51 (22.7)	15 (19,5)	*0.025*
	Consumes occasionally	134 (59.6)	37 (48,1)	
	Consumes	40 (17.8)	25 (32,5)	
Dried meat and meat products	Does not consume	153 (68)	38 (49,4)	*0.009**
	Consumes occasionally	55 (24,4)	27 (35,1)	
	Consumes	17 (7.6)	12 (15.6)	
Coffee	Does not consume	8 (3,6)	3 (3,9)	*0.914
	Consumes occasionally	20 (8,9)	8 (10,4)	
	Consumes	197 (87.6)	66 (85,7)	
Beer	Does not consume	126 (56)	18 (23,4)	*0.001**
	Consumes occasionally	76 (33.8)	44 (57,1)	
	Consumes	23 (10.2)	15 (19,5)	
Red wine	Does not consume	140 (62,2)	39 (50.6)	*0,201
	Consumes occasionally	73 (32,4)	33 (42,9)	
	Consumes	12 (5.3)	5 (6,5)	

*Pearson's Chi-Square Test $Fisher Freeman Halton Exact Test**p<0.01*p<0.05

There is no statistically significant difference between individuals genders and their consumption rate of cereal bar, breakfast cereal, whole grain bread derivatives, types of nuts, banana, pineapple, red plum, freshly squeezed fruit juice, onion, pea, tomato, freshly squeezed vegetable juice, cow The consumption rates of milk, soy milk, soy-containing products, yoghurt, probiotic yogurt, probiotic-effect instant drinks, cheese varieties,

breakfast olives, ice cream, boza, red meat, coffee and red wine ($p > 0.05$). A statistically significant difference was found between the ratios of raw green leafy vegetables consumed by males and females ($p=0.002$; $p<0.05$). The rate of consuming raw green leafy vegetables (64.9%) of females is higher than that of males (44.2%). The rate of consuming pickled cabbage or cucumber (36.9%) of female individuals was higher than that of male individuals (20.8%), and a significant difference was found between consumption depending on gender ($p=0.034$; $p<0.05$). According to kefir consumption rates, 44.9% of female individuals consumed kefir, while this rate was 26% for males, and a statistically significant difference was found in kefir consumption depending on gender ($p=0.013$; $p<0.05$). When the ratio of white meat types consumed by individuals according to their gender was examined, 47.1% of female individuals occasionally consumed white meat varieties, while the rate of male individuals consuming white meat varieties was 67.5%, and a statistically significant difference was found between them ($p=0.025$; $p<0.05$). The rate of consumption of sucuk (fermented meat product) was found to be higher in men than women (32.5%, 17.8%, respectively) and a statistically significant difference was found between ($p=0.025$; $p<0.05$). A statistically significant difference was found between the consumption of dried meat and meat products according to their gender ($p=0.009$; $p<0.05$). The rate of consuming dried meat and meat products was higher for males (50.7%), and the rate of not consuming dried meat and meat products for females (68%). The rates of consumption and occasional consumption of beer (19.5%, 57.1%, respectively) of male individuals are higher than the consumption and sometimes consumption rates of women (respectively; 10.2%, 33.8%), and a statistically significant difference was found between consumption rates by gender ($p=0.001$. $p<0.05$).

4. DISCUSSION

The discussion part of this study, which is a cross-sectional study conducted to determine the relationship between the consumption frequency of probiotic and prebiotic foods and body mass index of individuals, will be evaluated by proceeding in the same order with the titles in the results, respectively.

4.1. Evaluation of Individuals' Demographic Characteristics According to Gender

The ages of the individuals participating in this study are between the ages of 18 and65, and the average weight in females is 61.96 ±11.70 kg, and in males it is 81.17±12.69 kg. While 54.6% (n=165) of the individuals did not consume alcohol, it was observed that 37.1% (n=112) consumed 1-5 units of alcohol per week. There are studies showing that polyphenols

and β - glucan found in alcoholic beverages obtained by fermentation, such as beer and red wine, may show prebiotic benefits (11, 12). However, there are factors affecting the health of gut microbiota due to regular alcohol use for a long time. In a study conducted with 89 alcoholic cirrhosis patients and a control group of 40, which examined the relationship between alcohol consumption and gut microbiota, 30.3% of the patients with alcoholic cirrhosis had excessive bacterial growth in the intestine, whereas this was not observed in any healthy individuals (13). The imbalance in the gut microflora and the decrease in stomach acid together with the excessive bacterial growth in the intestine affect the metabolism by causing nutrient absorption, gas, diarrhea and, in rare cases, constipation.

4.2. Evaluation of Probiotic and Prebiotic Food Consumption Frequency of Individuals

The most common types of probiotic and prebiotic foods consumed by the individuals surveyed are; Whole grain bread derivatives, dried fruits, onion, tomato, peas, cow's milk, yogurt, types of cheese, breakfast olives, ice cream, white meat varieties and coffee. Among the foods that are consumed least or not at all include; Oat-based foods, Cereal bar, breakfast cereal, pineapple, red plum, fresh-squeezed fruit juice, fresh-squeezed vegetable juice, soy milk, soy-containing products, probiotic yogurt, probiotic ready drinks, kefir, sucuk (fermented meat product), dried meat and meat products, beer and red wine. The variables that affect the association of the survey results with BMI are the specific selection of the foods in the survey to show probiotic or prebiotic characteristics, and the individuals participating in the survey show different socio-demographic, socio-economic and socio-cultural characteristics. In addition, the fact that the frequency and prices of the foods in the survey vary in our country is among the reasons for consuming or not consuming these foods.

4.3. Evaluation of Probiotic and Prebiotic Food Consumption Frequency of Individuals According to their BMI Levels

The average weight of 302 individuals participating in this study was 66.86±14.59 kg and their average BMI was 23.56±4.28 kg/m^2. While the average weight in females is found to be 61.96 ± 11.70 kg and the average BMI is 22.98 ± 4.32 kg/m^2, the average weight in males is found to be 81.17 ± 12.69 kg and the average BMI is 25.25 ± 3.70 kg/m^2. The effects of probiotic foods and prebiotics containing dietary fiber on energy homeostasis and nutrient absorption also play a role in body weight control. In a study where 87 individuals with BMI ranging from observed 24.2--37.0 kg / m2 and with visceral fat gain were given Lactobacillus gasseri SBT2055 for 12 weeks, patients were observed to have lower BMI and body mass, waist and hip circumference, cisceral and subcutaneous fat

mass decrease compared to the place group (52). There are studies showing that the application of different Lactobacilli strains on obese individuals reduces fat mass, T2D and insulin resistance risk(Table 1). However, in a study examining the correlation between BMI and gut concentrations of Lactobacillus reuteri, Bifidobacterium animalis, Methanobrevibacter smithii and Escherichia coli species, a higher prevalence of Lactobacillus in obese subjects were observed compared to lean individuals (respectively; 32%, 20%; p=0.06) and BMI>25 individuals had higher Lactobacillus prevalence compared to individuals with a BMI of <25 (respectively; 32%, 20.8%; p=0.06) (141). Therefore, these two different results obtained from different studies show us that there may be a dose-dependent relationship between certain bacterial species in the gut and BMI.

No statistically significant difference was shown between individuals BMI levels and the consumption of Oat-based foods, cereal bar, whole grain bread derivatives, types of nuts, banana, pineapple, red plum, fresh-squeezed fruit juice, dried fruit, raw green leafy vegetables, onion, peas, fresh-squeezed vegetable juice, cow's milk, soy milk, soy-containing products, yogurt, probiotic yogurt, instant drinks with probiotic effect, cheese varieties, breakfast olives, kefir, ice cream, boza, red meat, white meat varieties, fermented sucuk(fermented meat product), dried meat and meat products, coffee, beer (p>0.05). The variety of foods included in the survey and the demographic, cultural and economic reasons underlying the reasons for consuming or not consuming these foods in daily life may be effective in the inability of finding a significant relationship between BMI and consumption frequency of these foods.

In this study, only the consumption frequency of probiotic and prebiotic foods was questioned regardless of the amount, and it is in line with the results of the above-mentioned studies that the amount of consumption, namely the portion size, has an effect on the BMI as well as the consumption frequency of the foods.

4.4. Evaluation of Probiotic and Prebiotic Food Consumption Frequency of Individuals According to Their Gender

When the probiotic and prebiotic food consumption frequencies of 225 female and 77male volunteers who participated in this study were examined; The rate of consumption of raw green leafy vegetables, cabbage or pickled cucumbers and kefir of women was significantly higher than that of men (p<0.05). The consumption rates of white meat, fermented sucuk (fermented meat product), dried meat and meat products and beer were found to be significantly higher in male individuals compared to women (p <0.05). In a study examining the gender differences in meat,

fruit and vegetable consumption, the consumption frequencies of these foods were compared between males and females in Finland and Baltic countries. According to the results of the study; in all countries included in the study, meat consumption rates of males were found to be higher than females. When fruit and vegetable consumption was examined, in all countries included in the study, fruit and vegetable consumption of females was found to be higher than males. In all three cases, the gender model has not been changed by other socio- demographic factors (15). In another study examining gender differences in fruit and vegetable consumption using data obtained from the National Cancer Institute's Food Attitudes and Behaviors Research, a questionnaire was applied to the participants asking how often they ate various fruits and vegetables in the previous month and participants have responded on a scale ranging from "Never" to "5 times a day or more." The result of the study resulted in females consuming more fruits and vegetables than males, similar to the other study ($p<.001$)(16). The results of both studies show similarities with the difference in the consumption of meat and vegetables, which are among the foods included in this study, by gender.

5. Conclusıons and Recommendatıons

The results obtained from this study conducted with 225 female and 77 male total 302 volunteers in order to examine the relationship between probiotic and prebiotic food consumption frequency and body mass index of individuals are given below.

The average weight of males was 81.17±12.69 kg, while the average weight of females was 61.96±11.70 kg. Average height was 179.23±5.54 cm for males and 164.28±5.69 cm for females. The average BMI of the individuals was 23.56±4.28 kg/m^2. Of all individuals, 8.6% were underweight (n 26), 57.6% were normal weight (n=74), 26.5% were overweight (n=80) and 7.3% were obese (n=22). Looking at the average BMI of male individuals, it was found to be 25.25±3.70 kg/m^2. According to BMI evaluation of male individuals, 0% were underweight (n=0), 46.8% were normal weight (n=36), 44.2% were overweight (n=34) and 7% (n=7) were obese.The average BMI of females is 22.98±4.32 kg/m^2. According to BMI evaluation of female individuals, 11.6% were underweight (n=26), 61.3% were normal weight (n=138), 20.4% were overweight (n = 46) and 6.7% were obese (n=15). 64.4% of the females participating in the study are single (n=146) and 35.1% are married (n=79). Considering the marital status of the males, 67.5% are single (n=57) and 32.5% are married (n=25).13.9% (n=42) of the participants have high school or less, 86.1% (n=260) have a university or higher education level. When the weekly alcohol consumption of the participants is examined; While 54.6% (n=165)

did not consume alcohol, 37.1% (n=112) consumed 1-5 units of alcohol per week, 6.0% (n=18) consumed 5-10 units of alcohol per week, and 2.3% (n=7) consumed 10-15 units of alcohol per week. A statistically significant difference was found between the BMI levels of individuals and their breakfast cereal consumption rates ($p = 0.015$; $p < 0.05$). As a result of the paired comparisons; it was found meaningful that these ratios were higher; underweight individuals consumed breakfast cereal occasionally (57.7%), while normal weight and overweight individuals did not consume it (51.1% and 58.8%, respectively) There was no significant difference between the breakfast cereal consumption rates of the other groups.A statistically significant difference was found between the consumption rates of pickled cabbage or cucumber according to the BMI levels of the individuals ($p=0.045$; $p<0.05$). As a result of the paired comparisons; The rates of obese individuals consuming pickled cabbage or cucumbers (50%) were found to be higher than the consumption rates of underweight and overweight individuals (19.2% and 26.3%, respectively). The rates of not consuming pickled cabbage or cucumbers (34.6% and 25%, respectively) of underweight and overweight individuals were also found higher than normal weight individuals (14.4%). There was no significant difference between the consumption rates of pickled cabbage or cucumber among the other groups. A statistically significant difference was found between the consumption of red wine according to the BMI levels of the individuals ($p=0.015$; $p<0.05$). As a result of the paired comparisons; The rates of red wine consumption of underweight, normal weight and overweight individuals (53.8%, 36.8% and 32.5%, respectively) were higher than obese individuals (9.1%). There was no significant difference between the red wine consumption rates of the other groups. A statistically significant difference was found between the ratios of raw green leafy vegetables consumed by males and females ($p=0.002$; $p<0.05$). The rate of consuming raw green leafy vegetables (64.9%) of females is higher than that of males (44.2%). The rate of consuming pickled cabbage or cucumber (36.9%) of female individuals was higher than that of male individuals (20.8%), and a significant difference was found between consumption depending on gender ($p=0.034$; $p<0.05$).

According to kefir consumption rates, 44.9% of female individuals consumed kefir, while this rate was 26% for males, and a statistically significant difference was found in kefir consumption depending on gender ($p=0.013$; $p<0.05$). When the ratio of white meat types consumed by individuals according to their gender was examined, 47.1% of female individuals occasionally consumed white meat varieties, while the rate of male individuals consuming white meat varieties was 67.5%, and a statistically significant difference was found between them ($p=0.025$;

p<0.05). The rate of consumption of sucuk (fermented meat product) was found to be higher in men than women (32.5%, 17.8%, respectively) and a statistically significant difference was found between (p=0.025; p<0.05). A statistically significant difference was found between the consumption of dried meat and meat products according to their gender (p=0.009; p<0.05). The rate of consuming dried meat and meat products was higher for males (50.7%), and the rate of not consuming dried meat and meat products for females (68%). The rates of consumption and occasional consumption of beer (19.5%, 57.1%, respectively) of male individuals are higher than the consumption and sometimes consumption rates of women (respectively; 10.2%, 33.8%), and a statistically significant difference was found between consumption rates by gender (p=0.001. p<0.05).

6. Comments

Intestinal microbiota, which has more than one important role in improving and enhancing human health, such as regulating energy metabolism in the human body, sorting out non-digestible dietary components, modulating the immune system and synthesizing essential vitamins, is recently seen as a key role in the treatment of many diseases, especially obesity and metabolic diseases related to obesity. The mechanism underlying in these key roles that microbiota plays in the human body is not yet fully understood, but more and more studies on both humans and animals show us more and more about microbiota.

The most important factor affecting microbiota health is a healthy diet. Studies report that the microbiota responds to different nutritional patterns in less than 72 hours, causing changes in the microflora. However, seeing the positive effects of a healthy microflora on the human body is possible with a healthy diet plan in the long term.

The effects of prebiotics and probiotics in improving and enhancing microbiota health are proven by studies. However, in most of the studies conducted in this direction, results are obtained by giving prebiotics and probiotics to patients in the form of supplements in the form of sachets, powder or tablets. There are not many studies on which foods contain prebiotics and probiotics naturally and in what amount they should be consumed. In addition, the effect of prebiotic and probiotic consumption on body weight has not been fully proven yet, and not enough studies have been done yet on these people. Increasing studies in this direction can positively affect public health by supporting the food sector to add probiotic and prebiotic bacteria to the basic foods that individuals consume in their daily lives and to make these foods functional and increase their accessibility. In addition, individuals can be made aware of the presence of natural foods with probiotic and prebiotic effects, and many diseases caused

by bad microflora can be prevented by nutrition.

Considering the diversity and availability of prebiotic and probiotic foods included in this study and the fact that the individuals participating in this study are from different segments in socio-demographic, socio-cultural and socio-economic terms, it can be explained that why there is not a statistically significant difference between most of the foods on the survey and BMI of the individuals. In addition, the fact that BMI does not give us enough information about the body fat ratios of individuals and working with devices that measure body fat and muscle amount and/or waist-hip measurements in such studies may be a more accurate method to reveal the relationship of the study with obesity. Finally; A study in which a nutritional questionnaire with more specific food groups (such as milk and dairy products with only probiotic effect or fibrous vegetables with prebiotic effect) is applied on individuals with the same demographic characteristics can give us more accurate and meaningful results about the effects of probiotics and prebiotics on body weight.

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