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Editor

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gece kitaplığı

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SILVER DIAMINE FLUORIDE IN PEDIATRIC DENTISTRY

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Introduction

Tooth decay is an microbial disease that being irreversible and leads to cavitation demineralization of inorganic parts of the hard tissues of the tooth and destruction of the organic part by cariogenic microorganisms (1). Although the frequency of being seen tooth caries in developed countries has decreased compared to previous years, today it is still one of the most common chronic diseases in both industrial societies and low-income countries (2).

Dental hard tissues are constantly affected by pH changes in the mouth environment. Falling the critical pH level for hydroxyapatite crystals in the mouth drops below 5.5 causes calcium and phosphate ions in the structure of dental hard tissues to dissolve and move away from the structure. This process is called as demineralization of dental hard tissues. If the factors that decrease the pH level in the mouth are eliminated within a short time, the ions dissolved as a result of the pH value rising again will settle on the enamel. This process is called as remineralization. The balance between demineralization and remineralization can be impaired due to many factors that affect the oral conditions (3,4). Thanks to the determination of the factors affecting the remineralization and demineralization processes and the understanding of the pathological process of dental caries, preventive applications have become more prominent especially in recent years.

The purpose of preventive applications is to early diagnose the newly formed lesions, to remineralize the demineralized areas before cavitation occurs and even to protect the dental hard tissues by preventing before occurring demineralization (5).

Many different materials such as ions, sugar alcohols, herbal agents, ozone, bioactive materials or nanotechnological products have been used to prevent dental caries from past to present (5,6). At the top of the most used materials is coming fluoride. Prevention of pellicle and plaque formation, the inhibition of acid production of plaque microorganisms, ensuring the formation of fluorapatite, which is a structure more resistant to caries from the hydroxyapatite structure in natural enamel, accelerating the precipitation of calcium and phosphate ions on the tooth surface, and bactericidal properties explain the anti-carious and remineralizing effect of fluoride (7).

Silver compounds also were first started to be used in the early 1840s in order to reduce the caries incidence of silver nitrate in primary teeth. Later, it has found use area as an anti-carious agent, cavity disinfectant and desensitizer in permanent molar teeth (8). Silver ion has been the subject of different remineralization studies, because of reducing the solubility of dental hard tissues in acidic conditions and providing remineralization of demineralized tissues (8,9).

When examinations are made for the common effect of silver and fluoride, it has been reported that 38% silver diamine fluoride solution prevents demineralization with a synergistic effect, minimizes mineral loss, slows collagen breakdown and shows bactericidal effect on cariogenic bacteria (10).

Silver diamine fluoride (SDF) is a low-cost, easy-to-apply agent using for caries control and appears to be appropriate the World Health Organization (WHO)'s millennium goals and the US medical institute's 21st Century medical care criteria (11,12). Restoration of caries by traditional methods is quite difficult in children, at a very young age or when they show limited cooperation, and in such cases, SDF can be used to slow and arresting caries' progression (13).

Silver Diamine Fluoride in Dentistry

SDF is a colorless, alkaline topical solution containing fluoride, silver and ammonium ions (14). Firstly, it was started to use as a therapeutic agent in Japan in the 1969. It has also been used in other countries such as Brazil, Australia and China for treating dental caries (15). In 2014, it was allowed to be used for dentin sensitivity by the FDA (Food and Drug Administration) in the USA (16).

Silver has been used by humans for thousands of years because of its antimicrobial properties and it is precious (17,18). In conducted studies, it has been reported that silver interacts with sulfhydryl groups of proteins and with DNA, affects hydrogen bonds, inhibits the respiratory process, inhibits DNA unwinding, cell wall synthesis and cell division. At the macro level, these effects cause the death of bacteria and inhibition of biofilm formation (19,20).

While silver salts having a long history of use in medicine and dentistry provide antimicrobial effects, on the other hand topical fluoride is effective in preventing tooth decay. Therefore, the combination of fluoride and silver has the ability to arrest caries and also prevent the formation of new caries (11). Ammonia, acts as a stabilizing agent for the solution (14).

There is 44.800 ppm fluoride in 38% SDF and this fluoride concentration is highest among the available agents for dental use. This fluoride ions support the remineralization of hydroxyapatites in enamel and dentin (15). Fluoride converts dental hard tissues to fluorapatite, which is more resistant than hydroxyapatite against acid attacks (21). When SDF is applied to the dental tissue, as a result of its reaction with hydroxyapatite, it creates silver phosphate and calcium fluoride as an additional by-product fluorapatite (20). Silver phosphate deposits on the tooth surface are insoluble and act as a fluoride reservoir against dissolution in calcium fluoride caused by various organic acids in the mouth environment (1).

SDF protects dentine against collagen breakdown as it inhibits some collagenases in dentin. This is very important because the collagen network serves as the scaffold for new remineralization cores (22).

Conducted studies show that silver diamine fluoride penetrates up to 25 µm into enamel and 50-100 µm into dentin (19,20). The SDF; it is reported that it involves almost 2-3 times more fluoride than sodium fluoride-phosphate, sodium fluoride and tin fluoride. Based on this, it can be said that silver diamine fluoride will be more effective than sodium fluoride and tin fluoride (23).

Clinical Use for Dental Treatment

Traditional tooth restoration treatment is very costly and at the same time, the technique applied during the operation and the patient's cooperation are of great importance for the success of the procedure. This makes treatment difficult for pediatric patients, too. Especially, the use of SDF in caries in young children or in patients with limited cooperations provides benefits in many ways. These benefits can be listed as follows (24).

• It is safety (no serious side effects have been reported in conducted many studies),

• It is effective (arrests about 80% of the caries on the treated teeth),

• It is efficient (it can be applied by healthcare professionals in different health institutions with a preparation that takes less than 1 minute),

• It is fastly (it can be treated as soon as caries are diagnosed as it is easy to apply),

• It is patient-centered (it meets the urgent needs of the patient in a single session since it is minimally invasive and painless),

• It is equitable (it is a suitable treatment for low income patients because it is low-cost).

General usage areas of SDF; arresting caries, prevention caries, treatment of hypersensitive teeth and disinfection of root canals (1,15,17).

The feature of arresting caries is due to the antimicrobial effect of SDF (10). In the guideline published by the American Academy of Pediatric Dentistry (AAPD) in 2017, the use of SDF is recommended for the treatment of active caries in patients with pediatric and special needs (25). In a study conducted by Yee et al. (26) in Nepal, they found that 38% of SDF was very effective in arresting caries, even with a single dose. In a pilot study that examined the caries-arresting effect of SDF in the occlusal caries cavities in 66 first permanent molar teeth, they stated that they had a significant success in arresting caries lesions in the controls 3 and 6 months

later (27). There are many studies examining the caries-arresting effect of SDF on primary teeth (28,29). In a study conducted by Clemens et al. (16), they examined the effect of SDF application on the primary teeth of 32 children having active caries who were untreated between the ages of 2-5. In this study, which examined the short-term results of SDF application, they reported that the arresting active caries lesions was very effective in primary teeth. In a study by Chu et al. (30) in China, they found that SDF is an effective agent in arresting dentine caries in primary teeth. In their systematic review of Chibinski et al. (22), in which they examined the effect of SDF on caries in primary teeth, they concluded that the use of SDF is 89% more effective than alternative treatments or placebo in controlling/arresting caries in primary teeth.

Direct SDF application to the healthy tooth surface in children helps to prevent caries formation. In a systematic review conducted by Oliveira et al. (31), it was concluded that the application of SDF in primary teeth effectively prevents dental caries compared with fluoride varnish, placebo and no treatment. In another systematic review, they said that 30% and 38% SDF is a potential for caries prevention in primary teeth and permanent first molars (32).

In a literature review of Chuo and Lo (13) with SDF on arresting caries in children reported that they prevent from new caries forming in primary teeth and can be an effective agent in arresting caries. They suggested that it can be used to arrest the caries progression in young children who are not cooperative and restorations can be done when the child becomes older and more suitable for dental treatment.

In a study where Llodra et al. (33) followed the decay-reducing effect of SDF for 36 months in both primary teeth and permanent first molar teeth in children of 6 years, they found that 38% of SDF is effective in both dentitions. Regarding the prevention of the formation of new caries lesions, SDF showed a higher percentage of efficacy in primary teeth (80%) than permanent first molar teeth (65%).

Many clinical studies have shown that SDF is effective in preventing caries and arresting the progression of existing caries. In addition to the results of these studies, in the 2016 report of the WHO on public health interventions against early childhood caries (ECC), they reported that SDF could arrest the progression of ECC (34).

Based on these effects of SDF on caries, it has been reported that it can be used to prevent pit and fissure caries and to increase the resistance against secondary caries formation in the restoration margins (19,35).

SDF application does not restore the tooth as form or function, so

conventional restorations can be used after caries arrested in cavitation lesions (36,37). Quock et al. (38) proposed the hypothesis that a restoration can be also performed after caries will be arrested by applying SDF without mechanical handpieces or dental drills. Silver-modified atraumatic restorative treatment (SMART) which is an adaptation of SDF application and Atraumatic Restorative Treatment, can be restored effectively in other visits of carious cavities arrested after SDF application (36). However, Alvear et al. (39) reported that SDF and glass ionomer cement may be applied to the same appointment in cases where patients cannot come to the next appointments or it is advantageous to use a minimally invasive procedure rather than doing nothing. The advantage of the SMART technique is that while the majority of bacteria in the cavity disappear with SDF, when a chemically-binding ART restoration is used, the remaining bacteria are also disconnected from the mouth environment, arresting the carious lesion, remineralizing and thereby protecting tooth tissue and pulp vitality (39).

In SDF application, both silver ions can precipitate proteins in dentin tubules, and fluoride ions can react with free calcium ions to form calcium fluoride deposits that can block dentin tubules (40). Thanks to these properties of silver and fluoride, SDF can be used in the treatment of dentin sensitivity, as it has the ability to block dentin tubules (41). In a study conducted by Craig et al. (40), they used it together with the idea that the use of potassium iodide will further decrease the dentin permeability after SDF application. As a result of this study, more satisfaction was observed in patients with teeth using SDF/potassium iodide than using alternative agent. They concluded that it was a clinically safe and effective desensitizer in studies where Castillo et al. (41) examined the short-term effect of SDF in sensitive teeth.

It is thought that SDF can also be used in teeth with Molar Incisor Hypomineralization (MIH) as it can reduce sensitivity by blocking dentin tubules (14,36).

It can be used where it is not important to darken the dentin with the silver component for irrigation of the root canal. Because of its inhibitory effects on bacterial cell wall synthesis, DNA unwinding, and cell division, it can effectively reduce microbial load within a root canal (1). In their study by Mathew et al. (42), in which they examined the antimicrobial efficacy of SDF, they found that 3.8% SDF was very effective in reducing bacterial density in circumpulpal dentine and canal wall. In their studies where Hiraishi et al. (43) examined the effectiveness of 3.8% silver diamine fluoride as an antibacterial agent against Enterococcus faecalis biofilms, they reported that they have the potential to be used as root canal irrigation.

Contraindications

It is contraindicated in the presence of a known silver allergy of individuals, in teeth requiring pulp treatment, such as irreversible pulpitis and necrosis, and also in cases parents do not allow it with concern for discoloration (17,18,37). It is still contraindicated in any desquamative gingivitis or mucositis that disrupts the protective barrier formed by the stratified squamous epithelium, as there may be irritation by contact during SDF application in the presence of open wounds such as herpetic gingivostomatitis and ulcerative gingivitis (18,37,44).

Clinical Application Protocol

It can be applied to the sound tooth surface (45) for preventing tooth decay, to the carious lesion (30) for arresting caries or to the surface of the sensitive tooth (41) for hypersensitivity.

After removing plaque and other debris from the tooth surface, it is recommended to use protective petroleum gel to prevent irritation that may occur as a result of SDF contact with skin, lips and other soft tissues (17). Teeth should be isolated with cotton rolls and tooth surfaces should be dried (18). It is not always necessary to remove soft caries in carious teeth before SDF application. It can only be done in the anterior region to reduce black coloration for aesthetic purposes (1). The UCSF (The University of California, San Francisco) protocol has been stated as follows about the penetration of SDF into the affected dentin by chemical preparation (37). The use of EDTA to remove superficial hydroxyapatite in the affected dentin can open the dentin tubules and allow greater penetration of SDF. Also hypochlorite application can break down bacteria and expose dentin proteins, but it is unnecessary for the effectiveness of this silver.

It is applied by putting 1 drop of SDF into the plastic dappen dish to the tooth/caries surface with a disposable micro brush (18). The ideal application time of SDF is 1 minute and it should be done gently using compressed air until the SDF is dry (17). Re-application may be considered when applying for a shorter period of time in young noncooperative patients (1). After removal of excess SDF fluid with cotton rolls or gauze after application, the region needs be isolated for another 3 minutes (1,17). After rinsing with water, equipments such as gloves, cotton roll and micro brush, should be placed in plastic waste bags as they can damage SDF glass and metal (18).

It is recommended not to eat and drink between 30 minutes and 1 hour after the operation (1).

In the literature, different application frequencies have been reported,

including one-time application (26), 3 times at weekly intervals (28), annually (30) and semiannually (46). In the studies of Duangthip et al. (28) where they examined the use of 30% SDF solution at different frequencies, they found that the use of 3 times (baseline, 12th month, 24th month) at yearly intervals was more effective than using 3 times (baseline, 1st week, 2nd week) at weekly intervals in active cavitation dentin caries. In another study where the frequency of application SDF was examined, they reported that the use of 38% concentration semiannually was more effective than using it annually to arrest caries (47). In addition to this, many clinicians recommended 6-month visits (6^{th} month, 12^{th} month, 18^{th} month, 24^{th} month) after SDF application on the first diagnosis visits of patients then at 1 and/or 3 month follow ups (1,37).

Safety and Adverse Effects of Sdf

Any fatal and systemic side effects of SDF used according to the manufacturer's recommendations haven't been reported in none of the reviews and experimental studies (41). It can cause gum and mucosal irritations, which can be called minor side effects, which usually resolve within 2 days (1). In a study conducted on young children, 1 week after the application, the prevalence of pain in the teeth and gums was 6.6%, the prevalence of gum swelling was 2.8% and the gum whitening was 4.7% reported by parents (48).

Painting of carious lesions in black after SDF application is an important disadvantage that may cause dissatisfaction with children and their parents (49). This dark coloration is due to the formation of silver phosphate (26) . In a survey study in the USA, it has reported that parents judged SDF staining on the posterior teeth to be more esthetically tolerable than the anterior teeth. In addition to this, even among those who found unsightly the painting on the anterior teeth, a lots of parents found SDF application acceptable to avoid advanced behavioral techniques (such as sedation and general anesthesia) (50).

Many studies suggest using potassium iodide after SDF application to control or reverse staining (24). In a study by Nguyen et al. (51), potassium iodine was used after SDF application to decrease coloration. It has been found that it significantly reduces color distortion caused by SDF.

Another disadvantage of SDF includes staining clothes and skin. It has a unpleasant metallic taste in the mouth (1). Due to the photosensitive of the SDF, the requirement to be maintained it in the dark container is one of its other disadvantages (52).

Although there is no acute toxicity reported about SDF when used as recommended, high fluoride concentrations (44,800ppm at 38% SDF)

can lead to fluorosis, especially in very young children, when repeated or over-administered (13).

It is very important to know the safety margin for dosing. Animal studies have been made to determine the lethal dosage (LD50) of SDF by oral and subcutaneous administration. The average is LD50 520 mg/kg by oral application and is reported as 380 mg/kg by subcutaneous application. One drop of 25 μL is quite sufficient to treat 5 teeth and contains 9.5 mg of SDF. Assuming that the youngest child with caries is 10 kg, the maximum dose will be 0.95 mg/kg. This shows us that using a full drop in a 10 kg child is about 400 times less than the lethal dose (18,37). The maximum limit is recommended as one drop per 10 kg of body weight per treatment visit at weekly intervals (37). Many researchers have suggested taking precautions and avoiding multiple and frequent applications on young children, although the amount of SDF applied is very little (13,24).

Conclusion

SDF is very useful in caries management in young children as it is an easy-to-use, low-cost, effective material that preventing caries and arresting its progress. Studies and clinical cases have found that SDF has a wide application in dentistry. With the further development of SDF application protocols and widespread use, it will be possible to obtain results at the social level in the management of caries.

We need to find ways to protect patients' oral health in cases where patients have limited access to treatment, or like the current world-wide outbreak of COVID-19. Many dental treatments produce aerosols and droplets that can be contaminated with the virus. In such pandemic situations, any procedure that produces aerosol should be avoided, but we need to design new ways to delay the spread of dental disease. In the acute phase of the pandemic, many countries ended all non-emergency dental treatments. However, there will be many situations where a number of non-surgical caries management approaches can be used to help reduce the initiation, progression of the caries and need for surgical procedures. The American Dental Association (ADA) and AAPD are constantly in contact with Centers for Disease Control and Prevention (CDC) for establishing a consistent guide between them. According to this guideline (53); dentists who treat children during this pandemic should enact to the highest standards of universal infection control procedures and advocate this behavior through their team. Opportunities to improve preventive dental behavior should be evaluated. Minimally invasive treatment and procedures that eliminate aerosol generation should be preferred as the risk of viral contamination will still remain high when practice restrictions begin to be eased. These procedures are; ART, fissure sealant applications, SDF and hall technique.

We believe that, it can be applied to large groups of patients thanks to its many positive features as low cost, limited equipment needs, easy applicability, effectiveness and safety in the areas where it is difficult to access dentists and healthcare services in non-cooperative or requiring special-care children, in the treatment of asymptomatic caries.

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Chapter 2

SURFACE CHARACTERISTICS OF IMPLANTS USED IN DENTISTRY

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1. INTRODUCTION

Titanium and its alloys are widely used today in implant manufacturing due to their mechanical strength, corrosion resistance and biocompatibility. Surface morphologies of titanium implants are critical in terms of long-lasting stability for the period following implant placement. Various surface treatments are employed to modify the surface morphology of titanium dental implants and to increase the effective surface area in bone-implant contact. Successful clinical use of dental implants requires functional integration with the surrounding tissues of the implant. The type and size of tissue implant interactions are determined by cellular and molecular events at the tissue implant interface.

Various conventional methods are used to modify the microtopography of the surface in order to increase mechanical adhesion between the implant and bone. These conventional methods include sandblasting, plasma spray spraying, etching, and anodization. Although sandblasting is a very popular method, it leads to Al and Si contamination depending on the type of sandblasting instrument used. At the same time, it creates a random surface roughness due to the fragile nature of the ceramics. Although methods that use calcium phosphate and TiO as sandblasting instrument have been developed to prevent this pollution, no solution has been found for distortion and unwanted geometrical irregularity problems on the implant surface. Considering all the foregoing, laser surface structuring is a promising method to create unique, regular surface structures. The laser surface configuration process is a rapid process without contamination and has the advantages of avoiding the application of any force to the implant surface. When the optimal structure of the implant surface is roughened using the laser method, this surface pollution is prevented because the laser can provide a contactless surface treatment. The laser method is one without sufficient surface roughness and surface contamination for osteointegration on implant surfaces.

Depending on the recent advances in surface technology, dental implant treatment is applied in several patients and progress is being achieved in the success rates of the treatment results.

2. Dental Implantology and Its History

The word “implant” consists of a combination of Latin words in (inside) and planto (planting, planting, placing). The first finding of dental implants was obtained in 600 BC after the excavation of a grave belonging to the Mayans (1). In ancient Egyptian records, animal and carved ivory teeth are one of the oldest examples of simple implantology (2). In ad-

dition, sea shells were shaped and placed in the maxilla bone and it was observed that they integrated with the bone because they contain calcium carbonate (3). It is claimed that in Chantambre (Essonne, France) there is an iron-implanted iron implant applied to a 2nd century Gallo-Roman (4).

The first recorded study in oral implantology was conducted by Maggiolo in 1809. An artificial root prepared from 18 carat gold was placed in the extraction cavity immediately after tooth extraction. Maggiolo stated that gold was used because it was tissue friendly and it was emphasized that the placed gold root should be waited for a month before the upper prosthesis is made (5,6).

In 1967, implants in dental form were started to be made and tested in apes. Blade-type implants made of chrome, nickel and vanadium were developed in 1968. Aluminum oxide and single crystal sapphire implants were developed in the 1970s (7,8).

The first clinical application of dental implants was carried out by PI Bränemark in 1965. Bränemark et al. explained the relationship between titanium and bone in 1985 with the term 'osseointegration'. Osseointegration has been defined as 'functional and structural association between living bone and implant surface' (1). In the following 5 years, the success rates were reported at values as low as 50%.

In the 1970s, implants were started to be made in larger and different designs, healing time was extended, and changes were made in surgical and prosthetic techniques (9).

Adell et al. announced the results of 15 years of implants applied in edentulous patients in 1981, exactly 16 years after Bränemark's invention. This publication is the first study to shed light on the birth of modern implantology and its widespread use in the world (3).

The Toronto conference held in 1982 has an important place in the development of modern implant dentistry. George Zarb understood the importance of osseointegration and was chosen as one of the researchers tasked with popularizing the topic (9).

3. OSTEOINTEGRATION CONCEPT

The development and continuity of the bone implant interface is achieved by direct apposition of the bone matrix and the implant surface without interfering with soft or fibrous tissue. This process is called osseointegration. Osseointegration is essential for the strong composition between the bone and the implant surface (10). In other words, osseointegration refers to life-long bone formation and adaptation to function and repair (11).

Today, one of the alternative ways to improve osseointegration is implant surface technology. Successful osseointegration of dental implants depends on bone-implant interactions at the contact point. Dental implant surfaces play a key role in these interactions. Implant surface properties significantly affect bone formation on the surface and maintaining bone continuity. Ensuring the continuity of the bone depends on constant adaptation to functional loading and repair of the damage that occurs after the implant and the bone interfacial overload (12).

4. Types of Dental Implants

The classification of dental implants can be made according to the material used, the shape of the implant, and where it is placed.

4.1 Classification by Material Used

4.1.1 Metals and alloys

* Titanium and titanium-6 Aluminum-4 Vanadium
* Cobalt-Chrome-Molybdenum alloys
* Iron-Chrome-Nickel alloys
* Other metals and alloys (gold, platinum, palladium etc.)

4.1.2 Ceramics and carbons

* Aluminum-Titanium-Zirconium oxides
* Calcium phosphate originated
* Hydroxyapatite
* Carbon and Carbon Silicone Compounds

4.1.3 Polymers and Composites

* Polytetrafluoroethylene
* Polymethylmethacrylate
* Ultramolecular weight Polyethylene
* Polypropylene, Polysulfate

4.2 Classification by Location

a- Intraosseous implants

b- Subperiosteal implants

c- Intramucosal implants

d- Transmandibular implants

e- Transdental (endodontic) implants

4.3 Classification by Implant Shapes

a- Cylinder

b- Screw

c- Blade

d- Vent

4.4 Classification by Surface Properties

a- Unprocessed surface implants

b- Machined surface implants

c- Coated surface implants

d- Combined surface implants

4.2.1 INTRAOSSEOZ IMPLANTS

The term refers to the placement of the implant in the slot opened with a bur in the alveolar crest in the toothless area by using the guidance of this extraction cavity after tooth extraction.

a) **Screw-Type Implants:** It is an implant type that has a shape very similar to the tooth root. These types of implants are placed into the bone with standardized special instruments. In these implants, the bone grows towards the screw grooves that provide mechanical fixation, and these implants provide better primary stabilization than others. It is stated that the forces coming in the scale screw type implants are transmitted to the bone through the inclined surfaces of the grooves (13). In another study, it was stated that screw-type implants transmit less stress to the bone and transmit it more homogeneously (14). It is the most commonly used implant type today.

a) **Cylinder-Type Implants:** As the name implies, it is cylindrical in shape and it is an implant type with a morphology close to the root form. The primary stabilization of such implants depends on the friction between the outer surface of the implant and the bone. By placing the implant diameter larger than the implant bed, friction is provided between the surfaces. The surface of the implants is covered with titanium plasma spray or hydroxyapatite plasma spray in order to ensure molecular retention at the bone level (15).

b) **Blade-Type Implants:** It is used especially in free end cases, distal of mental foramen in mandible and thin crests where cylindrical implants are difficult to apply. In order to increase the amount of surface with bone and activate new bone formation, these implants are produced with titanium plasma spray and hydroxyapatite coating, with the exception of the neck parts. Since blade implants cause excessive bone loss when they need to be removed from the tissue, they are not preferable today (13).

c) **Vent-Type Implants:** These implants are intended to create a wider anchorage surface and reduce the implant volume, causing the least possible bone defect in the implant bed. The bone that develops in the holes in the implant body acts as a kind of shock absorber at physiological loads and increases the slip resistance at the implant-bone interface (16).

4.2.2 SUBPERIOSTEAL IMPLANTS

They are implants placed on the alveolar crest almost like a saddle. It was first placed under the periosteum on the bone cortex by the Swedish dentist G.S. Dahl in 1943 (17).

4.2.3 INTRAMUCOSAL IMPLANTS

These are implants in the form of buttons in the mucosa in order to increase retention of full or partial removable dentures. Intramucosal implants are also called submucosal or subdermal implants (18).

4.2.4 TRANSMANDIBULAR IMPLANTS

These are the implants placed in the submental region in the anterior part of the lower maxilla, and the upper and lower cortical bone crossing vertically (17). They are used especially when the lower maxilla is severely damaged due to an accident or a surgical operation.

Dental implants are used in various different indications, from the restoration of a single tooth to complete edentulousness, rehabilitation of congenital or acquired maxilla-facial deformities (19).

4.2.5 TRANSDENTAL (ENDODONTIC) IMPLANTS

They are also called endodontic stabilizers, transradicular implants or transdental fixations (18). It is known as grooved or non-grooved pin-shaped implants that pass through the root canal of the tooth and settle in the periapical bone in order to stabilize the mobile teeth (17).

5. INDICATIONS AND CONTRAINDICATIONS OF DENTAL IMPLANTS

Dental implants are used in the rehabilitation of all tooth loss, from patients with a single tooth deficiency to patients with total edentulism. However, it cannot be applied in uncontrolled systemic diseases that do not allow surgical procedures, patients undergoing radiotherapy, pregnant women, and advanced psychiatric patients. Sufficient bone thickness, height and quality are needed to place the dental implant. In addition, high muscle connectivity, insufficient adherent gums, and inadequate oral hygiene are contraindicated situations, as they negatively affect surgical success and post-operative prognosis.

6. TITANIUM ALLOYS USED IN DENTAL IMPLANTS

Titanium was discovered in 1790 by Reverend William Gregor. Gregor named this structure he found as Menachin. Three years later Klaproth found TiO_2, which was widely dispersed, and named this metal as the titan, which is the son of the sky and the earth, inspired by mythological titans (20).

Production of titanium starts with a concentration of 98%. $TiCl_4$ is obtained by exothermal reaction with the addition of carbon and chlorination. With the following distillation process, by-products such as iron, vanadium and silicon are generated. Under an inert gas atmosphere, TiCl4 is reduced with magnesium or sodium in a steel reactor. As a result of the exothermal reaction that occurs, metallic titanium called titanium sponge is exposed. The titanium alloy obtained is transformed into forms such as plate, rod, wire, and block with hot-cold exchange processes (21,22).

Titanium and its alloys are compatible with noble metal alloys in terms of elasticity modules. The relatively low cost and easy availability make titanium indispensable in some applications, such as dental implants. Titanium metal is highly reactive and has a high affinity for oxygen. When the newly turned metal surface comes into contact with air or moisture, an oxide film layer is formed on the surface almost instantly. Oxide in most aqueous media is typically TiO_2, but Ti_2O_3 and TiO can also be found. The naturally occurring oxide layer is 10 nm thick and invisible to the eye, but chemically very resistant. This thin surface oxide also creates an effective barrier against hydrogen. The oxide layer of titanium is mostly disrupted by hydrofluoric acid (23). As an extraordinary biocompatible metal, titanium does not cause allergic reactions and is a relatively radiolucent element. In addition, it has very good mechanical properties in terms of tensile strength, hardness, elastic modulus and thermal permeability (24).

There are some difficulties in the usage of titanium in dental restorations. These can be listed as follows: casting requires special methods, it

is difficult to process, and its surface is very hard. Titanium reacts with oxygen, nitrogen, hydrogen and some other elements. Therefore, it requires special casting under vacuum. Titanium and its alloys are poured under high vacuum or pure gasous atmosphere (25,26). The presence of high fluoride and low pH increases the solubility of titanium. It shows high resistance to chlorine salt solutions and oxidizing acids such as nitric acid and chromic acid. It is also resistant to oxidizing organic acids and lactic acid. It is damaged by the reduction of hydrochloric, sulfuric and phosphoric acid (24). One of the unique properties of alloys is the potential for passivation. In the alloys showing fast and stable passivation, low ion emission occurs with appropriate corrosion behavior. Therefore, titanium and its alloys are generally resistant to corrosion (24).

In dentistry, two different titanium derivatives are used, namely Ti-6Al-4V and pure titanium (CpTi). To these, cast and cold-formed titanium alloys can also be added. The production patterns, composition, structure and properties of various titanium types are completely different from each other (27).

6.1 Commercially Pure Titanyum (CpTi)

CpTi, which is not available in alloys, can be found in 4 different forms as Grade 1, Grade 2, Grade 3 and Grade 4 depending on oxygen and iron content (27).

Table 1: Chemical Composition of (% weight)

ELEMENT	Grade1	Grade2	Grade3	Grade4
Nitrogen (max.)	0.013	0.3	0.5	0.5
Carbon (max.)	0.10	0.10	0.10	0.10
Hydrogen (max.)	0.10	0.10	0.10	0.10
Iron (max.)	0.20	0.30	0.30	0.50
Oxygen (max.)	0.18	0.25	0.35	0.40
Titanium (max.)	99.48	99.31	99.19	99.94

CpTi contains 0.18–0.40% oxygen. Oxygen ensures that the metal in the solution remains in one phase. Elements such as oxygen, nitrogen and carbon have more solubility in the hexagonal α phase than the cubic form

β-phase. These elements transform the solid form into titanium and help stabilize the α-phase. Transition elements such as molybdenum, cobalt, nickel, niobium, copper, palladium and vanadium are the most widely used β stabilizers. The α-stabilizers increase the allotropic conversion temperature but decrease the β stabilizers. β phase titanium alloys are more resistant but more fragile than α-phase alloys (27).

6.2 Ti-6Al-4V

The small amount of aluminum and vanadium alloy added to titanium significantly increases the resistance compared to CpTi. While aluminum acts as the α-stabilizer, vanadium plays a role in β- stabilization. With the addition of these elements to titanium at the temperature at which the α-β transformation occurs, the transformation is suppressed and in this way both α- and β- forms are formed at room temperature. Therefore, Ti-6Al-4V has a two-phase structure of α and β grains. These alloys are still the most widely used biomaterial among different titanium alloys because they are easy to obtain, suitable for working conditions and have strong mechanical properties at low temperatures (27).

The mechanical properties and biocompatibility of titanium are excellent. The elastic modulus is closer to the elastic modulus of the bone than other implant materials; thus, the force distribution is more regular at the bone implant interface (17).

Although titanium implants are thermodynamically stable, there is a small amount of ion release. Today, Ti-6Al-4V alloy is generally used in implant applications. It is known that the titanium released as a result of corrosion remains well below the titanium values taken with food. Titanium is easier to bend mechanically than titanium alloys. Titanium implants with normal root-shape allow for designs with a thinner cross section as they are approximately 1.5 times stronger than compact bone, and processed Ti-6Al-4V alloy implants 6 times stronger (25).

Comparison of some features of Ti-6Al-4V with CpTi is shown in Table 2. CpTi's elastic modulus is 110 GPa. The elasticity module of Ti-6Al-4V is between 85 and 115 GPa. These values are quite high compared to the bone whose elasticity modulus is 17-28 GPa (27).

Table 2 : Comparison of some characteristics of Ti- 6Al-4V and CpTi

CHARACTERISTIC	TITANIUM	Tİ-6A1-4V
Density (gr/cm$^{3)}$	4.5	4.5
Cast temperature (^{0}c)	1700	1700
Tensile stress (MPa)	520	100
Proportional stress (MPa)	350	920

Elastic modulus (GPa)	110	85-115
Hardness (VHN)	200	-
Fleexibility (%)	20	14

7. SURFACE CHARACTERISTICS OF DENTAL IMPLANT

In order to provide osseointegration, the quality of the bone to be implanted, the tissue compatibility, design, surgical technique and load transmission of the implant material are thought to have an important function as well as surface properties. This is so partly because the response of bone tissue is different according to the surface properties of the implant used. It is stated that the implant surface should have a healing effect, regardless of bone quality, quantity and anatomical region (28).

During bone development between bone and implant, four different stages occur. These are surgical implant placement, healing dynamics, early loading period, and late loading period. All implant design and surface properties affect these four phases. While implant design plays an important role in primary stabilization, its surface feature is helpful. However, in the initial healing period, the surface feature is primarily effective (29).

The roughness values obtained after the treatment change in a wide range according to the type and quality of the treatment applied to the surface. Main parameters such as grain size, grain shape and spray pressure, acid composition and temperature in acid etching change the average surface roughness in a wide range (30,31). Surface roughness is often expressed by the value of the "average surface roughness" indicated by the 'Ra' symbol.

Today, one of the alternative ways to improve osseointegration is implant surface technology. Successful osseointegration of dental implants depends on bone-implant interactions at the contact point. Dental implant surfaces play a key role in these interactions. Implant surface properties significantly affect bone formation on the surface and maintaining bone continuity. Osseointegration is achieved through cellular processes involved in bone formation on alloplastic surfaces. Ensuring the continuity of the bone depends on continuous adaptation to functional loading and repair of the damage that occurs after overloading the implant bone interface (32).

Surface modifications are applied to biomaterials to improve mechanical, chemical and physical properties. The purpose of surface modifications in implants produced for both dental and orthopedic applications is to create biological, mechanical and morphological tissues that support osseointegration (33).

The characteristics of the biomaterial surface direct the processes related to the biological response. The biomaterial surface plays a critical role in biological interactions for four reasons:

- The biomaterial surface is the only part that comes into contact with the biological environment.
- The surface area of the biomaterial is always different from the main substance in terms of morphology and composition.
- Surface properties control biological responses.
- Surface properties affect the mechanical stability of the interface (33).

The first response of living tissue to any biomaterial is rejection. Accordingly, the biomaterial is normally considered a foreign substance by the host tissue. The biological acceptance of these impurities by living tissues is mainly controlled by surface and interfacial reactions between organic matter and inorganic matter. The surface is not just free finishing of matter. The surface is the contact and boundary zone with other substances. A physical system that includes homogeneous components such as solid, liquid and gas and can be easily distinguished from each other is called phase. The boundary that these phases are in contact with is called the interface (33).

Implant success in bone-related applications depends on the biological, physicochemical and mechanical properties of the implant material. Due to the tissue response and environmental reactivity, the implant surface is in constant change. This process is associated with long-term implant stability. In other words, implant integration is determined by the performance of the system, mainly occurring at the tissue implant interface. Most of the important interactions between tissue and implant occur at the interface created between the material surface and the biological environment. These interactions for implants generally consist of protein-surface and cell-surface phenomena (34). The structure, shape, topography, chemistry, surface energy and mechanical properties of the material can determine the orientation of the molecules and which molecules will be absorbed onto the surface, thereby affecting normal bone cellular development (35).

The success of titanium implants depends on the direct contact of the implant with the alveolar bone that surrounds it. The 2-6 nm thick oxide layer on the surface of the titanium implants ensures that the implants have high corrosion resistance and biocompatibility properties. The surface properties of the implant regulate the migration of specific cell types from the host tissue to the surface of the implant and determine the function of these cells. Therefore, topographic properties and rough structure of the implant surface are important variables that affect bone and soft

tissue accumulation on the implant surface. In general, soft tissues adapt to the shiny and polished surface of the neck of the implant, while the rough structure in the body of the implant causes more bone to form on the surface. The methods used to process the implant surface are divided into three main groups (36).

1. Addition of substance to the surface: Plasma spray methods (Titanium plasma, titanium dioxide), hydroxyapatite (HA) and other coatings.

2. Removal of substance from the surface: Using particles that hit the surface and acid etching methods.

3. Surface arrangement: Roughening can be done without removing or adding material from the surface (electron thermal roughening, laser and ion implantation).

According to the researchers, as the bone quality decreases, the need for treated surfaces increases, whereas there are no significant differences in areas that can be accepted as quality (37). When supported by the determination of bone physiology before the operation, it is possible to choose the most suitable implant surface that can be used.

Several methods have been developed to increase the surface roughness of dental implants and to improve osseointegration. These methods are divided into 4 main categories (38):

1. Physical methods
2. Chemical methods
3. Biochemical methods
4. Laser Surface Modifications

7. 1. PHYSICAL (MECHANICAL) METHODS

Changes in surface morphology and roughness are used to affect the cell and tissue responses of the implants. In addition to their mechanical bonding, corrugated (grooved) surfaces can form a “contact guide”, so that the cell movement direction is affected by the morphology of the base material.

The surface treatments applied to the implant material affect the surface energy as well as the roughness of the surface. Therefore, in determining the degree of attachment of the implant to the tissue, surface roughness as well as surface energy should be taken into account. In the literature, it has been suggested that there is a relationship between surface roughness and surface energy and contact angle (39). In general, increased roughness reduces the angle of contact.

Mechanical methods are methods in which the surface is shaped by physical forces. The most widely used mechanical techniques are machining, turning, titanium plasma spray and sandblasting.

7.1.1.Turning

Turning, which has been widely used in the past, is done with a stainless steel cutting instrument. However, studies have shown that treated surfaces are required to be modified to rough surfaces in order to increase implant stabilization and expand the surface area (40). In addition, surface morphology has been shown to play a role in cellular behavior.

7.1.2. Machining

It refers to making anisotropic some traces and obvious surface irregularities created by turning process. Heterogeneous topography affects bone cells and reduces the speed of osteointegration. As the depth of the cavities increases, the trend of unidirectional cell growth also rises. They show a great trend following cell orientation during cell growth. These behaviors lead to a longer recovery time. In a study, it has been shown that implants with a machine surface have a lower breakdown torque value than treated implant surfaces, which reduces osteointegration. The initial healing time of implants with machine surface is shorter than treated implants. Lower resistance was observed at the bone-implant interface of implants with a machine surface. (41)

Bone growth occurs preferentially on surface irregularities in dental implants with a machine surface. On the turned implant surfaces, cell retraction occurs during the growth of cells in the wells of the substrate, such as the contact guide (42).

On the machine surfaces, the fibroblasts take planar shape and there are growth trends along the machine tracks. This behavior is very different from surfaces with homogeneous roughness. On homogeneous surfaces, epithelial and fibroblast cells take a round shape and grow in all directions (41).

7.1. 3 Titanium Plasma Spray (TPS)

Titanium plasma spray is one of the most widely used implant surface coating methods. TPS surface is obtained by coating the titanium implant surface with titanium particles by thermal spraying. With this method, the surface area of the implant is increased approximately 6-10 times. In TPS surface implants, the surface thickness is about 20-30 microns, while the surface roughness is about 15 microns. In studies conducted, it is stated

that rough surfaces are more physiologically advantageous than polished surfaces. The wettability of the rough surfaces is better. This is important for bone healing. In addition, implant-bone connection was found to be stronger in implants with rough surfaces (43).

Leize et al. (44) observed that when the TPS coated implants removed for various reasons were examined with an electron microscope, a chemical connection was established with the bone and calcium phosphate crystals grew into the rough surface. In this study, the presence of titanium particles that are very close to the rough titanium surface and whose diameter varies between 5-50 nm is shown.

TPS is often used in cases where higher surface roughness is intended (Ra> 2 μm). It is stated in some research that the TPS method can increase the surface area up to 6 times. For this reason, TPS implants are often recommended in areas with low bone density. However, increased surface roughness is also a negative factor due to the surface exposed to oral liquid and bacteria. The relationship between existing porous areas facilitates migration of pathogens into deeper bone areas and jeopardizes implant success due to peri-implantitis. Today, there is consensus on the clinical advantages of moderately roughened TPS implants, and several implant systems have moderate roughness (45).

6.0.4 Sandblasting

Another method applied for roughening the titanium surface is sandblasting the surface. Sandblasting agents are applied to the surface at high speed with compressed air. Different sizes of roughness are obtained on the titanium surface according to the size of the particles. These blasting materials used should be chemically stable and biocompatible and should not interfere with osseointegration of implants. Commonly used ceramic particles are alumina, titanium dioxide and calcium phosphate (37).

Titanium dioxide (TiO_2) particles create an average roughness of 1-2 μm in dental implants. There are many clinical studies reporting that TiO_2 roughened implants provide higher bone levels and long-term success compared to turned surfaces (9).

Calcium phosphates such as hydroxyapatite and ß-tricalcium phosphate are other materials used in roughening. These materials have biocompatible, osteoconductive and resorbable properties. There are also studies reporting the superiority of these materials over the treated surfaces (46).

The sandblasting method causes the formation of a thin superficial layer with stretching. The level of compression stresses is a function of the size and hardness of the particles as well as the pressure and sandblast-

ing time. Now the superficial layer with compression stress increases the fatigue strength of the material. The surface roughness obtained after the blasting process is larger than the acid etched surface and smaller than the oxidized surface (41).

The success of the blasting process depends not only on the nature of the application, but also on the type of metal used. Softer pits are formed on noble metals and sharper lines on non-noble metals. Many of the micromechanical techniques other than sandblasting require expensive equipment and precise technique and contain harmful chemicals. It has been reported that the blasting technique increases the bond strength. With sandblasting, the alloy surface is cleaned and the surface area increases. As a result of all these processes, an increase in the wettability of the metal surface is observed (47).

7.2. CHEMICAL METHODS

Chemical methods are applied to make modifications in the chemical structure of the titanium surface.

7.2.1 Acide etching

Another widely used roughnening method is roughening the titanium surfaces by etching them with strong acids such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4) and nitric acid (HNO_3). It is reported that 1.5-2 μm diameter micro-cavities are formed on the implant surface by acidification. There are also studies reporting that acidification significantly accelerates osseointegration (48).

Acid-etched dental implant surfaces show a superficial morphology that varies with the acid composition percentage, etching time and process temperature. It is possible to control the roughness, pore size, number and distribution in micrometer and nanometer scale during acid etching (41).

Acidic surface type facilitates osteogenic cell attachment and allows cell migration on the implant surface. Some dental implant manufacturers have reported that dental implant surface morphology similar to this surface causes fibrin adhesion and facilitates the osteointegration process. Acid-etched implants have higher disassembly torque than machine surface implants. It may mean that osteointegration is faster in acid-etched implants than machine surfaces (41,42).

In experimental studies, acid etching has been reported to provide more bone-implant contact and reduce bone resorption more than flat surfaces or TPS-roughened surfaces (49).

The dual aciding process converts macro structured surfaces into micro structures. This has been reported to provide a surface roughness that

accelerates the adhesion of platelet genes and greater release of extracellular genes, and bone apposition (48).

7.2. 2 Sandblasting and Large-Grid Acid-Etching

Acid-etching, and acid-etching together with sandblasting, is one of the oldest methods used in surface treatments. Acid-etching is also applied to the vast majority of implants available on the market that are subjected to sandblasting. Silica, resorbable ceramic, alumina and titanium dioxide are frequently used in sandblasting procedures. In addition, it is important that acid-etching after sandblasting removes residues on the surface. Acid-etching aims to increase the topographic profile of the surface and to remove residual products formed during the processes. Hydrofluoric, nitric, sulfric acid or combinations of different acid solutions are used in acid-etching processes applied after sandblasting. The advantage of this method is the total surface increase achieved due to the selective removal caused by electrochemical differences in surface topography as chemical analyzes on unsuccessful implants cause this type of particles to decrease the osteoconductivity of titanium, regardless of the biocompatibility of the biomaterial (50).

7.2.2.1 Sandblasted Large Grid Acid-Etched (SLA) implants

SLA implant surfaces were introduced by Straumann in 1997 as sandblasted and acidified titanium surfaces (51). SLA surface is not a coating surface. Macro roughness is created by spraying large grains of sand onto the implant. By applying the acid to the surface, 2-4 μm micro pits are obtained. SLA implant surfaces are moderately rough surfaces. The degree of roughness is the same across the surface of the implant. Martin et al. (51) showed that alkaline phosphatase activity in osteoblast-like cells is higher in SLA surfaces than TPS surfaces.

Li et al. (52) compared the osseointegration of SLA surfaces with acid treated lathe surfaces and found the torque resistance of SLA surfaces to be higher. Buser et al. (31) histologically examined bone-implant contact on different implant surfaces and found that the most bone-implant contact was on SLA surfaces after HA-coated surfaces.

7.2.2.2 Hydrophilic Surfaces

Recently, hydrophilic profiles of surface-modified titanium implants have been emphasized. Dynamic wettability of the titanium implant surfaces with chemically cleaned microstructure and cell response at the beginning are increased. Buser et al. (31) reported that implants with hydrophilic properties initially increased bone apposition to the implant surface. Changes in physicochemical properties have been reported to strengthen

protein absorption and cell attachment by integrin-mediated mechanisms. Accordingly, hydrophilic TiO_2 surfaces are thought to increase protein absorption first and then bone apposition (53). It is reported that this special production process produces a chemically active surface with a small amount of hydrocarbons and carbonates on the surface. In addition, Rupp et al. (54) reported an increased surface energy resulting in an increased water / biomaterial contact zone.

7.2.2.2.1 SLA Active Surface Implants

Hydrophilic properties are added to the SLA implant surface. It should be stored in its special package containing saline solution until it is placed. Thanks to its hydrophilic feature, the implant surface, when placed in the tissue, attracts blood to the micropore on it (31). It was launched in 2005.

SLActive® implants are obtained by chemically modifying these surfaces, treating them in N2 gas, and maintaining their isotonic NaCl solution, maintaining their hydrophilic properties. Sandblasted, double acid roughened and chemically modified SLActive® implants of Straumann® company are available.

7.2.2.3 Osseotite Implants

Implant surfaces were subjected to acid roughening twice. Hydrochloric and sulfuric acid are used. The top of the implant is left as a machine-prepared surface. The aim here is to reduce the risk of peri-implantitis. Osseotite implants are minimally rough. The longest follow-up study on osseotite dual-acid-etch surfaces was 6 years and success rates were 95-99% (55). There was also a 97-99% success rate in immediate loading (56). In a study comparing machine surface implants with osseotite implants, the success rate was found to be 95% for Osseotite in a 3-year follow-up, while it was 86.7% for other flat surface implants (57).

7.2.2.4 Frialit 2 Implants

These implants are designed on different surfaces such as Deep Profile Surface (DPS), TPS and Cell Plus. Cell Plus surfaces are acid-etched and grit blasted at high temperature and are moderately rough.

Frialit 2 implant surfaces are sandblasted and acid-roughened. Therefore, they are similar to SLA surfaces. In a 5-year study performed by Gomez Roman et al. (58) with 696 Frialitis 2 single implants, the success rate was 96%. In the same study, the same researchers applied 124 implants after attraction and achieved 97% success in 6-year follow-up (59). Krennmair et al. conducted a 3-year study with 146 Frialitis 2 single implants. This study showed a 97% success rate (60). In another study, Wheeler et

al. (61) applied 802 implants and achieved 96% success in 1-3 years follow-up. In a study with bone grafts, 94.8% success was observed in 2 and 3 years of follow-up.

Surfaces obtained by aciding / sandblasting technique are the most interesting type among rough surfaces and these surfaces are still being investigated.

6.1.3 Oxidized (Anodization) Dental Implant Surface

Depending on the anodization conditions such as the basic electrical potential (voltage), electrolyte and temperature used, the oxide layer on the implant surface can be micro or nano-sized porous. Tubular oxide structures can be formed by controlled anodic oxidation of a metal substrate. Optimized anodization parameters are essential for ideal self-organized nanotubes. Generally, rough layer structure and morphology are rapidly affected by electrochemical conditions (41).

Anodized surfaces are obtained by applying high voltage to the titanium model in the electrolyte (strong acids such as H_2SO_4, HNO_3). Anodization micropores of various diameters are formed on the surface and do not show cytotoxicity. In addition, higher cell adhesion and proliferation are observed compared to the treated surface (62,63). As a result of anodization, the oxide layer on the titanium surface becomes 600-1000 nm or more.

The anodized surface has been reported to increase bone formation in the early stages of healing. This difference is attributed to surface morphology, roughness, chemical composition and a thicker oxide layer (41).

Sandblasted or acid-etched implants have pores that allow for more uniform surface roughness and better cell adhesion than implants that have not been subjected to surface treatment. However, contamination is an important problem during sandblasting and etching (41).

7.2.3.1 Ti Unite Implants

In Ti Unite implants, the surface is anodized. Electrochemical anodic oxidation is performed to the titanium with electrodes in galvanostatic mode. Thus, the surface becomes pseudo-micro pits are formed. Since some types of phosphoric acid are used as electrolytes, the implant surface contains phosphorus ions, which has a bioactivity reducing effect. Two different combinations were used on the implant surface. The thickness of the oxide layer varies along the implant surface, the upper areas have minimal roughness (0.5-1 μm) and a thin oxide layer, while the oxide layer (more than 10 μm) and hardness (more than 2 μm) in the apical region are increased (64). Its clinical application began in 2001.

7.3 BIOCHEMICAL METHODS

7.3.1 Coating with calcium phosphate

Another modification commonly applied on dental implant surfaces is to cover the surface with calcium phosphate, which is generally composed of hydroxyapatite. Calcium phosphate ($CaPO_4$) has been suggested to be used with implantable materials because of its similarity with its content of bone mineral, its ability to form carbonate hydroxyapatite on the implant surface, and its ability to form a suitable scaffold for bone making, to bind and store endogenous bone morphogenetic proteins (65).

Today, the mechanism of the bioactive capacity of $CaPO_4$ is not fully known. There are two views on this subject. The first is that the intercalated carbonate apatite layer is caused by the ion solubility in the bioceramic material. Second, high affinity of $CaPO_4$ concentrations to growth factors is shown as a factor (66).

7.3.1.1BioHorizons Implants

Ti alloy (Ti-6Al-4V) is used. There are two types of surface properties. One is HA coated surface and the other is RBT (Resorbable Blast Texturing) surface. These surfaces are obtained by sandblasting on titanium with calcium phosphate.

Piattelli et al. (46) investigated osteoblast adhesion and osteoid matrix formation on machine-prepared surfaces and Ti surfaces roughened using HA and RBM. They found that more osteoblast and osteoid matrix formation occurred in RBM implants.

7.3.2 Hydroxyapatite (HA) Coating

Ceramics have higher texture-friendliness than metals, but have less biomechanical properties. Therefore, when ceramics are used as dental implants alone, breakage problems are encountered under occlusal loads.

It is a technique developed by taking advantage of the osteoconductive effect of hydroxyapatite and by considering that implant anchorage and bone growth will be increased in Type 3 and 4 bones with low bone quality (67).

In coating dental implants with HA, several methods such as plasma spray, spray storage, sol-gel coating, electrophoretic precipitation are used. But the clinically applied method is only the plasma spray method. In this method, HA ceramic particles are injected into a high temperature plasma torch and then sprayed onto the titanium surface. Then titanium and HA combine to form a film. This thickness varies between 1-2 μm and 1-2 mm. In order for the coating to hold onto the surface mechanically, the titanium surface must be roughened before coating (63).

Although there are positive short-term results for HA coatings, there are some disadvantages as well. These are the smoothness formed in the coating, leaving residual stress residues at the junction with the titanium and negative changes in the composition and crystallization of the sprayed powder. In addition, some clinical failures are seen in HA-coated implants with plasma spray technique. These are ruptures at the implant-coating junction as a result of the delamination (fatigue) of the coating, the release of impurities, and the clinical failure of the implants as a result. It is also reported that the plasma spray technique is not effective in short implants with complex structure and that HA coating has a negative effect on the success of the implant by increasing the microorganism attitude (63,68).

7.3.2.1 BioHorizons Implants

They measured the percentage of bone contact on machine-prepared, TPS, HA coated surfaces with SBM (Soluable Blasting Material) and found the % of BIC (bone-to-implant contact) highest on SBM sanded surfaces. This was followed by HA and TPS surfaces, and showed that the lowest value was on the surface prepared by the machine (69).

7.3.2.2 SwissPlus Implants

It has a medium rough surface. Pure Ti surface is roughened by sand-blasting with HA particles.

7.3.3 Fluorinated Surfaces

A new surface modification associated with sandblasted and acid etched surfaces is fluoridated surfaces that lead to changes in the chemical structure of the surface. Fluorinated surfaces are a chemical modification in which fluorine, one of the basic elements of bone, is added to the surface in order to promote osteogenesis (70).

Titanium is very easy to bind to fluorine ions and forms TiF4, which dissolves immediately. When titanium is modified with fluorine in this way, it has both surface roughness and accelerating osseointegration effect of fluorine (63). Fluorine also has properties to increase bone regeneration. It increases the number of growth factors that cause calcification. It is effective on osteoprogenitor cells or undifferentiated osteoblasts that synthesize most growth factors (71).

An important feature of this implant surface is its ability to react with calcified tissue and thus calcium and phosphate ions.

Today, dental implants that are modified with fluorine and stated to be potential bioactive are Osseospeed® implants belonging to Astra-Tech® company.

7. 3.3.1 Tioblast and Osseospeed Implants

Optimal surface roughness in thioblast implants was achieved by spraying titanium dioxide on the implant surface. Osseospeed implants are the fluorine modified form of the Tioblast surface. Fixture Microthread-Osseospeed implants have a surface structure formed by being exposed to diluted hydrofluoric acid after roughening with titanium sandblasting technique. When comparing the osseospeed surface with the thioblast surface, it was found that the amount and speed of osteoblast formation was significantly higher in the cell culture experiments, and 3 times more bone sialoprotein was formed after 14 days. In addition, there was a significant increase in bone-implant contact amount (70).

In a study by Rasmusson et al. (72), Branemark (machined surface), Astra Tech ST (Ti grit blasted surface), Astra Tech Microthread (Ti grit blasted surface) implants were used. Considering the BIC amounts, the highest percentage has been shown to be in the Astra Tech Microthread implant, especially in the neck region. In a study by Cooper et al. (73), it was revealed that when mineralization and calcium phosphate levels were measured in TPS, Machined, Tioblast surfaces, TPS surfaces are very low compared to Tioblast surfaces.

7.4 Laser Surface Modifications

Lasers for surface modifications have some unique properties. Electromagnetic radiation of a laser beam is absorbed in the first atomic layer for opaque materials. Laser shaping is based on the ability of a high-intensity laser beam to focus on a precise spot on the surface, where the high beam intensity evaporates the material. In fact, the applied energy can be left very precisely at the desired point on the surface. With this approach, pits smaller than 1μm can be made. With the control of beam movement, it is possible to obtain pre-designed shapes both by using optical elements and by moving the sample. Lasers can make the desired changes on the surfaces of organic and inorganic materials very quickly (74).

Laser surface structuring is the laser production of uniform regular geometries on the surfaces in order to create the desired specific changes in the technical properties of the surface. There are very few studies on the surface modifications of laser biomaterials. Considering the published studies, it is confirmed that the wettability characteristics of laser irradiated material surfaces can be affected.

It has been shown that it is possible to effectively produce a fully wettable surface using the contact angles generated in CO2 laser processes. Laser surface structuring method is a promising method that can clean the

surface roughness required to reach full osteointegration on implant surfaces (75).

7.4.1 Advantages of Laser Surface Treatment in Implants

The most important advantages compared to laser surface configuration alternatives are:

- Chemical cleaning
- Controlled thermal penetration and distortion,
- Controlled thermal profile and thus the location and shape of the zone affected by controlled heat,
- Less machine labor,
- Contactless operation that can be controlled remotely.

The disadvantage of laser surface configuration compared to other conventional methods is the high cost of the device. Laser technology is indispensable in dentistry, especially in dental implant production and dental implant surface configuration due to its several advantages. It would be beneficial in applying and developing laser dental implant production technology in dentistry without delay. Laser diode devices with 50 W to 250 W laser power are sufficient for surface roughening of dental implants.

8. CONCLUSION

Implant success in bone related applications depends on the biological, physicochemical and mechanical properties of the implant material. Due to the tissue response and environmental reaction, the implant surface is in constant change. This process is associated with long-term implant stability. In other words, implant integration is determined by the performance of the system, mainly occurring at the tissue-implant interface. Most of the important interactions between tissue and implant occur at the interface created between the material surface and the biological environment. These interactions for implants generally consist of a protein-surface and cell-surface pair. The structure, shape, topography, chemistry, surface energy and mechanical properties of the material can determine the orientation of the molecules and which molecules will be absorbed onto the surface, thereby affecting normal bone cellular development.

In addition to surgical technique, implant material and implant design, surface properties are known to be important in ensuring osseointegration. Research shows that implant surface features play a role in bone healing response. Among the surface preparation methods, morphological methods have more pronounced effects than physical-chemical methods. It has been

shown that rough surfaces positively affect this response. Among the methods used to obtain rough surface, aciding / sandblasting and hydroxyapatite coating techniques show more successful results than others.

Dental implant surface technology has made great progress in order to achieve full osseointegration and improve the results expected from implant treatment, but there are still cases that result in implant failure despite the high success rates of implant treatments. More studies are needed to improve implant surface treatments.

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Chapter 3

PEDIATRIC ZIRCONIA CROWNS

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Early childhood caries (ECC) affects all parts of deciduous teeth and can destroy their structure. For this reason, ECC is a serious public health problem that affects chewing function of children and their psychology (H. Lee, Chae, Lee, Choi, & Nam, 2019). Restoration of large carious lesions and tooth fractures with conservative methods is one of the most common problems that dentists face (Dimova-Gabrovska, Dimitrova, Yordanov, Yankova, & Peev, 2019). In such cases, an ideal restoration should be durable, easy to place, aesthetic and inexpensive. Especially in large and multisurface cavities in deciduous teeth, full coverage restorations are recommended (Townsend et al., 2014).

For many years, many treatment options which have different advantages and disadvantages, associated different aesthetic, functional and technical limitations, have been tried for completely covering the deciduous teeth (Ashima, Sarabjot, Gauba, & Mittal, 2014; Waggoner, 2006).

Based on the opinion of specialists and fewer scientific studies about clinical techniques applied to teeth with a multi-faceted lesion, the American Academy of Pediatric Dentistry (AAPD), recommends full coverage methods such as stainless steel crowns (SSCs), resin composite strip crowns, open-faced SSCs and recently pre-veneered SSCs for the treatment of primary teeth (Salama, 2018).

Since 1950, in posterior primary and permanent teeth of children, SSCs are the most preferred method of full coverage restoration (Taran & Kaya, 2018). In posterior primary teeth, SSCs are reported to perform better than Class II amalgam restorations as well as Class I and II composite fillings (Seale, 2002). SSCs are known as the most protective and durable restorations in the primary dentition (Seale & Randall, 2015).

Changes in crown design overtime allow for easier adaptation and improved morphology. SSCs are flexible enough to allow crimping and trimming for a good fit. Retention in SSCs is achieved by adapting the crown to the cervical undercut region of the primary molar teeth. In addition, SSCs are quite durable and relatively low priced. It requires minimum technical sensitive during cementation (Taran & Kaya, 2018).

Despite its advantages, such as durability, longevity, and reducing recurrent caries, SSCs do not meet the aesthetic demands of parents, patients, and pediatric dentists (Townsend et al., 2014).

Due to the greater involvement of the parents in the decision-making process in the clinic and the greater demand for aesthetic restorations, the aesthetic treatment of primary teeth has become mandatory (Salami, Walia, & Bashiri, 2015). When parents are asked about their greatest concerns about treatment, 57% of the most concerning issues are aesthetics;

followed by cost, toxicity, and durability (Zimmerman, Feigal, Till, & Hodges, 2009). In the same survey study, pediatric dentists reported that 87% of parents had aesthetic anxiety, even if the tooth to be treated was posterior (Zimmerman et al., 2009).

There are many treatment options for the treatment of carious teeth in pediatric patients, from SSCs to its various modifications, and from resin strip crowns to increasingly popular zirconia crowns (ZCs) (Garg, Panda, Shah, & Panchal, 2016).

Resin strip crowns are undoubtedly the most aesthetic option since their colour and shape can be controlled. However, it is both time-consuming and requires adequate isolation and sensitive technique (Kupietzky, 2002). Also, loss of some or all part of the strip crowns, or colour changes caused by endodontic pastes can lead to aesthetic dissatisfaction. (J. H. Lee, 2018). Pre-veneered SSCs are cemented with moisture-tolerant glass ionomer cement and do not require as much isolation as strip crowns (MacLean, Champagne, Waggoner, Ditmyer, & Casamassimo, 2007). However, it requires a more aggressive preparation and passive fit. It also has a resin face that can debond. Open-faced crowns require a more conservative preparation and are more tolerant of moisture in cementation. However, isolation is needed while placing the resin face. Other disadvantages are that the studies supporting their use are limited and the composite face can debond (Holsinger, Wells, Scarbecz, & Donaldson, 2016).

Pediatric ZCs, introduced in 2010 and gaining popularity due to aesthetic concerns, have become a new alternative to resin strip crowns and pre-veneered SSCs in deciduous teeth. Clinical acceptability and parental satisfaction of ZCs are higher than other full-coverage restorations (J. H. Lee, 2018; Townsend et al., 2014).

ZCs, also known as “ceramic steel”, have been used in dentistry for the last decade since they provide aesthetic and close strength to existing metal crowns. It is commonly used in forms such as crowns, fixed partition prostheses, implant abutments, fixture, inlay, onlay, CAD/CAM in the restoration of permanent teeth. (Ashima et al., 2014; Ramires-Romito, Wanderley, Oliveira, Imparato, & Correa, 2000). Zirconia is a crystalline zirconium dioxide, which is similar to tooth colour and has mechanical properties similar to metals. Today, ready-made ZCs are also available for both deciduous incisor and molar teeth (Planells del Pozo & Fuks, 2014).

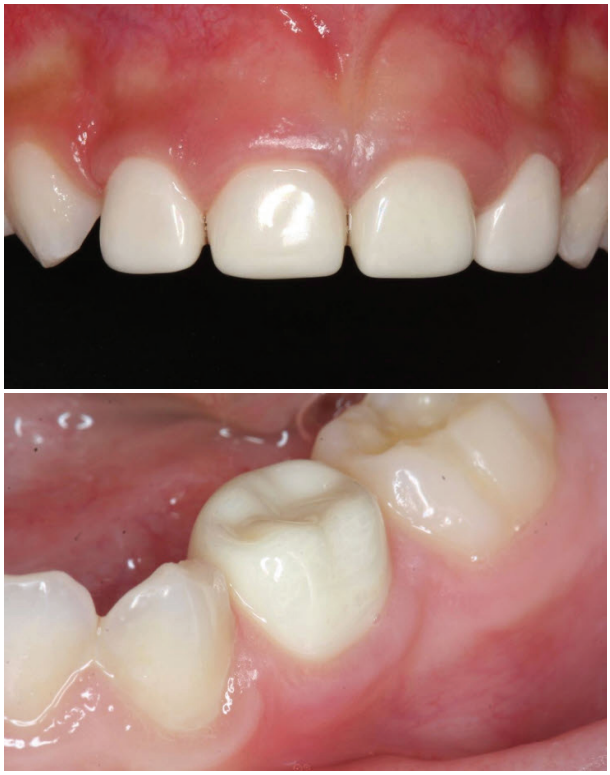

Fig 1. *Deciduous incisor and molar teeth with pediatric ZCs (Casian & Hoang)*

Pediatric ZCs play an important role in meeting the increasing aesthetic demand in anterior and posterior restorations (Christensen, 2011). Case reports and clinical studies on ZCs have shown very high success rates in terms of retention, durability, and periodontal health (Azab, Moheb, El Shahawy, & Rashed, 2020; Holsinger et al., 2016; Taran & Kaya, 2018). In addition, while ZCs exhibit high biocompatibility and strength; it also causes less wear away on the opposite primary and permanent tooth (J. H. Lee, 2018).

Many companies have introduced prefabricated primary molar crowns made of zirconium. Some of the pediatric ZCs available on the market are NuSmile Zirconia crowns, E Z Pedo crowns, Kinder Zirconia pediatric crowns, Cheng Zirconia pediatric crowns.

The advantages of ZCs are that they offer excellent aesthetics, cover the treated teeth completely, lack a debonding component and require less sensitive technique during cementation than resin strip crowns (Clark, Wells, Harris, & Lou, 2016). However, ZCs have several disadvantages. These crowns are visibly thicker than SSCs. It cannot be modified in any way, and the manufacturer recommends sitting passively during cementa-

tion. The thicker of these crowns requires more preparation. In this case, the possibility of pulp exposure increases. Like all ceramics, these crowns are not capable of flexing and may break during cementation (Townsend et al., 2014). There are other disadvantages such as not being able to bend the crown to hold it mechanically, change its colour or shape. Also, ZCs are more expensive (Clark et al., 2016; Holsinger et al., 2016).

A key feature sought in modern dental materials for crown restorations is that they should be biocompatible and hypoallergenic. All kinds of prefabricated metal crowns made of chromium-nickel alloys show a major disadvantage in this regard. Nickel is a proven allergen for most of the population (Randall, 2002). Despite the decrease in the number of modern alloys, it has been proved that ion release due to irritations can lead to burning sensation around the mouth, metallic taste and swelling of the tongue (Dimova-Gabrovska et al., 2019; Kodaira et al., 2013). Keinan et al. confirmed these evidences and detected a high rate of metal ions in the cement of teeth restored by prefabricated metal crowns (Keinan, Mass, & Zilberman, 2010).

In a systematic review conducted in 2015, various prefabricated pediatric crowns and different types of filling materials (glass ionomer, composite, amalgam) were compared for efficacy and safety. This study has shown that SSCs are more effective in a period of 12-24 months and are the most suitable restorative method compared to traditional methods (N. P. Innes et al., 2015).

In a study comparing dental preparations of SSCs and ZCs, it was reported that 100% retention was achieved in all crowns despite different preparations. In the same study, ZCs have been shown to require more aggressive tooth preparation, including subgingival reduction compared to SSCs (Clark et al., 2016). While SSC requires a snap-fit, zirconia crowns required passive fit (Mathew, Roopa, Soni, Khan, & Kauser, 2020). In another study, in the 36-month follow-up of the ZCs performed in the primary incisors, the survival rate in the mouth was found to be 76% (Seminario et al., 2019). The small size of the crowns and the less amount of tissue remaining after widespread caries can cause a lower survival rate, especially in the primary incisors.

The long-term health of periodontal tissues around the crowned teeth depends on marginal contacts, well-adapted seating, absence of cement remnants in the sulcus and the prevention of plaque associated with them (Madrigal López, Viteri Buendía, Romero Sánchez, Colmenares Millán, & Suárez, 2014; Taran & Kaya, 2018).

In a study, the gingival health was better in the ZCs than the SSCs in the third and sixth months after the restoration. However, during the

12-month follow-up, it was reported that healthy gingiva was seen in both groups. With these results, it can be said that the ZCs are more biocompatible, the smooth and polished outer surface causes less plaque accumulation and less irritating the periodontal tissues (Abdulhadi, Abdullah, Alaki, Alamoudi, & Attar, 2017).

Similarly, Taran et al. reported that there was less plaque accumulation in ZCs compared to SSCs (Taran & Kaya, 2018). Frequent manipulation of the SSC by crimping and trimming will lead to surface irregularities. While irregularities on the polymeric surfaces of SSCs support bacterial adhesion and biofilm formation; the smooth and polished surfaces of the ZCs did not support this accumulation (AlShaibah, El-Shehaby, El-Dokky, & Ala'a, 2012; Mathew et al., 2020; Townsend et al., 2014).

Prefabricated crowns tend to microleakage due to their open margins. The reduction of micro-leakage depends on the cement to be used. Traditional cements contain zinc phosphate and polycarboxylate (Stepp, Morrow, Wells, Tipton, & Garcia-Godoy, 2018; Zmener, Pameijer, & Hernandez, 2014). However, glass ionomer and resin-modified glass ionomers showed less coronal microleakage and less clinical failure (Waggoner, 2015).

As mentioned before, a problem encountered while restoring deciduous teeth with ZCs is that they must be placed passively on the prepared tooth. In this case, ensuring adherence remains completely dependent on the luting cements (Beldüz Kara & Yilmaz, 2014). In daily clinical practice, debonding of the crowns is annoying for children, parents and dentists. However, it also involves extra visits, extra costs and undesired situations such as accidental swallowing of crowns. In A research, it is reported that packable glass ionomer cements are more retentive than bioactive cements in the bonding of pediatric ZCs (Azab et al., 2020).

Managing the behaviour of pediatric patients during their treatment is often not easy. It is necessary to apply the restorations as quickly as possible. It should be long-lasting, not damage the surrounding tissues, and satisfy the parents in aesthetic and costs. For this reason, crown restorations are often preferred as they are easy, fast, effective, and painless treatment options (Dimova-Gabrovska et al., 2019; Garg et al., 2016; N. Innes, Evans, & Hall, 2009). However, choosing the type of crown depends on the aesthetic expectation of the parents, the behaviour of the child, and the controllability of moisture and bleeding in the area (Ashima et al., 2014; Waggoner, 2006).

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Chapter 4

MAGNETIC RESONANCE IMAGING OF THE MENISCUS:

DIAGNOSIS AND CLASSIFICATION

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INTRODUCTION

Accurate and prompt diagnosis of meniscal tears is of critical importance in planning treatment and reducing morbidity. Meniscal injury is a predisposing factor for damage of neighboring joint cartilage and leads to early degenerative osteoarthritis. Prevalence of asymptomatic tears -generally horizontal tears- increases with age. With the help of clinical information and exclusion of other potential causes, symptomatic meniscal tears can be reliably diagnosed (1). Since entering clinical practice in the 1980s, magnetic resonance imaging (MRI) has become the first-line noninvasive imaging method for diagnosing intraarticular knee pathologies. While arthroscopy is the reference standard imaging method, MRI has high sensitivity (93% for medial meniscus, 79% for lateral meniscus) and high specificity (88% for medial meniscus, 96% for lateral meniscus) in determining meniscal tears (2). However, despite these advantages, MRI has limitations in identifying small posterior horn tears and tears involving less than one-third of the lateral meniscus (LM). Additionally, high false-positivity rates have been reported for longitudinal tears of the medial meniscus (MM) posterior horn.

This article aims to review MRI parameters, normal meniscal anatomy, anatomical variants and pitfalls in detecting meniscal tears, and to summarize the diagnosis and classification criteria of various types of tears with MRI.

IMAGING METHODS

The use of high-spatial-resolution MR imaging facilitates the detection of meniscal tears. Spatial resolution can be improved with the use of knee coils, large matrix, small FOV (field of view) and thin sample thickness. Typical parameters that can be used include: <16 cm FOV, matrix of at least 192×256 (phase coding x frequency coding) and sample thickness of 3-4 mm (Table 1). Compared to conventional spin echo (SE) and fast spin echo sequences (FSE) (short ETL (<5) and long bandwidth), it is stated that these two do not differ in the rate of detection of meniscal tears. Although two-dimensional (2D) and three-dimensional (3D) FSE techniques detect tears with almost similar accuracy, low sensitivity of 3D FSE imaging has been mentioned, especially in LM tears involving the root. Classically, it is recommended that the use of proton density (PD) weighted sequence be preferred to T2 weighted images in the diagnosis of meniscal tear. It has been indicated that the hydrogen nuclei within the tear is related to macromolecules rather than being free, which ensures shorter T2 relaxation time. However, it has also been stated that coronal T2 weighted images have high accuracy compared to PD weighted images in detecting medial meniscus root tears. Therefore, it is important to evaluate all plans and sequences when interpreting MRI of the knee (3).

Table 1: Example for a routine MR imaging protocol of the knee

Orientation	Sequence type	Fat Suppression	Repetition time/echo time(ms)	FOV	Matrix	BW
Sagittal	PD FSE	No	2850/22	14	384X224	31.25
Sagittal	T2 FSE	Yes	5050/70	14	384/256	41.67
Axial	PD FSE	Yes	3250/30	12	448/224	41,67
Coronal	T1 FSE	No	550/min ful	14	320/224	31.25
Coronal	PD FSE	Yes	2850/22	14	320/224	31.25

NORMAL ANATOMY

The main function of the menisci is to absorb shock, disperse axial and compressive forces of the body to the lower extremities, facilitate joint lubrication, and spread synovial fluid. Menisci are crescent-shaped structures composed of fibrocartilage. Each meniscus has a concave-shaped upper surface corresponding to the femur condyle and a flatter lower surface corresponding to the tibial plateau. Its periphery is thicker while its free side is thin. Type 1 longitudinal collagen fibers traversing the meniscus resist axial forces and prevent meniscus extrusion. Thinner radial fibers extending perpendicularly hold longitudinal fibers in place. Each meniscus has an anterior horn, corpus, posterior horn, and roots. The anterior and posterior roots adhere to the central portion of the tibia plateau and provide biomechanical function with neutral meniscal position. The close relationship between the anterior cruciate ligament (ACL) and LM anterior root result in a striated or comb-like appearance at MR imaging. Insertion of the MM parallel to the ACL is an anomaly observed in 2% of the population and may be misinterpreted as a meniscal tear. Furthermore, slightly lower insertion of the MM anterior horn to the anterior margin of the tibia may also be confused as pathologic subluxation. Menisci show low signal intensity in MRI; however, globular or linear increased intrameniscal signal intensity may be observed in children due to normal vascularity and in adults due to internal mucinous degeneration or acute contusion after trauma. Anterior and posterior horns and the corpus appear triangular (bow-tie) on both coronal and sagittal images. The MM is a wider “C” shape to conform to the tibia plateau and its width increases from the anterior towards the posterior. In sagittal images, while the anterior horn of the MM is shorter than the posterior horn, the anterior and posterior horn of the LM appear identical in size. The medial meniscus is less mobile since it is firmly attached to the deep medial collateral ligament and joint capsule. The lateral meniscus is more loosely attached to the capsule due to the presence of the popliteal tendon and meniscal fasicles (3).

ANATOMIC VARIANTS AND PITFALLS

Anatomic variants and pitfalls which may mimic meniscal tear include discoid meniscus, meniscal flounce, meniscal ossicle, and chondrocalcinosis.

Discoid meniscus

Discoid meniscus is an anatomic variant defined as a meniscus which spreads widely on the joint surface of the tibia. It is seen in 1%-5% of the normal population and is 10-20 times more common in the LM than in the MM. Watanabe classified this variant as *i)* complete: the entire meniscus is disc-shaped and completely covers the tibial plateau; *ii*) partial: the meniscus covers <80% of the tibial surface; and *iii*) Wrisberg: the posterior horn is thick, the normally expected posterior meniscal ligament is absent, and may cause snapping knee syndrome. Later, a ring-shaped variant with more closely involved roots was added to this classification(4). Measurements used to identify discoid meniscus appearances include: meniscal width of at least 15 mm on a midline coronal image, and three or more consecutive sagittal slices showing continuity between the anterior and posterior horns of the meniscus. Discoid meniscus is often an incidental finding and is prone to tearing. Tears are more common in the complete variant (Image 1). Increased vascularity of the disc-shaped meniscus and extensive intensity in the disc make it difficult to differentiate from tears. Linear intensity reaching the surface in two or more images is considered a meniscal tear. However, due to its poor positive predictive value (PPV) (57-78%), diffuse intrameniscal signal intensity reaching the articular surface should be noted and reported as a possible tear (5).

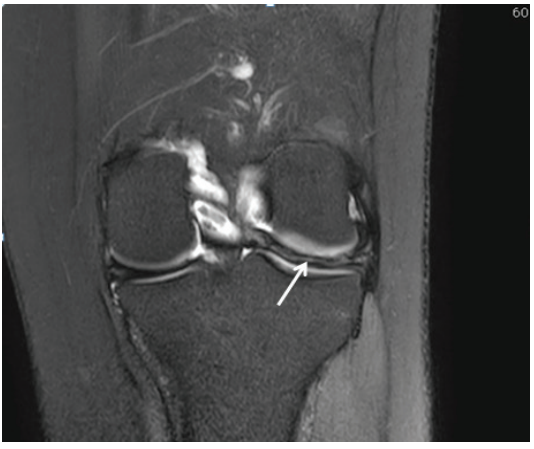

Image 1: Coronal T2 weighted image through the meniscal body shows the complete discoid lateral meniscus which covers almost the entire tibial plateau region and is associated with the accompanying horizontal tear (arrow)

Meniscal flounce

Meniscal flounce refers to a rippled appearance observed in the free margin of the MM when the knee is in flexion and may be present in 0.2% -0.3% of asymptomatic knees. On coronal images it may be confused with radial tears. It is more easily identified at arthroscopy due to the position of the knee and relaxation as a result of anesthesia (6).

Meniscal ossicle

Meniscal ossicle is a rare entity with a tendency for association with the posterior horn of the MM. It may be developmental, degenerative, or posttraumatic in origin. On radiographs it may be mistaken for a loose body, or it may be mistaken for a tear in MRI due to high signal intensity. While it may be asymptomatic, it may cause a mass effect or symptoms due to an accompanying tear and may require arthroscopic resection (7).

Chondrocalcinosis

Chondrocalcinosis is characterized by increased signal intensity similar to meniscal ossicle and also causes difficulty in diagnosing tears. Prevalence increases with age and ranges between 5%-15%. Correlation with radiographs may help to reduce the number of false-positivity (8).

MRI DIAGNOSIS OF MENISCAL TEARS

Prevalence of meniscal tears increases with age and they are often associated with degenerative joint disease. Tears are especially more common in posterior horns, especially in the MM. However, anterior horn tears are also frequently encountered in younger patients, following acute trauma. Isolated tears of the anterior horn are uncommon and account for 2% of MM tears and 16% of LM tears (3).

MRI is a highly sensitive and proven method in determining meniscal injury. Low signal intensity is expected in normal menisci. MR imaging criteria for diagnosis of meniscal tear include meniscal distortion in the absence of prior surgery or increased intrasubstance signal intensity unequivocally contacting the articular surface. If increased intrasubstance signal intensity does not extend to the articular surface in only one image, chance of determining tear is <55% in arthroscopy, while extension is observed in two or more consecutive coronal or sagittal images this likelihood is close to 96%. Increased signal intensity observed in one image should be confirmed in another image. If this signal is present on a single image, the PPV for tear is 43% in the MM and 18% in the LM, and this finding should be reported as “possible tear” (9).

CLASSIFICATION OF MENISCAL TEARS

A comprehensive report must include localization, orientation, and

spread of the meniscal tear. Which joint surface it extends to (femoral or tibial) must be indicated. Clear and detailed reporting of the tear is important for preventing unnecessary surgery or planning a better surgery. Meniscal tears can be treated conservatively, with surgical repair, or partial or total meniscectomy. While longitudinal tears are suitable for repair, partial meniscectomy may be required for horizontal and radial tears. Therefore determining the morphology and type of tear is important for planning treatment. While there is currently no standardized classification system, according to orientation, the most common types of tears are horizontal, vertical (longitudinal, radial), and complex (root, complex, displaced, and bucket-handle) tears.

A widely used meniscal grading system (grade I-III) used in many fields in the past is now considered obsolete in routine imaging (10). However, since there is still no definite classification system, clinicians are unable to share common terminology. Therefore, a standard knee MRI report must mention in detail whether or not the tear is stable-unstable, its orientation, spread, and which articular surface it extends to (Table 2). While stable tears have the potential to recover with conservative treatment, unstable tears often require surgical repair (11).

Horizontal Tear

Horizontal tears run parallel to the tibial plateau. It involves either the central free edge or one of the articular surfaces and extends peripherally, dividing the meniscus into top and bottom halves (Image 2). These tears usually affect patients over 40 years of age, without prior trauma, and with an underlying degenerative joint disease. Typical MRI appearance is a horizontal signal line that contacts the meniscal surface or free age. Parameniscal cyst formation is more common in these types of tears and likely develop secondary to its correspondence with joint fluid in complete tears (12).

Vertical Longitudinal Tear

Vertical longitudinal tears extend perpendicular to the tibial plateau and parallel to the long axis of the meniscus and divide the meniscus into central and peripheral halves (Image 3). In contrast to horizontal and radial tears, they do not extend to the free edge of the meniscus. These tears are often encountered in young patients following trauma, and tend to occur in the peripheral half and the posterior horns. Typical MRI appearance is a vertically oriented line of high signal intensity contacting one or both articular surfaces. As a close relationship between peripheral longitudinal tears and ACL tears has been reported, ACL should be closely evaluated in these types of meniscal tears. In addition, peripheral longitudinal tears of the LM posterior horn may be difficult to diagnose due to complex posterior attachments. In this circumstance, evaluation of sagittal T2-weighted

images may be more helpful (13).

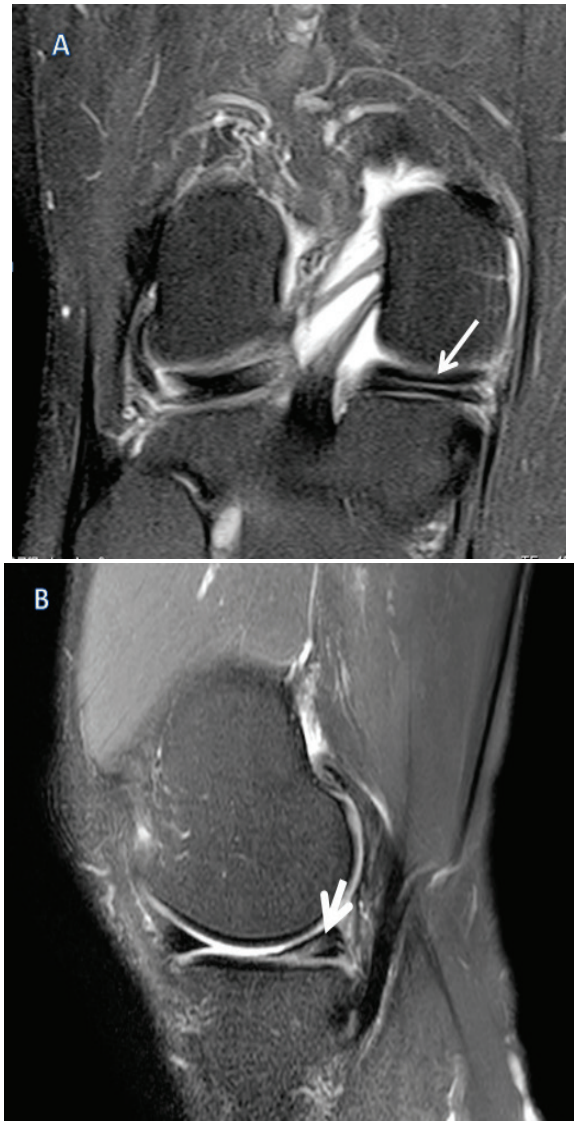

Image 2: On coronal T2-weighted image (A): horizontal tear (long arrow) separates the medial meniscus posterior horn into top and bottom halves. In sagittal PD weighted image (B): horizontal tear (short arrow) associated with the inferior articular surface runs oblique to the medial meniscus posterior horn

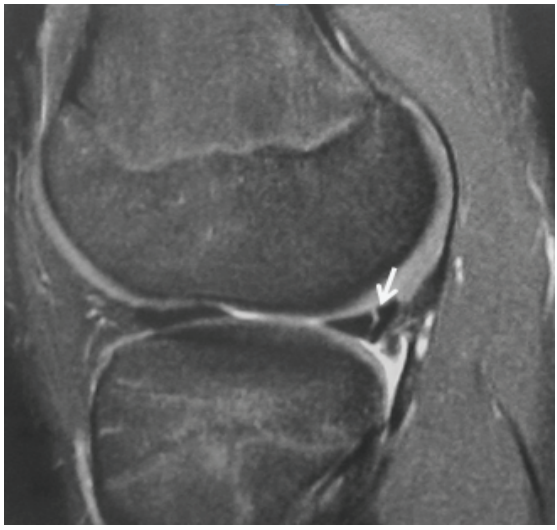

Image 3: On sagittal PD weighted image, a vertical longitudinal tear (arrow) in the medial meniscus posterior horn separates the meniscus into central and peripheral halves

Vertical Radial Tear

Vertical radial tears extend from the free edge toward the periphery. In contrast to horizontal and longitudinal tears, radial tears result in dramatic loss of function and may result in meniscal extrusion. Because they are located within the avascular "white zone", likelihood of potential recovery is low. Radial tears often involve the posterior horn of the MM or the junction of the anterior horn and body of the LM. In axial images, they appear as vertical clefts running perpendicular to the free edge. They are recognized by various findings and signs relative to the imaging plane in MRI (Image 4). These signs include the "truncated triangle," "cleft," "marching cleft," and "ghost meniscus" and may increase detection rates of radial tears up to 89% (14).

Root Tears

Root tears are typically radial tears. Complete root tears may be highly associated with meniscal extrusion, especially in the medial meniscus (15). Roots are best viewed in coronal fluid-sensitive sequence in which magic-angle and pulsation artifacts are less perceived. On coronal MRI, the root tear must be observed to extend over its respective tibial plateau on at least one image. On sagittal MR images, root tear should be suspected if the posterior root of the MM is not detected just medial to the posterior cruciate ligament. In addition, it should be kept in mind that there is increased incidence of lateral root tears in the presence of ACL tear (16).

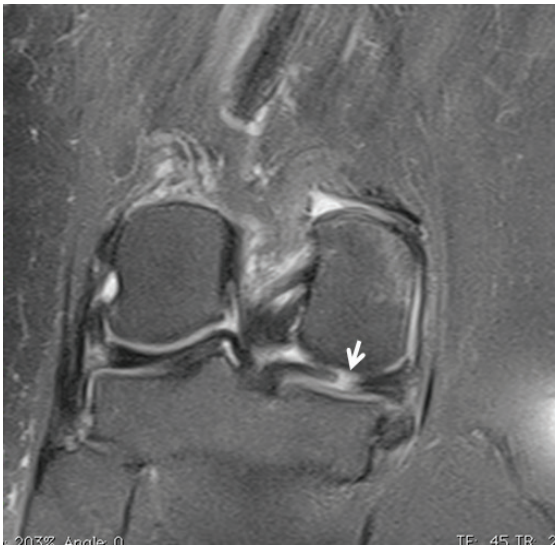

Image 4: Coronal T2-weighted MR image shows a radial tear (arrow) exhibiting "cleft" sign located in medial meniscus posterior horn

Complex Tear

Complex tears may be a combination of two horizontal, longitudinal, and radial tears or of all three. The meniscus often appears fragmented and tears are observed to extend in more than one plane (Image 5).

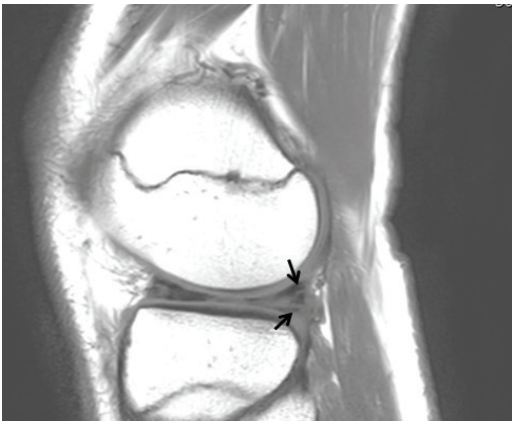

Image 5: Sagittal PD weighted MR image shows a complex tear of horizontal and vertical orientation in the posterior horn of the LM

Displaced Tear

In bucket-handle tears, flap tears, or free fragment tears, the meniscus section may appear displaced. These types of tears may require surgical repair or debridement since they lead to mechanical obstruction. Very small fragments may not be visible in arthroscopy. Therefore, identifying these fragments with imaging is vital in order to prevent potential knee locking

in the future or ongoing knee pain. Flap tears, which are six to seven times more frequently in the medial meniscus, where fragments are displaced posteriorly or near the PCL in two-thirds of cases, or migrate to the intercondylar notch or superior recess in the remainder of cases (17).

Lateral meniscus fragments show distribution along the posterior joint line and lateral recess. In cases without prior history of surgery, presence of foreshortened meniscus may require investigation of displaced meniscal fragments (18).

Table 2: Classification of Meniscal Tears

Vertical	Longitudinal	Incomplete: signal intensity which only extends to one articular surface Complete: linear signal changes which extend to both articular surfaces	Stable Unstable
	Radial	Meniscal root tear	Generally unstable
Horizontal/ Oblique Horizontal	Incomplete: linear signal changes which do not reach the meniscal surface Complete: linear signal changes which involve one or more meniscal surfaces		Stable Stable/unstable
Complex	Horizontal and vertical tears Bucket-handle tear Displaced tear		Unstable

Bucket-handle tear

Bucket-handle tears are characterized by central migration of the inner portion of a longitudinal tear. It is seven times more frequent in the medial meniscus (19). There are at least five different MRI signs including: "an absent bow tie, a double PCL, a fragment within the intercondylar notch, a double anterior horn or flipped meniscus, and a disproportionally small posterior horn" (Image 6). These signs are sensitive but have low specificity. Although bucket-handle tear is rare in the lateral meniscus, when it is located just posterior to the ACL it may present a double-ACL sign (20).

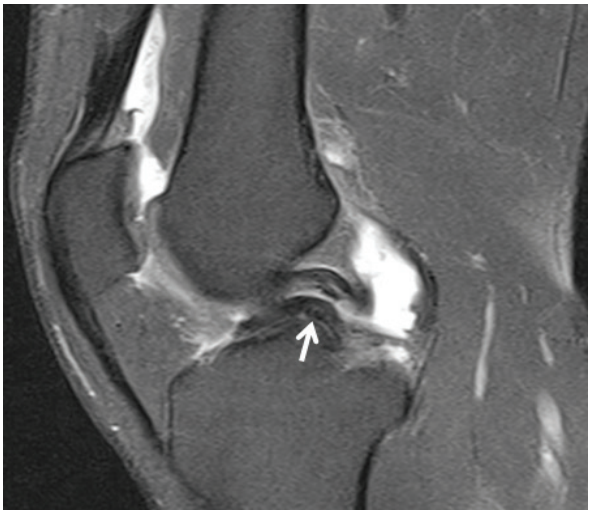

Image 6: Sagittal T2-weighted image demonstrates a double-PCL sign (arrow) due to bucket-handle tear of the medial meniscus

INDIRECT SIGNS OF MENISCAL TEARS

Indirect signs are used to increase the reader's diagnostic confidence in meniscal tears of images together with artifacts or equivical images. Although indirect signs have low sensitivity, they have high specificity and high PPVs. These signs include: parameniscal cyst, meniscal extrusion, and subchondral bone marrow edema

Parameniscal Cyst

Parameniscal cysts can be distinguished from bursae and ganglion cysts with their close association with the torn meniscus and continuity with the tear. They occur as a result of entrapment of fluid in the tear caused by the "check-valve" mechanism. This sign has over 90% PPV (21) (Image 7).

Meniscal Extrusion

Meniscal extrusion refers to when the meniscus extends at least 3 mm past the edge of the tibial plateau. In the presence of hypertrophic bone spurs, measurement is made by determining the outer margin of the tibial plateau

while excluding osteophytes. Meniscal extrusions are closely associated with root tears. However, meniscal extrusions may also show involvement with complex tears, large radial tears, and severe meniscal degeneration (22).

Subchondral Bone Marrow Edema

In contrast to nonspecific edema often associated with degenerative changes, linear subchondral bone marrow edema, which is characterized by superficial edema that is adjacent to meniscal attachment sites, parallels the articular surface, and is less than 5 mm deep, may indicate presence of a meniscal tear. This sign is present in 60% of MM tears and over 90% of LM tears (23).

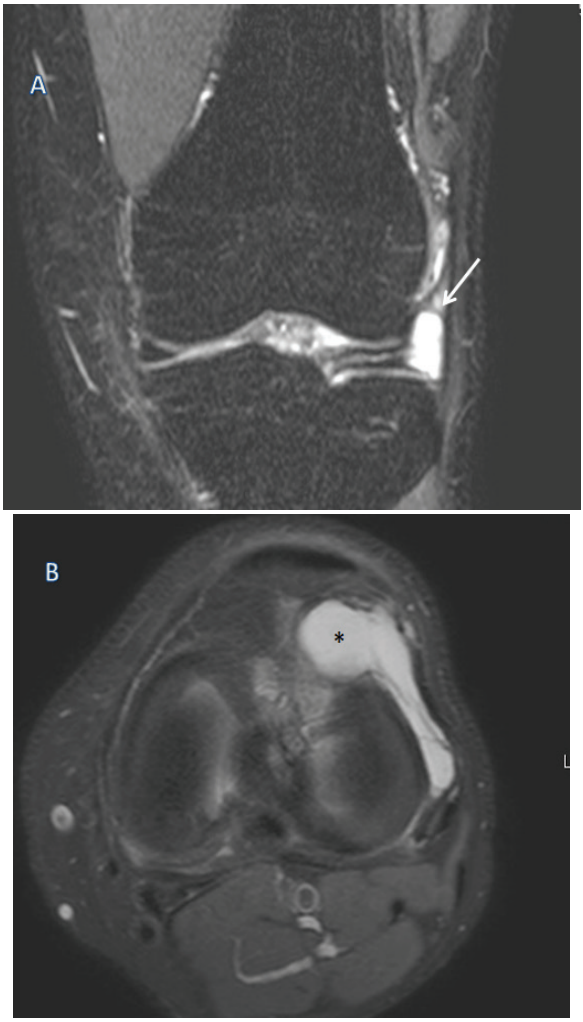

Image 7: Coronal T2-weighted MR image (A) shows parameniscal cyst (arrow) accompanies horizontal tear of the lateral meniscus. Axial PD weighted MR image shows (B), a large parameniscal cyst (asterisk) extending towards the anterior

is also observed adjacent to the lateral meniscus.

CONCLUSION

Magnetic resonance imaging is the preferred imaging modality for evaluating meniscal lesions with high accuracy. However, in order to avoid errors of interpretations and pitfalls, there are several factors that should be taken into consideration. The technical platform and the sequence parameters, the awareness of the normal meniscal anatomy, and the knowledge of the patterns of the tears may influence the accuracy of diagnosis. It also allows for the characterization of a wide variety of tear patterns, guiding surgical planning.

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Chapter 5

Analysis of Antiinflammatory Compounds Carrying Pyrazoline Ring

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Introduction

Pyrazole is a heteroaromatic compound that includes two neighbouring nitrogen atoms in a five membered ring. NH-pyrazoles show both weak basic and weak acidic properties. The basic property is originated from C = N and the acidic property is due to the single bonded nitrogen atom (Castillo and Portilla, 2018). Pyrazolines are products formed as a result of catalytic hydrogenation of pyrazoles. Pyrazolines are stronger bases and less stable compounds comparing to pyrazoles (Alex and Kumar, 2014). Pyrazolines turn into tautomers (figure 1) by mere heating and by acid catalysis. 1-Unsubstituted Δ^2-pyrazolines transform to Δ^1-pyrazolines. Δ^1-pyrazolines turns into cyclopropane with nitrogen output. N_1-H or N_2-H Δ^3-pyrazolines turn spontaneously into Δ^2-pyrazolines because of being very unstable (Alkorta and Elguero, 2015).

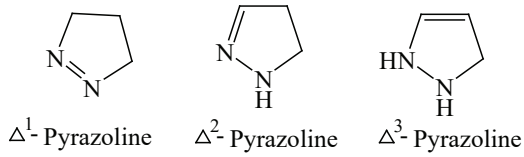


Figure 1. *Tautomers of pyrazoline*

Since 2-pyrazolines are stable and common type of pyrazolines, the relationship of antiinflammatory activity and pyrazolines will be explained through this pyrazoline isomer. Pyrazolines are compounds that attract medicinal chemists, having a lot of different pharmacological activities such as antimicrobial (Özdemir, Turan-Zitouni, Kaplancıklı, Revial, Demirci and İşcan, 2010), antidepressant (Salgin-Goksen, Yabanoglu-Ciftci, Ercan, Yelekci, Uçar and Gokhan-Kelekci, 2013; Mathew, Suresh and Anbazhagan, 2014; Evranos Aksöz et al, 2017), antiinflammatory (Rahman and Siddiqui, 2010; Küçükgüzel and Şenkardeş, 2015), antioxidant, anticancer, antitubercular (Rahman and Siddiqui, 2010), antiviral, antidiabetic (Bhutani, Pathak, Husain, Kapoor and Kant, 2015), and cholinesterase inhibitor (Tok, Koçyiğit-Kaymakçıoğlu, Sağlık, Levent, Özkay, Kaplancıklı, 2019). The pyrazoline ring, located in the structure of antiinflammatory drug-active substances such as phenazone (antipyrine), metamizol (dipyrone), aminophenazone (pyramidone, aminopyrine, amidopyrine), propyphenazone (isopropylantipyrine), and ramiphenazone attract the attention of researchers when designing new antiinflammatory drugs.

The antiinflammatory activities of the compounds can be detected by *in vivo* and *in vitro* methods. The most widely used *in vivo* methods are the carrageenan induced paw edema method (CIPEM) (Winter, Risley, and Nuss, 1962; Joshi, Mandhane, Diwakar, Dabhade and Gill, 2010; Bardalai and Panneerselvam, 2012**;** Fernandes, Kumar and Kumar, 2013) formalin-induced paw edema method (Hamdy and Kamel, 2012) and xylene-in-

duced ear edema method (Wang, Chen, Tang, Wang, Li, and Zhang, 2014; Karim et al., 2019). However, antiinflammatory activity can also be determined using cell culture (Khalil, Ahmed, El-Nassan, Ahmed and Al-Abd, 2012; Cai et al, 2019). Nurkenov et al. (2019), determined antiinflammatory activity by inhibition of lipopolysaccharide (LPS)-induced production of antiinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF) in monocytic MonoMac-6 cell as well as NF-κB-dependent production of alkaline phosphatase in the transfected monocytic THP-1 Blue cells. *In vitro* determination of antiinflammatory activity can be made by using COX-1/COX-2 assay kits (Chandel, Kumar, Singla, Kumar, Singh and Gill, 2019), albumin denaturation method (Rao, Jayachandran, Srinivasa and Shivakumar, 2005; Reddy, Raju, and Sridhar, 2016; Chavan and Hosamani, 2018; Chandel et al., 2019) and membrane stabilization assays (Waghmare, Lingampalle, Patil and Asrondkar, 2017; Chandel et al., 2019).

COX enzymes and binding properties

Compounds in the pyrazoline structure usually show their antiinflammatory activities by inhibition of the cyclooxygenase enzymes (COX), especially they act as selective COX-2 inhibitors (Abdelsayed et al, 2014; Abdellatif, Elsaady, Abdel-Aziz and Abusabaa, 2016; Chandel et al., 2019). COX (EC 1.14.99.1) catalyze the prostaglandins biosynthesis which are autocoids derived from arachidonic acid (Rouzer and Marnett, 2009; Ricciotti and FitzGerald, 2011; Paiotti, Marchi, Miszputen, Oshima, Franco and Ribeiro, 2012). Prostaglandins are important mediators in inflammation. Although prostaglandin levels are very low in normal tissue, prostaglandin production increases in acute inflammation (Ricciotti and FitzGerald, 2011). Prostaglandin synthesis and, accordingly, inflammation can be controlled by inhibition of cyclooxygenases (COX-1, COX-2) (Rouzer and Marnett, 2009).

Human COX-1 is homodimer of 576 aminoacids while hCOX-2 is homodimer of 581 aminoacids. COX-1 includes three high mannose oligosaccharides, while, COX-2 includes four high mannose oligosaccharides. Fourth oligosaccharide is responsible from degradation of COX-2 (Rouzer and Marnett, 2009). COX-1 and COX-2 enzymes have 60% sequence homology (Rouzer and Marnett, 2009; Idris, Amin, Selvaraj, Jamari, Kek and Salleh, 2018). Active site of COX enzymes consists of a narrow entrance at the membrane binding domain and a hydrophobic channel. Although these active sites are similar, binding cavities of the two enzymes are different (Idris et al, 2018). COX-2 enzymes' binding cavity is larger than COX-1 enzymes binding cavity. So, inhibitors that include bulky groups interact with COX-2 but not with COX-1 (Idris et al, 2018; Yousif, Mahdi and

Raauf, 2019). Monomer of COX-2 enzyme consist of three domains: large catalytic domain, epidermal growth factor (EGF) domain and the helical membrane binding domain. The catalytic domain has cyclooxygenase and peroxidase activities. Tyr 385 and Gln 203 are important amino acids for enzyme activities. Tyr 385 is important for cyclooxygenases and Gln 203 is important for peroxidases (Luong, Miller, Barnett, Chow, Ramesha and Browner, 1996). Active site of COX-2 includes 22 amino acids and three regions (aromatic (cavity A), aliphatic (cavity B) and selective regions (cavity C)) (figure 2) (Yousif, Mahdi and Raauf, 2019).

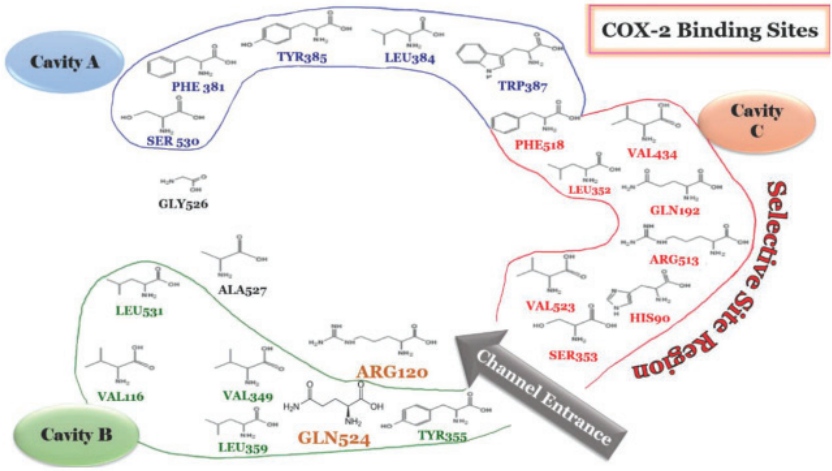


Figure 2. *Active site of COX-2 receptor (Yousif, Mahdi and Raauf, 2019)*

COX inhibitor complexes form hydrogen bonds with Arg 120, Tyr 355, His 513 and Glu 524 aminoacids. These bondings form a gate for ligand entrance to the COX active site (Llorens, Perez, Palomer and Mauleon, 1999).

Examination of antiinflammatory pyrazolines

Abbas and Naseer (2014) synthesized 3,5-diphenyl pyrazoline derivatives (figure 3), which include acetyl or propanoyl groups at the first position of pyrazoline ring and 4-alkyloxyphenyl at the fifth position. They investigated antiinflammatory and cytotoxic activities of these compounds. They used indomethacine as standard drug to detect antiinflammatory activity. They found compound **2c** more active than indomethacine. Compound **2c** includes propyloxyphenyl at the fifth position and propanoyl at the first position of pyrazoline ring. They found activity of **1c** close to the standard drug. Compound **1c** carries methoxyphenyl at the fifth and acetyl at the first position of pyrazoline ring.

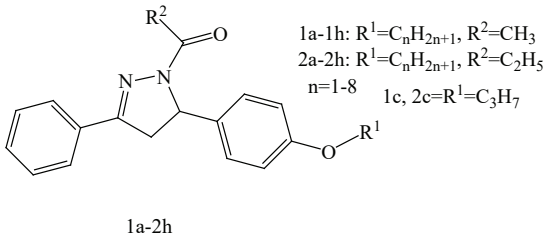


Figure 3. *Some 3,5-diphenyl pyrazoline derivatives*

Abdelsayed et al. (2016) designed and synthesized 1,3,5-trisubstituted pyrazoline derivatives. Evaluating the antiinflammatory, analgesic and ulcerogenic activities of their compounds, they detected antiinflammatory and analgesic activities. Antiinflammatory and analgesic activities are determined *n vivo* by using CIPEM and hot plate test in mouse, respectively. *In vitro* cyclooxygenase inhibitory activity is determined by using ovine COX-1 and COX-2 and EIA kit. Their compounds showed COX-2 inhibitor activity but not COX-1 inhibitory activity. Compounds **4h**, **6e**, **7a**, **7e** and **9** displayed better antiinflammatory and analgesic activities than celecoxib. Their compounds did not have ulcerogenic activity. They made molecular docking for their compounds and they determined that compounds **7a**, **7e** and **9** displayed similarity in binding mode to selective COX-2 inhibitor, SC-558. Formulas of their compounds and SC-558 are showed in figure 4. Compounds **7a**, **7e** and **9** made hydrogen bonding with (His90) and (Arg513) of hydrophilic pocket of COX-2 enzyme. This binding is specific for interaction with COX-2 enzyme. They stated that for an excellent antiinflammatory and analgesic activity, fluorophenyl at third and nitrophenyl at fifth positions of the pyrazoline is necessary. Acetylphenylamino substituent that binds to carbonyl at the first position of pyrazoline is valuable for the activity. Replacing this group by chloromethyl, cause inactivity.

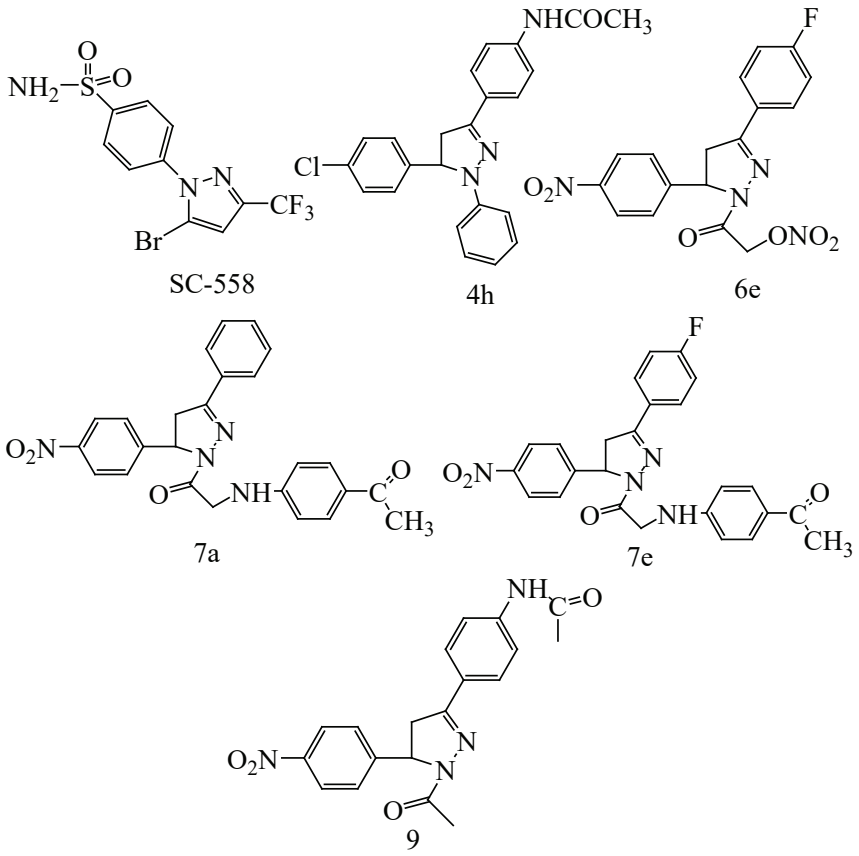


Figure 4. *Formulas of* **SC558**, **4h**, **6e**, **7a**, **7e** *and* **9**

Abdel-Aziz and Gamal-Eldeen (2009) described synthesis of galloyl pyrazoline derivatives in which they determined anticancer, antioxidant and antiinflammatory activities. They detected antiinflammatory activity by using the cell culture supernatant and Griess reagent. The amount of nitric oxide increases during inflammation; therefore, nitrite accumulation was measured in this test. They found **14b** and **14f** (figure 5) as the most active antiinflammatory agents. They detected that substitution with furyl or methoxy phenyl decreased nitric oxide inhibition.

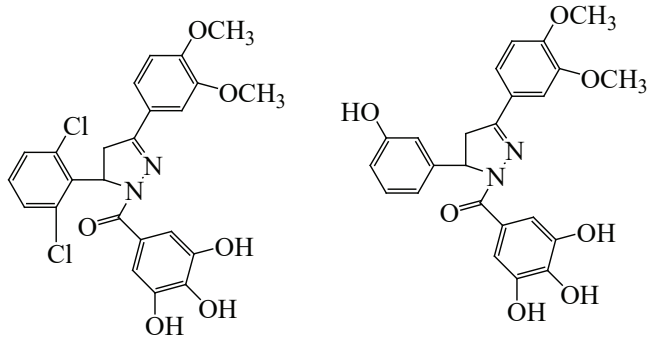
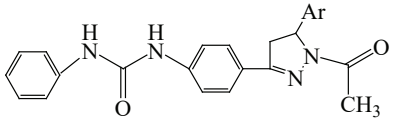


Figure 5. *Some galloyl pyrazolines*

Amir, Ali and Somakala (2016) prepared pyrazoline derivatives that include urea and sulphonamide groups and evaluated the antiinflammatory, analgesic, ulcerogenic and lipid peroxidation activities of these compounds. They observed that compound **3e**, having a urea pharmacophore, with a 4-fluorophenyl group at fifth position of pyrazoline, is the most active antiinflammatory pyrazoline derivative. Replacement of 4-fluorophenyl with 3-chlorophenyl, 4-chlorophenyl and 3-nitrophenyl caused reduction of the activity. Compound **4c**, a pyrazolyl sulphonamide derivative, with a 3-chlorophenyl at fifth position of pyrazoline showed the best antiinflammatory activity of this group. For this group compounds, having furyl at the same position caused decline of the activity.

3a, **3b**, **3i**, **3l**, **4c** and **4l** of their compounds (figure 6) showed significant antiinflammatory and analgesic activities. They found **3i**, including urea pharmacophore, with high analgesic activity and low ulcerogenic potential.



3a, 3b, 3e, 3i, 3l

Ar= a: phenyl, b: 2-chloro phenyl, e: 4-fluorophenyl, i: 3-nitrophenyl, l: 3-antracinyl

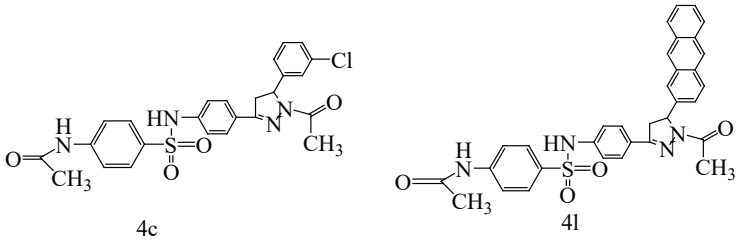
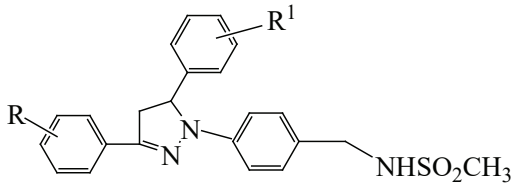


Figure 6. *Formulas of* **3a**, **3b**, **3i**, **3l**, **4c** *and* **4l**

1-(4-Methyl sulphonyl amino methyl) phenyl-3,5-diaryl-pyrazolines (figure 7) have been synthesized by Waghmare et al. (2017). To detect antiinflammatory activity, they used HRBC membrane stabilization method. Their compounds, **3b** (EC50= 4.47± 0.06), and **3c** (EC50= 4.57± 0.07) exhibited very good antiinflammatory activity comparing to diclofenac sodium (EC50 = 13.24). In addition, activities of **3d** (EC50= 6.95± 0.46), **3e** (EC50 =10.11±0.08) and **3f** (EC50 =9.45± 0.12) were found remarkable.



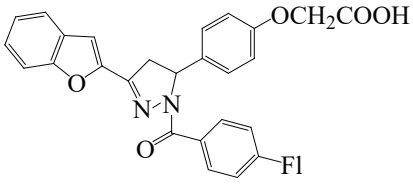
3b-3f

b: R=3-Cl, R^1=4-OCH_3 c: R=4-Cl, R^1=4-Fl d: R=4-Cl, R^1=H

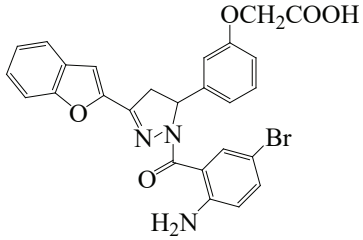
e: R=H, R^1=4-OCH_3 f: R=4-Fl, R^1=H

Figure 7. *Some 1-(4-Methyl sulphonyl amino methyl) phenyl-3,5-diaryl-pyrazoline derivatives*

Babu, Sridevi, Joseph and Srinivasan (2007) synthesized benzofuranyl pyrazoline derivatives and investigated their antiinflammatory properties. They used ibuprofen as standard drug. Ibuprofen's inhibition of edema volume was 91.9%, however, their most active compounds **4g** and **5m** (figure 8) showed 83.4% and 80.5% inhibition.



4g

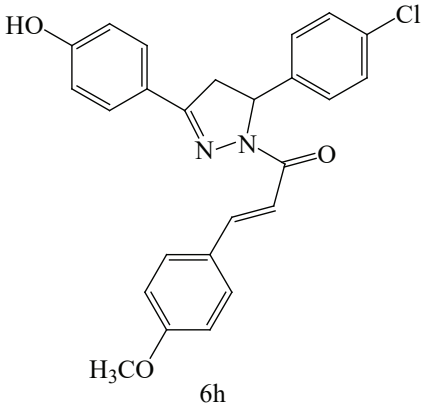


5m

Figure 8. *Formulas of* **4g** *and* **5m**

Bhadoriya and Jain (2018) synthesized pyrazolinyl chalcone derivatives. They screened their compounds antiinflammatory activity by using CIPEM. They investigated the interactions of these compounds with COX-2 enzyme. The most active compound of them were found **6h** (figure 9) with 63.58±1.24 (after 3h) and 67.31±1.20% (after 5 h) inhibition potential. They compared activity of **6h** with indomethacin (71.25±1.23 (after 3h), 73.21±1.12 (after 5h). Compound **6h** carries a p-chloro phenyl at fifth position of pyrazoline and a p-methoxy moiety in the chalcone part of the compound. They reported that an electron withdrawing group like

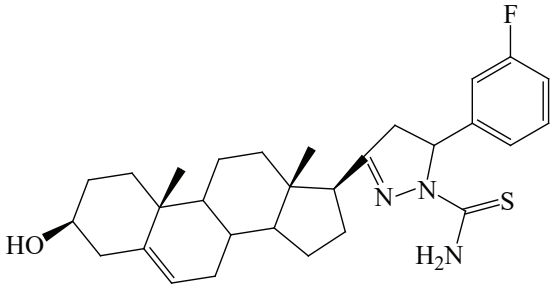
chloro in an aryl ring and a bulky methoxy group on another ring caused the binding with the receptor. Hydroxyl group of compound **6h** interacted with amino acid residues of receptor and made hydrogen bonding. At the same time, phenyl rings of compound interacted with LEU-352, GLY-526, ALA-527 and LEU-531 residues. Also, phenyl ring of the compound made π-π interaction with the amino acid phenyl rings.



6h

Figure 9. *Formula of* **6h**

Cai et al (2019) prepared steroidal pyrazolines and screened them for antiinflammatory activity. They found compound **4g** (figure 10) as the most effective agent with a comparable activity of dexamethasone. They detected the nitric oxide production in LPS-induced RAW 264.7 cells and toxicity of the compounds against RAW 264.7 cells. Compound **4g** inhibited nitric oxide synthase (iNOS), IL-6, TNF-α and COX-2 in LPS-induced RAW 264.7 cells. They reported that the electron donating groups (-OCH_3) connected to the aromatic ring reduced the inhibitor potency, and the presence of electron withdrawing groups (-F, -Cl) caused a better inhibitor potency.

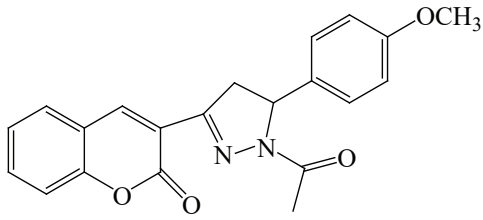


4g

Figure 10. *Formula of* **4g**

Chandel et al. (2019) tested coumarine based pyrazoline derivatives

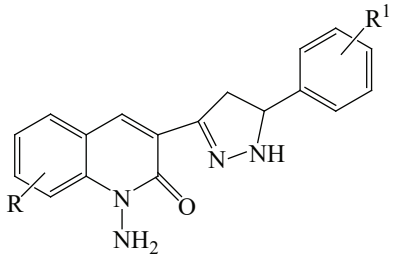
for their antiinflammatory activity. They designed their compounds according to some rules. COX-2 inhibitors have some structural similarities. These compounds carry a five or six membered heterocyclic or carbocyclic structure in the center. Usually, this central structure is binded to 1,3-aryl groups or two vicinal aryl rings. They synthesized seven compounds according to these rules, the most effective of which is **7a** (figure 11). Compound **7a** had a perceivable antiinflammatory and analgesic potential with a safety up to 2g/kg dose. They used ovine COX-1\COX-2 assay kit and membrane stabilization assay for *in vitro* activity. To detect the *in vivo* activity, they used CIPEM. *In vivo* analgesic activity was determined using writhing test. Compound **7a** showed good binding properties to COX-2 enzyme (Gold score: 88.25). Acetyl group of pyrazoline interacted with Ser 516. Coumarin's carbonyl made hydrogen bonding with Tyr 341, the aromatic ring in coumarin made π-H hydrophobic interaction with Val 509.



7a

Figure 11. *Formula of* **7a**

Fernandes et al. (2013) synthesized quinolinyl pyrazoline derivatives (figure 12) and screened these compounds for antiinflammatory activity using CIPEM. Their compounds were compared with diclofenac sodium as standart. Standart showed 64.52% inhibition. Their compounds inhibition potential is ranged from 24.39 to 48.78% after 2 h.



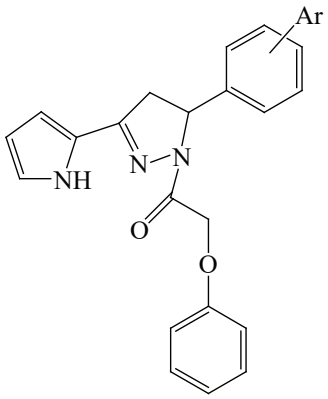
AJP1-AJP8

R= H, 6-NO2; R^1=3-NO_2 , 3,4,5-OCH_3,4-CH_3,4-OH, 2-Cl, 2-NO_2

Figure 12. *Some quinolinyl pyrazoline derivatives*

Fernandes, Revanasiddappa, Ishwarbhat, Kumar, D'Souza and Alva (2017) synthesized pyrrolyl pyrazoline derivatives (figure 13). They used *in vitro* testing methods to detect the antiinflammatory activity. They used

protein denaturation by bovine serum albumin and protein denaturation by egg albumin methods. Their compounds showed moderate antiinflammatory activity for both methods.

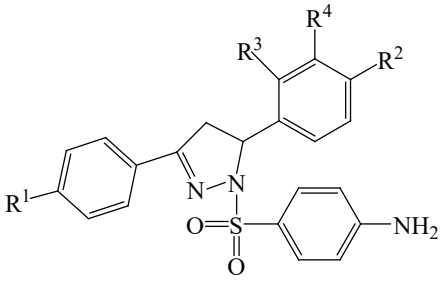


3a-3j

Ar: C_6H_5, 4-Cl, 4-OCH_3, 2,5- $(OCH_3)_2$, 4-OH, 4-NO_2, Furfuryl, 4-F, 4-Br, 4-Hydroxy-3-methoxy-phenyl

Figure 13. *Some pyrrolyl pyrazoline derivatives*

Gonjare, Awati, Kumbhoje, Patil, Patil and Kondawar (2013) synthesized p-aminophenyl sulphonyl pyrazoline derivatives (figure 14) and screened antiinflammatory activity of these compounds with CIPEM. Their compounds antiinflammatory activity (30-34% inhibition for 4 h) were less than the standart drug ibuprofen (51% inhibition for 4 h).



SP1-SP6

SP1:R^1= Cl, R^2= OCH_3, SP2:R^1= Cl, R^2= CH_3, SP3:R^1= OCH_3, R^2= CH_3, SP4:R^1= Cl, R^2= OCH_3, R^4=OCH_3, SP5:R^1= Cl, R^2= Cl, R^3=Cl, SP6:R^1= Cl, R^2= OH

Figure 14. *Some p-aminophenyl sulphonyl pyrazoline derivatives*

First, Joshi et al. (2010) synthesized morpholine bearing pyrazolines (figure 15), then using phenylisothiocyanate/triethylamine in diethylether, they obtained 4,5-(dihydro-3(2-hydroxyphenyl)-5-(4-morphinophenyl)-N-phenyl-pyrazol-1-carbothioamide derivatives. These compounds were tested for analgesic and antiinflammatory activities. To

detect the analgesic activity, they used acetic acid induced writhing response test. Antiinflammatory activity is evaluated by CIPEM. They found analgesic activities of **6a**, **6b**, **6g**, **7a**, **7d** and **7g** comparable to diclofenac. Most of their resulting products showed higher antiinflammatory activity than their starting pyrazolines. They said that presence of bromo (**6g**, **7g**) increased the activity for the starting and the resulting pyrazoline derivatives. They didn't find a direct relationship of the increase of the activity with the presence of electron donating or electron withdrawing groups.

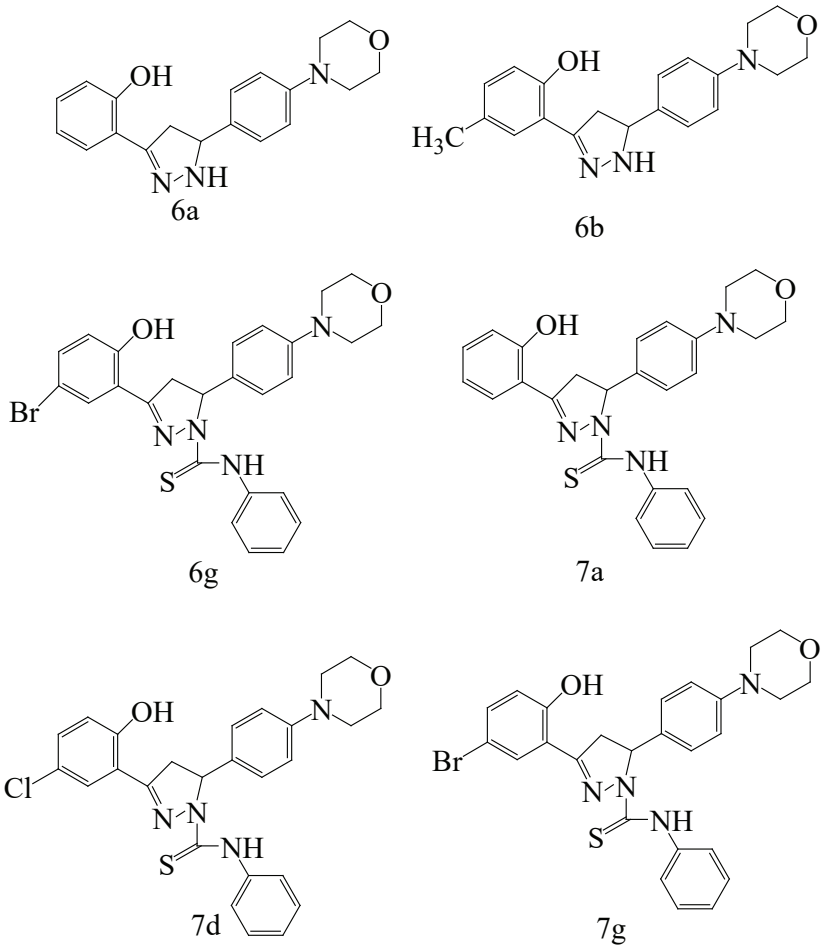
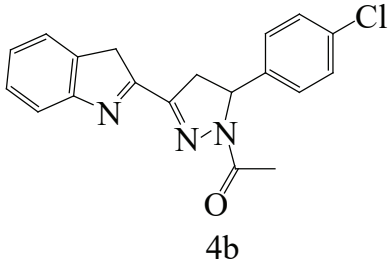


Figure 15. *Some morpholine bearing pyrazolines*

Indolyl pyrazoline derivatives were synthesized by Kale, Narute and Kalyankar (2014). They screened analgesic and antiinflammatory activities of their compounds. They determined analgesic activity by measuring the response of animals to the hot-plate test and antiinflammatory activity by employing CIPEM. They compared activities of chalcones and different pyrazoline derivatives. Cyclisation of chalcones to pyrazolines caused in-

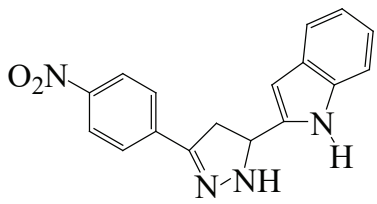
crease in the antiinflammatory activity. p-Chloro substituted phenyl ring at the five position of pyrazoline caused increase in the activity, substitution of dimethoxyl group at meta and para position of same phenyl ring caused decrease in the activity. Compound **4b** (figure 16) showed maximum anti-inflammatory activity. They stated their compounds would be selective for COX-2 enzyme, not for COX-1 enzyme because of having bulky groups.



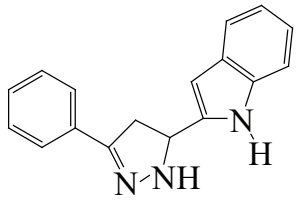
4b

Figure 16. *Formula of* **4b**

Shroff and Daharwal (2017) synthesized indolyl pyrazoline derivatives and determined their antiinflammatory activity by CIPEM. Compound **7d** and **7g** (figure 17) showed closer activity to indomethacin. p-Nitro phenyl substitution at C3 position of pyrazolines and unsubstituted N1 orient favored antiinflammatory activity. Fused cyclohexanone with pyrazoline at C3 and C4 caused poor activity.



7d



7g

Figure 17. *Formulas of* **7d** *and* **7g**

Khalil et al. (2012) synthesized 5-aryl-3-cyclopropyl-4,5-dihydro-1H-pyrazole-1-carbothioamides (**2a-h**) and 5-aryl-3-cyclopropyl-4,5-dihydro-1H-pyrazole-1-carboxamides (**2i-p**) and screened these compounds for *in vitro* antioxidant and antiinflammatory activities. They used lipopolysaccharide-stimulated macrophage cells to detect the nitric oxide (NO) production. These cells produce chemical mediators released in inflammation such as nitric oxide. Overproduction of nitric oxide indicates acute and chronic infection. In this test, nitric oxide content (nitrate, nitrite and stable end products of L-arginine-dependent NO synthesis) is assessed. Decline of the nitric oxide content showed antiinflammatory activity. All compounds caused reduction of nitric oxide production. Especially, carbothiamide derivatives, **2e** and **2g**, and a carboxamide derivative

2n (figure 18) caused the most reduction of nitric oxide levels.

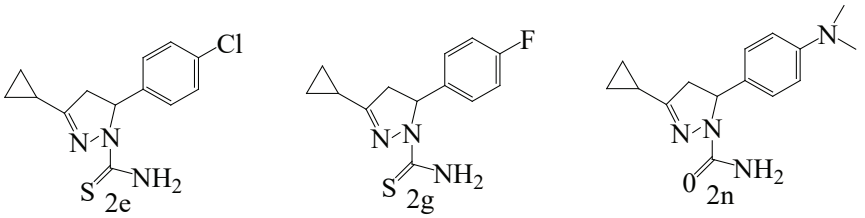
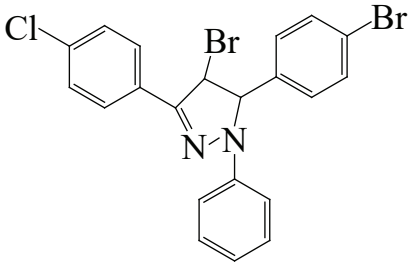


Figure 18. *Formulas of* **2e, 2g** *and* **2n**

4-Bromo-3,5-diaryl-1-phenyl-2-pyrazoline derivatives were synthesized by Kumar, Rout and Sahoo (2013) and tested for antiinflammatory activity using CIPEM. Their compounds displayed antiinflammatory activity ranging from 55.77% to 94.27%. Compound **3g** having 3,4-dimethoxy phenyl at C-5 of pyrazoline nucleus showed better inhibition (94.27%) than diclofenac (93.63%). Compound **3l** (figure 19), having a p-chloro phenyl at C-3 and a p-bromo phenyl at C-5 position of pyrazoline, showed high inhibition (89.24% inhibition). Synthesized compounds had low toxicity up to 2 g/kg for rats.

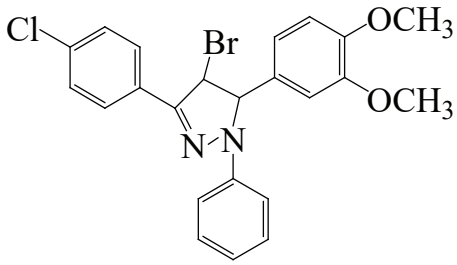


3l

Figure 19. *Formula of* **3l**

Kumar, Rout, Panda and Raju (2011) reported synthesis of 3,5-diaryl-1-phenyl-2-pyrazolines and evaluated their analgesic and antiinflammatory activities. They used CIPEM in Wistar rat to detect the antiinflammatory activity, acetic acid-induced abdominal writhing method and hot plate method for analgesic activity. Compound **3h** (figure 20), having p-Cl and m,p-OCH_3 groups in the phenyl ring at C-3 and C-5 respectively of pyrazoline nucleus, exhibited the best antiinflammatory (68.75±1.376 for 3h, 83.67±1.574 for 5 h) and analgesic activities (63.23±0.715 for writhing method, 57.45±0.721 for hot plate method). These activities are comparable to that standart drug diclofenac (70.35±3.55 for 3 h, 87.17±1.77 for 5h; 72.33±1.09 for writhing method, 67.45±0.09 for hot plate method). Existence of electron withdrawing groups at the phenyl rings of C-3 and

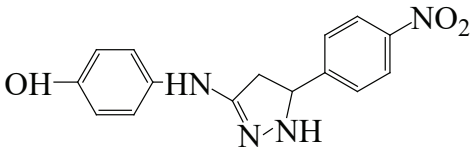
C-5 of pyrazoline exhibited significant antiinflammatory activity.



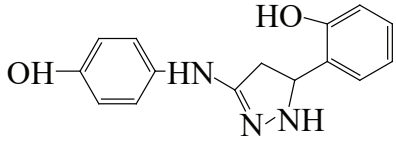
3h

Figure 20. *Formula of* **3h**

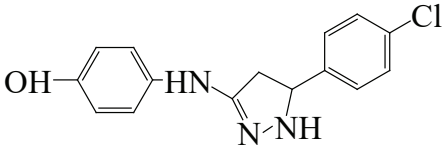
Sahu, Banerjee, Samantrayl, Behera and Azam (2008) synthesized 3,5-diaryl-2-pyrazoline derivatives and tested these compounds for their analgesic, antiinflammatory and antimicrobial activities. Analgesic activity was determined by tail-flick test while antiinflammatory activity was determined by CIPEM. An increase in analgesic, antiinflammatory and antimicrobial activities has been observed when C-5 of pyrazoline ring included 4-NO_2 (**2c**), 2-OH (**2e**) and 4-Cl (**2f**) phenyl (figure 21).



2c



2e



2f

Figure 21. *Formulas of* **2c, 2e and 2f**

Sridevi, Balaji, Naidu, Kavimani, Venkappayya and Suthakaran (2008) synthesized indoquinoxalinyl pyrazoline derivatives and investigated antioxidant, antiinflammatory and antihistaminic activities of these compounds. Ibuprofen reduced edema volume by 92.54%, while QVI 13 and QVI 14 (figure 22) decreased 83.89% and 80.49% respectively. All compounds showed significant antioxidant activity.

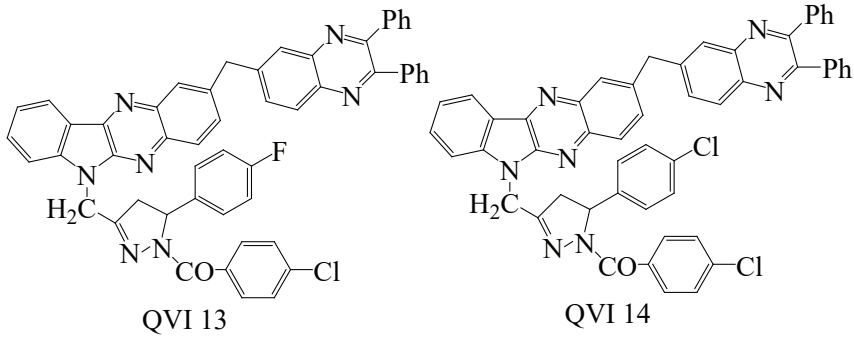


Figure 22. *Formulas of QVI 13 and QVI 14*

Suthakaran, Somasekhar, Sridevi, Marikannan, Suganthi and Nagarajan (2007) synthesized thirty benzofuropyrazoline derivatives and screened 7 of these compounds for antiinflammatory activity which was detected by CIPEM. They compared their compounds to ibuprofen (91.93% inhibition). The compounds with the highest antiinflammatory activities, PY-7A and PY-13B (figure 23), exhibited 83.89% and 80.49% inhibition of edema, respectively. They stated that the presence of p-fluoro and 2-amino groups caused good activity.

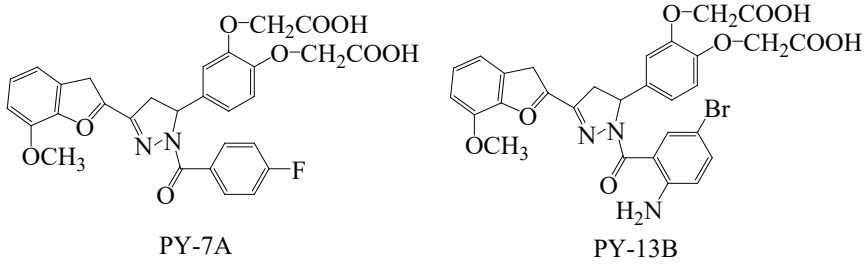


Figure 23. *Formulas of* **PY-7A** *and* **PY-13B**

Conclusion

In the light of the current literature, the antiinflammatory activity of the compounds carrying the pyrazoline ring, and their interactions with the COX enzymes that play a significant role in this activity and how the functional groups associated with the pyrazoline ring affect the activity are mentioned. In general, the presence of fluorine and chlorine atoms has been observed to increase the antiinflammatory activity. These atoms mostly make hydrogen bonding with COX enzymes. It is a great advantage that this ring system can be easily synthesized. For optimization of physicochemical properties of pyrazolines, in silico methods can be used. By evaluating all these data together, new and effective antiinflammatory drugs can be developed in the

pyrazoline structure. It appears that pyrazolines will remain important in the development of new antiinflammatory drugs.

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Chapter 6

Colorectal carcinoma in pediatric patients: A comparison with adult tumors, treatment and outcomes

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Introduction

Colorectal cancer (CRC) is the third most common cancer in adults, and is the second leading cause of cancer-related deaths in the United States. In contrast, colorectal adenocarcinoma is a rare pediatric tumor representing only 1% of all pediatric malignancies, with an incidence of approximately 1 per million (1).

There is good evidence for the management of colorectal cancer in adults, but since the tumor is extremely rare in the pediatric age group, it may be difficult to develop a treatment plan when diagnosed with CRC. CRC studies in the pediatric age group are limited with a small number of patients, without major institutional experience or without prospective studies which can lead treatment. In the literature, studies on this subject are generally in the form of small case series. There are several studies comparing pediatric colorectal cancer with adults. Many studies in the literature have defined age limits for early colorectal cancers as 30, 40 and 50 years. Because of this rarity, clinical management and treatment strategies often result from experience with adults. But one should not forget that children are never smaller versions of adults. They have different structural and functional properties. Therefore, the signs, diagnosis and treatment of their diseases also require certain changes.

The symptoms present in children are nonspecific and may mimic many benign gastrointestinal diseases. However, due to the low awareness of the disease, the diagnosis is usually delayed until the disease is at an advanced stage, resulting in poor prognosis compared to adults(2). In this review, we aimed to briefly review the incidence, epidemiology and risk factors, clinical presentation, treatment and outcome of colorectal carcinoma in children, adolescents and young adults by comparing them to the adult age group and to raise awareness about colorectal carcinoma in the pediatric age group.

Incidence and epidemiology

The lifetime prevalence of CRC is around 2.4-5% in the whole population. This ratio rises with certain risk factors present in an individual(3,4). CRC is estimated to account for 2.1% of all neoplasms in adolescents and young adults aged 15 to 29 years(3,5). Based on Surveillance, Epidemiology and End Results (SEER) data, which lists cancer incidence and survival based on the US population, there is a slight male predominance for colorectal cancer in children in general, but this is not evident in patients under 20 years of age(6). Earlier pediatric literature reviews reported a male predominance of 3: 1 as a closer view for pediatric patients with CRC. In the Saab et al. review, when all reported patients are considered, a

male-female ratio of 1.08:1 was found(3).

Using data from the Italian cancer registry network (AIRTum), a recent study of the Italian TREP project on rare pediatric tumors found that the incidence of colorectal cancer for children between 10 and 14 years and children between 15 and 17 years was 0.09 per million and 0.72, respectively. Patients younger than 10 years of age was not observed in their records(7,8). The incidence of colorectal cancer is lower in the group under 10 years of age (9,10). In most of the published literature, it is seen in the second decade of life (10,11).

According to the Turkey 2014 cancer statistics, colorectal cancer is seen in 0.5 of 100,000 men in the group under 19 years of age, and is seen with a rate of 0.7 per 100,000 women(12). Sultan et al. reported an increased incidence of pediatric CRC in the non-white population, including hispanic people, but found no association with survival. Socioeconomic factors have not yet been reported in the pediatric population(10,12).

Etiology and Risk Factors

In adults, most CRCs are thought to be caused by pre-existing adenomas. In 1990, Fearon and Vogelstein proposed a model of successive steps for the development of colorectal cancer. Their recommendations suggest causality between progression from epithelial hyperplasia to adenomas and invasive carcinomas, based on the findings of predictable accumulation of repetitive genetic mutations in the process of development of colonic adenoma to carcinoma(13).

Although this model appears to be applicable to most adults with CRC, it may not apply to childhood conditions of this disease(3).

However, the situations where this “adult CRC” model is probably valid in pediatric cases, is familial polypoid syndromes of the gastrointestinal tract, such as familial adenomatous polyposis (FAP) syndromes(3).

Hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP) are the most important cancer susceptibility syndromes associated with CRC(14).

Colorectal cancer occurs sporadically in most children and adolescents, but has an identifiable predisposing condition at an average rate of 10%, making up about 5% of FAP cases(11).

In addition to genetic syndromes susceptible to CRC, one of the most important risk factors in adults is a first-degree relative with colorectal adenoma or carcinoma. The importance of familial history in children is not clear.

Gastrointestinal polyposis syndromes

Most childhood polyps, called juvenile polyps, are benign and do not carry a long-term risk of malignancy(15). However, careful assessment should be made for a condition predisposing carcinoma in the case of polyps in children with a family history of CRC, multiple or recurrent polyps, or adenomatous polyps. Hamartomatous polyposis syndromes are a rare disease group with autosomal dominant inheritance and constitues less than 1% of all hereditary colorectal cancers. Two hamartomatous polyposis syndromes are associated with an increased risk of CRC: juvenile polyposis and Peutz-Jegher syndrome(16)**.**

In the literature review by Saab, R et al., gastrointestinal polyposis conditions in childhood probably accounted for 26 (7.2%) of 361 CRC cases(**3).**

Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is characterized by multiple (> 100) premalignant colorectal adenomatous polyps and is caused by germline mutations inactivated in 5q21 in the tumor suppressor adenomatosis polyposis coli (APC) gene. Lifetime CRC risk in FAP patients is 100%(17,18).

In general, adenomas tend to develop close to puberty, but may occur in early childhood. In addition, CRC has been reported in FAP patients younger than 5 years of age, although the mean age for the development of adenoma and carcinoma in FAP is 16 and 39 years, respectively(3,19). Once diagnosed, annual sigmoidoscopy is recommended starting at the age of 10. Prophylactic colectomy should be performed in the late 10s and early 20s(20).

Church et al(21). investigated families with FAP to determine the age of onset of colorectal cancer. They estimated that at least 14 individuals from a different family were diagnosed with CRC before the age of 20 (9-19 years) and the incidence was one case per 157 families.

Juvenile Polyposis Syndrome

Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder characterized by multiple hamartomatous polyps throughout the gastrointestinal tract. Individuals with JPS are at risk for colorectal and gastric cancer. Unlike JPS, sporadic juvenile polyps of the colon occur in up to 2 percent of children under 10 years of age, they are usually solitary and not

associated with an increased risk of cancer(22). Solitary polyps are usually found in the rectosigmoid area in children under 10 years of age, but they may develop at any age(14). Patients typically present with rectal bleeding and anemia, usually between 4 and 30 years of age. They may also present with intussusception, bowel obstruction, rectal or polyp prolapse, abdominal pain, or protein-losing enteropathy(3).

Peutz-Jeghers Syndrome

Peutz-Jeghers syndrome (PJS) is an autosomal dominant syndrome characterized by mucocutaneous pigmentation and intestinal hamartomatous polyps. Pigmentation is typically observed around the lips, but may also affect the buccal mucosa, hands, feet, reproductive organs, and areas around the nose and eyes. This rather typical presentation should always be considered by the pediatrician. Hamartomatous polyps in PJS may involve the entire intestinal tract or may be confined to a particular site. PJS usually presents a risk for intestinal and extraintestinal cancers presenting after childhood(14).

Although hereditary gastrointestinal polyposis syndromes produce a small number of CRCs in children, it is important to bear in mind that these conditions may occasionally occur as childhood CRC. Therefore, in addition to having a complete colonoscopy in children presenting with CRC, obtaining a detailed family history is critical for identifying those with familial polyposis conditions.

Hereditary Nonpolyposis Colon Cancer

Hereditary Nonpolyposis Colon Cancer (HNPCC), also known as Lynch syndrome, is a rare autosomal dominant inherited condition that constitutes 1-5% of all CRCs. It is caused by DNA mismatch repair (MMR) gene mutations such as MLH1, MSH2, MSH6, or PMS2. Mutations in these genes lead to microsatellite instability (MSI)(23).

In families with a history of HNPCC, other tumors, including colorectal cancer, cancers of the stomach, small intestine, hepatobiliary system, ovary, endometrium and upper urinary tract are more common(23).

Affected patients are typically diagnosed with CRC earlier than patients with sporadic CRC. However, since these patients have an estimated 80% risk of developing colorectal cancer, it is important to identify affected families to establish intensive surveillance programs(3).

In the SAAB et al(3) study, HNPCC diagnosis was found in 16 (4.4%) of 361 patients under the age of 30. However, the prevalence of HNPCC in children and adolescents with colorectal cancer is not fully known.

Other Risk Factors

Ulcerative colitis (UC) is clearly associated with CRC development. Age and prevalence of colonic involvement at the time of initial diagnosis are strong independent risk factors for subsequent CRC development. Those diagnosed before <15 years of age and those with entire colon involvement are at highest risk(24). Jackman et al(25). found that CRC was twice as common in children with UC, as in adults with UC, and that CRC developed in 6 (6.3%) of 95 children with UC.

Clinical Presentation

In the pediatric age group, similar to adults, colorectal cancer occurs with complaints such as abdominal pain, hematochezia, changes in intestinal habits, weight loss and anemia.

In a review of 77 pediatric patients with CRC diagnosed at St Jude Children's Research Hospital over a 40-year period, 83% presented with abdominal pain, 65% with weight loss, and 80% with other bowel symptoms (such as altered bowel habits or hematochezia). Other commonly reported symptoms in children included nausea and vomiting, weight loss, abdominal distension or abdominal mass, changing bowel habits, and anemia(2).

In children and adolescents, coloretal cancer also occurs with acute abdominal conditions, such as acute obstruction, severe pain mimicking perforation or appendicitis. It has been reported in the literature with a higher incidence than in adults.

In the study of Kaplan et al(26). on the clinicopathologic and prognostic differences in colorectal cancer patients in three different age groups (Child/Adolescent, Young Adult and Adult), abdominal pain was the most common symptom in all age groups (n = 378, 65.6%).Abdominal pain (72.0% among adolescents, 48.3% among young adults, 73.4% among adults, $p < 0.001$), vomiting (20.0% among adolescents, 8.6% among young adults, 24.0% among adults, $p = 0.002$) were more common symptoms in adolescents and adults, compared to young adults.Although not statistically significant, rectal bleeding (24.0% in adolescents, 41.1% in young adults, 40.1% in adolescents, $p = 0.125$) and changes in intestinal habits (24.0% in adolescents, 31.9% in young adults, 32.1% in adults, $p = 0.706$) were less common in adolescents than in other groups. The prevalence of weight loss (20.0% in adolescents, 13.2% in young adults, 20.3% in adults, $p = 0.239$) was similar between groups.

Signs and symptoms of CRC depend on the location of the tumor. While a tumor in the left colon usually shows a change in intestinal habits

and hematochezia, a tumor in the right colon is usually associated with chronic anemia or abdominal mass. Since gastrointestinal symptoms such as abdominal pain, constipation or diarrhea are rarely associated with CRC in children, it is likely to be overlooked. Patients presenting with these characteristics are often not suspected of being malignant by the physician or the patient and this explains the delayed diagnosis in this age group(27). It is shown in the literature that the median delay time (duration between onset of symptoms and diagnosis) is 3 months for patients younger than 20 years(28). Less than 20% of children/adolescents have localized disease and the prevalence of metastatic disease is increased in this age group. It is also assumed that diagnostic delays may partially explain advanced diseases in diagnosis(14).In addition, CRC is rarely considered in the differential diagnosis of a patient with gastrointestinal symptoms in childhood. In contrast, the same symptoms in a 60-year-old patient will lead the patient to an emergency colonoscopy.

Diagnosis and Staging

Once the diagnosis is suspected, the evaluation process is similar to adult patients. It typically begins with digital rectal examination, which is difficult for the child age group to accept. Abdominal x-ray, barium enema, computed tomography (CT) and colonoscopy which will eventually show a narrowing of the lumen of the colon or an abdominal mass. A colonoscopy with biopsy alone can provide a confirmatory diagnosis and represent the gold standard. Evaluation of the entire colon is necessary to rule out other polyps or synchronous lesions.Evaluation of distant metastatic diseases including chest, abdominal and pelvic CT scan and bone scan is important to define the stage of the disease. Plasma carcinoembryonic antigen (CEA) and, to a lesser extent, tumor markers such as CA 19-9 and CA 242 may be amplified. These markers can be used to monitor outcome and evaluate treatment response in patients with metastatic disease. However, in many young patients, the diagnosis is made by the surgeon in the operating room after an emergency laparotomy due to obstruction, bleeding or perforation.

The staging guidelines for CRC are intended for adults, and these guidelines, based on surgery and pathology, are widely adopted in children. The current staging is based on the tumor, node, metastasis (TNM) system developed by the American Joint Cancer Committee (AJCC). Old staging systems such as the Dukes and Astler-Coller classifications are less used nowadays.

Biological and pathological characteristics

The biology of tumor development in hereditary colorectal cancer is likely to be similar regardless of patient age. There is extensive literature

on the biology of adult CRC for sporadic CRC. In contrast, little has been published about the biology of sporadic CRC in patients younger than 21 years. In adults, CRC originates from the intestinal mucosal surface; usually caused by adenomas that are thought to progressively advance invasive carcinoma for a decade or more. The applicability of this model to children with sporadic CRC is unknown(13,29).

Microsatellite instability, a common mutation in repetitive DNA sequences, was found in 45-50% of those tested in pediatric series(30,31).

Pathologically, childhood CRCs, similar to the adults, most commonly have adenocarcinoma histology (2,11). The presence of mucinous component, signet cell appearance, degree of differentiation and surgical margins are important in pathological examination. All of these are important in predicting the biological behavior of the disease[3].

In their population-based study, Sultan, I et al(11). compared the characteristics of colorectal cancer seen in children and adolescents with the adult age group. Although adenocarcinoma NOS was the most commonly reported histological subtype in both pediatric and adult groups, negative histologies were significantly higher in pediatric cases: mucinous adenocarcinoma and signet ring cell carcinoma were 22% and 18%, respectively. This rate was 10% and 1% in adults.Adenocarcinoma of the polyps in the series was seen in 10% of children/adolescents, and 5.6% of patients >20 years of age. The most common sites of involvement in children/adolescents were rectum/anal canal (27%) and transverse colon (26%), while the most common tumor sites in adults were rectum/anal canal (30%) and ascending colon (27%).

Poles, G. C(32) in their study using the National Cancer Database grouped the patients as pediatric (≤21 years), young adults (22–50) and older adults (>50).In the pediatric group, the tumor was most commonly seen in the left colon (34.8%). Mucinous histology was seen in 17.8%, 9.7%, 8.7% respectively in the groups and was more common in the pediatric group ($p<0.001$). Microsatellite instability was 41.3%, 23.9%, 26.7% in the groups, respectively, and was again higher in the pediatric group($p<0.001$). Poorly differentiated tumor rate was 29.5%, 16.9%, 15.3%, respectively, and was higher in the pediatric group($p<0.001$).Pediatric patients presented more frequently with stage 3 and 4 disease than the other two age groups(62.0%,49.7%, 37.3%; $p< 0.001$). They obtained more aggressive tumor biology results in children.

Differences in tumor biology and differences not fully captured by histology presenting at a later stage in children may explain the poor outcomes observed(3,32).

There are limited number of studies in the literature on the biological behavior of colorectal cancer in childhood.

Treatment

The literature on the treatment of colorectal cancer in the pediatric age group is limited. In the absence of pediatric prospective clinical trials, treatment guidelines are calculated from adult trials. Following the principles of adult treatment, the main point of treatment is full surgical resection. Close collaboration with surgeons and oncologists is essential to improve outcomes for children and adolescents with colorectal cancer. In most cases, en bloc resection of the colon section containing the tumor, with the mesentery and lymph nodes (radical colectomy) is sufficient([2]6,32). Without surgical resection, treatment is not possible. Adjuvant multi-agent chemotherapy based on the fluorouracil spine is typically used in high-risk localized diseases as well as in advanced-stage diseases. It is performed based on the individual staging of the patient and taking into account the biological characteristics of the tumor(3).

Overall survival for stage I disease in adults is approaching 90% by surgery alone(33).If a malignant polyp is completely removed and pathological evaluation shows a positive histology, no further treatment is needed, frequent screening for the development of other polyps should be conducted(34).Although there is no clear data for children, most survivors of pediatric case series had low-stage disease(26,29,32). In most low-stage pediatric CRC patients, surgery alone is probably sufficient. Follow-up plan for children with fully resected low-grade disease should be made according to those recommended for adults(3).

For patients with stage II disease, there is less data to suggest an optimal approach, even in adult trials. Adequate lymph node removal is extremely important for accurately staging stage II cancers[34]. Based on adult data, chemotherapy may be considered, although postoperative adjuvant is an alternative option in patients with stage II CRC who do not have high risk. For pediatric patients with stage II CRC, it is difficult to make recommendations regarding chemotherapy, and the current practice is to generally follow these adult guidelines(3).

In stage III - IV patients, surgical treatment alone is ineffective and adjuvant chemotherapy is required. Due to the lack of pediatric clinical trials, the optimal treatment for children in such cases is not known[3]. In spine of the treatment protocols, similar to adults, is fluorouracil. Combination of fluorouracil with folinic acid and oxaliplatin or irinotecan has shown survival benefits in adults. When added to these chemotherapy regimens, the addition of cetuximab or bevacizumab increases survival(33,34).

It should therefore be used as a primary treatment in pediatric patients with advanced disease, based on the observed efficacy of these agents.

In the study by Poles, G. C et al.(32), where the pediatric age group was compared with the adult age group, 62% of the pediatric patients had stage 3-4 disease, meaning advanced stage disease was much more common than the adult age group. Segmental resection was performed in 54% of the patients and total colectomy or proctocolectomy was performed in 22.3% of the patients. While segmental resection rate was lower than the adult age group, total colectomy was performed more frequently than the adult age group. In relation to the stage of the patients, 70% had received adjuvant chemotherapy, this rate was 59% for patients between the ages of 20 and 50.

Kaplan et al., in a study in which patients with colorectal cancer were divided into three different age groups (Child/Adolescent, Young Adult and Adult), 80.6%, 75.6%, 89.7% of the patients underwent R0 resection, respectively, and 62.5% of the child/adolescent group received adjuvant chemotherapy. The rate of metastatic patients at the time of diagnosis was 25.8% in the child/adolescent group, 26.7% in the young adult group, and 15.0% in the adult group($p < 0.001$)(26).

In a retrospective study of 40 years at St Jude hospital, Hill DA et al. found tumor progression or recurrence in 51 of 77 patients. The 10-year cumulative locoregional and distant recurrence incidence was 48.2% and 37.8%, respectively(2).

There are currently no specific pediatric CRC treatment algorithms, so adult protocols are used, but as prospective studies are inadequate, it is unclear whether this is the best option or how age affects individual treatment decisions. Given that tumor biology is probably different, we may get different responses to treatments(35).

Survival

Results in patients with colorectal cancer often appear to be worse in the pediatric group than in adults[2,26,32]. These poor results point to a more biologically aggressive disease in childhood and adolescence. Although all available data are based on retrospective investigations, the advanced stage of diagnosis, poor histological type and possibly a natural difference in the biology of the disease are thought to contribute to this(3,29).Knowing these factors can help explain the poor outcomes in pediatric CRC patients.

Poles, G. C et al.(32), in their study comparing the pediatric age group and adult age group, found no significant difference in survival in terms of stage 1 and stage 2 disease, but reported lower life expectancy in the

pediatric age group in stage 3 and 4 patients .

In the Kaplan et al study, the median follow-up was 33.6 months; median disease-free survival in adolescent, young adult and adult groups was 29.0, 29.9 and 61.6 months, respectively($p = 0.003$). Median overall survival was 32.6 months in adolescents, 57.8 months in young adults and was not evaluated in adults($p = 0.022$).Both disease-free and overall survival were shorter in the pediatric age group(29).

In the population-based study of Sultan, I et al(11)., 5-year and 10-year survival for children and adolescents was 40% + 4.2% and 31% + 4.4%, respectively; the 5-year and 10-year survival estimates for adults were 60% ± 0.10% and 54% ± 0.1%, respectively (p: 0.001).An important point in their study was that although there was no significant difference in survival (1973 to 2005) for children and adolescents, an improved outcome was observed in adults over time.

Adult CRC series have demonstrated that surgeons' experience and expertise are prognostic variables. The same situation may affect the survival of patients in the pediatric group(29,36).

Conclusion

In conclusion, CRC is rare in the pediatric age group and is often at an advanced stage when diagnosed. Although some pediatric patients with CRC have predisposing diseases, most childhood CRCs are sporadic. Colorectal cancer has different clinicopathological and prognostic characteristics among different age groups. Surgeons should be aware of these differences in childhood and manage patients according to these observed differences in order to achieve better results in the treatment process. Because colorectal cancer is rare in childhood, clinical management and treatment approaches are usually planned based on experience from the management of adult patients. Early diagnosis in pediatric patients is necessary to capture the disease at an earlier stage before progressing to advanced stages and is critical for extensive lymph node dissection and aggressive surgical resection, complete resection and appropriate staging. Complete surgical resection is the most important prognostic factor and therefore resection should be performed by an experienced surgical oncologist.A better understanding of disease biology requires further studies, a better understanding of the underlying molecular mechanisms of CRC will set targets for molecular therapeutic approaches to this disease. Finally, it is our responsibility to take measures that can have a positive impact on the outcome of the disease in the pediatric age group.

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Chapter 7

MULTIDISCIPLINARY APPROACH TO CHILDREN WITH DISABILITIES: THE ROLE OF THE PHYSICIAN AND NURSE

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Introduction

The problem of disability has existed in society for centuries. it is as old as human history. Disability is a part of humanity. Most people may experience temporary or permanent disability at some point in their lives and may experience increasing difficulties in their function (WHO 2011).

Although the efforts to prevent disability have been developed in parallel with technology, there has not been a complete solution. The number of people with disabilities sadly is increasing due to wars, terror incidents, natural disasters, traffic accidents, home accidents, drugs, food and chemical poisoning, and many other reasons (Bilge, Ekti Genç, & Nişli, 2005).

Disabilities are an umbrella term, covering impairments, activity limitations, and participation restrictions. Impairment is a problem in body function or structure; an activity limitation is a difficulty encountered by an individual in executing a task or an action; while a participation restriction is a problem experienced by an individual in involving in life situations.

Disability is thus not just a health problem. Disability is a part of humanity. Most people may experience temporary or permanent disability at some point in their lives and may experience increasing difficulties in their function. It is a complex phenomenon, reflecting the interaction between features of a person's body and features of the society in which he or she lives. (WHO 2011).

Most people may experience temporary or permanent disability at some point in their lives and may experience increasing difficulties in their function (WHO 2011).

In other words, handicap is defined as a social disorder resulting from inadequacy or disability that causes deviation from the individual performance or situation expected by society. It is possible to define disability as the social and environmental consequences of impairments (Minaire, 1992).

1. Handicap, impairment, disability

The concepts of Handicap, Impairment, Disability are used interchangeably and their definitions vary. However, these concepts have different meanings (Çağlar 2011). The World Health Organization (WHO) has developed several international classifications in order to prevent this confusion and to establish a standard, common language that provides worldwide communication in health and medical care across various disciplines and sciences (WHO 2011). First, in 1980, the WHO developed a classification system called the International Classification of Impairments, Disabilities, and Handicaps (ICIDH).

ICIDH handles disability in 3 categories:

Impairment: A mental, physical or functional disorder of the body. Inadequacy refers to the temporary, sustained loss or abnormality of the psychological, physiological or anatomical structures and functions in the tissues, organs, extremities, functional system or body mechanism.

Disability: A decrease or loss of functional capacity as a result of the disability. Inadequacy is the lack or excess of expected behavior and functions involving daily activities.

Handicap: It is defined as a social disorder caused by a disability that causes deviation from the individual performance or situation expected by society. Disability is the social and environmental consequences of impairments (Minaire 1992).

In order to change the social perception of disability and stigmatization caused by ICIDH disability classification, WHO developed the International Functioning, Disability and International Classification of Health system (WHO, 2011). Since 2011, the United Nations (UN) has been replacing the ICIDH classification system. List of types of disability identified according to ICF (2011) and recommended for use in research:

1. Seeing
2. Hearing
3. Learning and applying what they have learned
4. Mental functions
• Attention function
• Memory function
• Thinking function
• High cognitive functions
5. Meet the daily requirements
• Manage daily requirements
• Completing the daily requirements
6. Communicating
• Receiving
• Production
7. Changing and keeping the body posture
8. Holding, moving
• Lifting and transporting things
• Using fingers
• Using hands and arms

9. Walking and moving

• Walk

• Navigating around

10. Self-care

• Bathing

• Physical care

• Toilets

• Suit up

• Eating

• Drinking

11. Basic life activities

• Education

• Work

12. Community, social life

• Community life

• Entertainment and leisure

• Religious and spiritual life (Mbogoni and Mc 2002).

ICF is a universal classification that deals not only with people with disabilities but with all people.

Health-related conditions associated with all health conditions can be identified using ICF. The ICF consists of two parts, each containing two components:

Part 1: Functions and Disability

(a) Body Functions and Structures

(b) Events and Participation

Part 2: Contextual Factors

(c) Environmental Factors

(d) Personal Factors (WHO 2011).

ICIDH's (1980) 'disease outcomes' have been translated by ICF (2011) into the classification of 'health components'. "Health components" describe the contents of health, while "results" describe the effects of health status or disease (WHO 2011).

In our country, the use of idioms that expressing disability (sick, disabled, impaired, deaf) shows that disability is negatively understood and labeled in an individual specific medical problem.

Although the ICF classification and the UN Convention on the Rights of Persons with Disabilities emphasized that humiliation of persons with

disabilities is first mentioned in the language and that labeling should be prevented consciously.

Due to Disability Act adopted in 2005 and Turkey's legislation is still used in the concept of disability. For this reason, disability is divided into 6 groups as orthopedic disability, visual disability, language and speech disability, mental disability and chronic diseases (Çağlar 2011).

The disability in a child's life is complicated as that of an adult's life, requiring a versatile and professional approach. Physicians and nurses working in hospitals, community health services, and rehabilitation centers have important initiatives such as prevention of disability, early diagnosis, and care for family and child. Although physicians and especially nurses have separate roles and duties in each field of study, they have unchanged roles of caregiver, advocate, educator, researcher, manager, and leader (Çavuşoğlu 2008). For this reason, all health personnel, especially physicians and nurses, should know the definition of disability and the legal rights of children with disabilities and their families.

The main objectives are to mature the character of the child and to prevent disability to affect his personality structure and to lead him to a social, high standards and honorable life without being excluded from the society. Although disability in childhood has different meanings for people from different groups, it is generally defined as a concept that includes changing situations and medical needs. In this context, a disability includes not only physical limitations, loss of sensory organs, but also chronic conditions such as asthma, Type 1 diabetes, cerebral palsy, mental retardation, and learning difficulties. Early childhood is an important opportunity for all children to achieve lifelong learning and social participation. For this reason, it is obvious that providing disabled children with interventions and services that can help them to realize their maximum potential (Arslan, 2017)

2. The status of disabled children in the world and in our country

According to the Global Burden of Disease (2004), 15.3% of the world population is estimated to be "(moderately or severely disabled)", 2.9% is "severely disabled", and 0.7% to 5.1% of children aged 0-14 are disabled. (WHO 2011). Regarding the numerical data of disabled people in the world; According to Namibia National Disability (2003) data, 1.62% of the population is disabled and 53.8% of the individuals aged 5 and over have mental, learning, vision, hearing, and communication disabilities (Eide et al. 2003). According to the New Zealand Handicapped Survey (2006), 17% of the population and 10% of children under the age of 15 are disabled, 39% of children with disabilities have chronic diseases, 21%

have psychological or psychiatric disabilities (HTTP:/ govt.nz/browse_ for_stats/health/ disabilities.aspx), according to the Canadian Disability Survey (2006), 14.3% of the population, 3.7% of children under 14 It was reported that 54.1% of the children had communication, 5% had chronic disease, 6.3% had learning, 3.1% had emotional, 1% had hearing, 0.8% had vision and 0.8% had physical disabilities (http://www.hrsdc.gc.ca/eng/disabilityissues/reports/disability_profile/2011/disability_profile.pd). Turkey Disability Survey (2002), the proportion of the total population according to the results with a disability is 12.29%.

According to the research on the problems and expectations of the disabled, 25.1% of the children in the 0-6 age group are language and speech disabled, 9.6% are hearing impaired, 7.4% are mentally disabled, 3.7% are orthopedically disabled, 3.7% are multiply disabled, 3.6% had chronic illness, 2% had mental and emotional disability and 1.4% had visual impairment. Turkey Statistical Institute (2010). According to the results of this study, when the disability rates are examined according to the age group, the disability rate increases with age.

Knowing the problems experienced by disabled individuals and children is a key point in determining the right approach and meeting the needs. In the literature, mental problems experienced by disabled people; Disability dilemma, exaltation of normal behavior, blame, group stereotype behaviors, anger, denial. In addition to mental health problems, health problems that affect the quality of life of people with disabilities, as well as health problems, should not be ignored. The problems experienced by the families and caregivers who have undeniable importance in the life of the disabled child, their socio-economic status, lack of knowledge, inability to cope, and susceptibility to depression are among the problems that remain current. The holistic approach to disability requires the evaluation of all situations with bio-psycho-social, economic, cultural and environmental dimensions and providing rational, effective services in this context. Children with disabilities have to be hospitalized in cases of deterioration of health, and their response to recovery decreases due to their fear, anxiety and destructive emotions. At this point, health professionals' approach to gaining a sense of trust, showing love and cooperating with patients to support healing. The use of play therapy in children to reduce the stress caused by illness and hospital experiences, increase their adaptation to experiences and support their normal development in this process is an important initiative (Atay, Eras & Ertem, 2011) because it reduces the negative effects of hospitalization, and negative child's feelings and misunderstandings regarding treatment and procedures, in addition to the play therapy is used to develop positive coping methods in stressful events (Öztürk, Onan, Güngör, & Alsan, 2016). On the other hand, children with disabilities do

not have the same facilities as their normal friends. This causes them to be isolated from society. For example, the attitudes of children towards disability are greatly influenced by their families' attitudes. Many children with disabilities are mentally healthy and have a normal life as long as their circumstances allow. Others become a major problem for both themselves and the community as a result of being neglected by their families, both in the hospital and at home. Children with disabilities often know the burden of their presence on the family and the feelings of the family towards them. They hold themselves responsible for the unrest in the family. Health professionals have important responsibilities in improving all these processes. Support services are provided to children with disabilities within the scope of medical rehabilitation are carried out under the leadership of health professionals.

And these are;

1-Health, social assistance and rehabilitation services,

2- Physical therapy, hydrotherapy, sports rehabilitation,

3-Psychosocial service,

4- Small steps program (for children with mental retardation),

5-Visual rehabilitation,

6-Speech therapy,

7-Community awareness seminar,

8-Social activities (Bilge, Ekti Young, & Nişli, 2005).

3. Habilitation and rehabilitation

The concept of habilitation; new skills are defined as a learning process in order to uncover and use maximum potential. Both the concepts of rehabilitation and habilitation are defined as the process of helping individuals with disabilities for the future. Rehabilitation refers to re-learning a skill or adapting it to disability and age. In this context, rehabilitation involves attempts to restore life skills to people who have lost pre-existing skills for various reasons (accident, illness, etc.) (Bastable, 2008). When we look at the conceptual framework of rehabilitation, it is seen that disability is based on a medical model. Within the scope of this model, individuals with disabilities are tried to be treated in order to reach the "normal" and according to cultural norms and the process of learning to do it comes to the forefront (Oliver, 2012). Habilitation defined the process of learning new skills in order to uncover and use maximum potential (Anderson, 2011). The best example for this is children with cerebral palsy. These children are children with developmental delays from birth and whose life skills develop differ-

ently from other children. Therefore, it is not enough to use the principles of rehabilitation approach in the care of these children. Because the aim here is not to normalize, but to gain new skills effectively and to ensure the active participation of the family in this process (Bastable, 2008).

Table 1. The principles of habilitation nursing are as follows:

Nurse perspective	The perspective of families	Child's perspective
Evaluation of the child according to the determined criteria Supporting the development of the child Educating the family to gain new skills	Supporting the family by considering the child's views and thoughts	To support the development of the child by giving priority to his / her expression and wishes

Nurses support the development of the child in habilitation practices, communication, motor skills and independence in daily living activities, positive feedback, encouragement, and many arguments, supporting actual social events through examples, both individually and in group-oriented studies.

Also, Nurses support the motor skills of the child; by guiding him in his physical activities, assisting in the use of assistive tools. The habilitation nurse not only provides services for the disabled child but also advises the family about the needs. The aim is to listen to the child, strengthen the relationship between them and help the family to support the child. In this way, it is emphasized that the relationship established between the family and the nurse is important in obtaining positive results for the child (Arslan, 2017).

Another dimension contributed by health professionals includes guidance in the process of diagnosing disability in children. Supporting children with disabilities in the right physical activities has been shown to have positive effects on their quality of life, and in this context, the referral of the physician to the family during the initial medical diagnosis process is vital. Again, the awareness levels of all health care providers in relation to physical education and sports are also very important (Kemeç, Tekkurşun Demir, & Koç, 2018).

4. Health problems of disabled children in the world and in our country

It is known that health institutions are not adequately fit to receive health services for disabled people, difficulties in access to health institutions, inadequate care and rehabilitation units in health services, failure to maintain continuity of treatment, and problems in health services other than disabilities are delayed (Durduran 2009). Durduran (2009) found that 8.2% of the families had problems in receiving health services from the health center and 31.8% had some complaints about health services. In Canada, 14.8% of parents with disabled children do not have access to health care workers for their children, 77% of these parents stated that they have a long list of health care requirements as a result of not meeting these needs. It was found that health care workers who are mostly needed by families were speech therapists, child psychologists and pediatricians (http://www.hrsdc.gc.ca/eng/disability_issues/reports/disability_profile/2011/disability_profile.pdf). Children with disabilities have many health problems outside the disability.

It has been determined that disabled children and their families have many physical, social and emotional problems (Thyen et al. 2003, Aktaş 2010). Respiratory infections are frequently seen in children with physical disabilities such as muscular dystrophy (Çavuşoğlu 2008, Durduran 2009, http: // www.stats. Govt.nz/ browse_for_stats / health / disabilities.aspx), aspiration pneumonia, dental caries in disabled children caused by cerebral palsy (Çavuşoğlu 2008, Çokpekin et al. 2003), mental retardation (Çavuşoğlu 2008), chewing, swallowing and speech dysfunction (Çavuşoğlu 2008, Erdoğanoğlu, Kerem Günel 2007), recurrent generalized seizures and sensory disorders (eg hearing, vision) (Erdoğanoğlu, Kerem Günel 2007, Çavuşoğlu 2008, Durduran 2009) and psychiatric disorders (http: // www. stats. govt.nz/ browse_for_stats / health / disabilities. aspx) . It has been determined that families need home care because of these problems (Thyen et al. 2003, Aktaş 2010). In a study conducted with home visits and nursing care to reduce the needs of children with disabilities, nursing care given to disabled children and their families was found to be effective in reducing the needs of families (Bilsin 2012). There are different practices in the world about meeting the home care needs of children with disabilities. In the USA, home visit programs are generally provided free of charge to individuals with low incomes, low education or disabled people (Bilukha et al. 2005, Astuto and Ailen 2009). In countries such as Spain, Germany and Belgium, home care is provided to all families regardless of the child's health and social problems and income level (Astuto and Ailen 2009, Bilukha et al. 2005). In South Africa, the constitution and the Convention on the Rights of the Child protect the basic health and education

rights of children with disabilities, and since 2004, health care for adults and children with disabilities has been provided free of charge (Saloojee et al. 2006).

In our country, there is a "Regulation on the Provision of Home Care Services which entered into force in 2005. However, this service includes individuals who are decided to receive home health care services at the discharging stage by their doctor and the number of health workers is insufficient. In the home care service regulation, "according to the demands of the patient and the needs of the service, including day and night, weekend and holidays should be in a way to provide 24-hour uninterrupted service when necessary" (http://www.ailehekimligi.gov.tr). There is a need for home care services provided by a health care team with the knowledge and skills to care for children with disabilities, including the nurse. Nurses have important roles in caring for disabled children and their families. One of these roles is to determine the problems of individuals in need of home care and to ensure that the problems are handled appropriately, to determine their strength, to support the use of these powers and to provide continuity in care. With home visits, nursing care is provided based on the needs of the families, the interaction of the family members with each other is observed, the family is evaluated, the child's behavior problems are detected in the early period, families are informed about the development, health and care of the child by the educated health worker, the family is provided support and health. services are facilitated (Bilsin 2012). The legal arrangements concerning the health services of children are specified in the Children's Rights and UN Convention on the Rights of Persons with Disabilities. (http: // www. ozida. gov.tr/.../Bm_Individual rights agreement. PdÇŞİmanman 2011). Under the UN Convention on the Rights of Persons with Disabilities, States recognize the right of persons with disabilities to the enjoyment of the highest attainable standard of health without discrimination due to their disability. At the same time, States should take all appropriate measures to ensure that persons with disabilities have access to health care, including health-related rehabilitation. In this regard, States should provide, strengthen and expand existing services, including the right to comprehensive education and care and rehabilitation, particularly in the areas of health, employment, education and social services. (Http://www.ozida.gov.tr/.../bm_engellıhaklarısozlesmesı.pdf).

In our country, the early diagnosis and prevention of diseases that may cause disability in article 11 of the Disability Law, which is titled as early diagnosis and preventive services, and to combat other inherited diseases within the scope of preventive health services leading to disability in Article 36 of the Law on Combating Hereditary Diseases Article 6 states that it is essential to provide home care services in relation to care services, and

Article 30 states that disabled people in need of care may receive care fees. (http: // www. legislation.justice.gov.tr/html/151 /.html^Fat 2011).

5. Needs of parents with disabled children

Having disabled children leads to major changes in parents' lives, parent roles, social environments, future plans, professional lives, family structure and functioning, and economic issues. These changes cause families to face many problems in terms of psychological, familial, social relations and financial aspects and cause intense stress in families. In some studies, it was found that mothers with children with disabilities had more family problems and more depressive symptoms (Mıdık Özpak, et al., 2017). The needs of families; information and education needs, emotional support needs, economic needs, care needs, socialization needs and family functions. In particular, areas where health professionals such as nurses and doctors should intervene;

1-Information and Training Needs:

Children with disabilities have special needs unlike other children, each child is special, and different. Knowing the characteristics of these children and being aware of their needs increases the quality of care as well as positively affects the development of the child. It has positive effects on the psychological status of the family directly and indirectly. Again, coping with difficult times, problems and fears of not knowing how to care can cause multiple traumas in the family. At this point, families should be supported in terms of education and information (Saçan & Gürkan, 2016).

2-Emotional Support Need:

The traumas caused by having disabled children and the bad conditions in them lead to the need for emotional support in many families.

3-Care Need:

The daily care of these children may take more time by parents than families with normal children. Family, relatives, caregivers and so on. If there is no support mechanism, the family may experience psychological and physical problems (Saçan & Gürkan, 2016).

4-The Need for Socialization:

Having disabled children is considered as the reason for getting away from society for some families. Attempts to isolate himself. the child is in need for socialization so this will damage the coping mechanism. In this sense, it is necessary to raise awareness and gain the fact that every individual is valuable (Saçan & Gürkan, 2016).

5-Needs for Family Functions:

These children may need to restructure their roles in the family for healthy upbringing and development. The responsibility of family members varies according to the communication relations, status and expected roles of the members. In line with expectations, parents may have difficulty in performing their roles. If necessary, the family should be supported with psychological support and behavioral therapies. (Scatter & Gürkan, 2016). The positive attitudes of the health care team also play an active role in providing quality care in the treatment process (Kemeç, Tekkurşun Demir, & Koç, 2018). It is also the responsibility of the team to correctly identify the needs of the family, to plan initiatives for the needs, and to provide the necessary guidance and consultations to provide care in cooperation with other disciplines.

Conclusion

Identifying the needs and problems of children with disabilities and their caregivers is the main step leading to a solution. It is also the responsibility of experts in many areas to address these needs and problems. In this context, nurses and physicians are also at a strategic point. Because they have a very effective role in the diagnosis and initiation of the treatment process. They have a significant impact not only on medical interventions but also on psychosocial determinants. It is known that children with disabilities have many health problems outside their disability. Taking into account the regional differences in the world, awareness raising activities should be carried out in cooperation with the prevention of disability, and children with disabilities should be given the same rights as non-disabled people in order to eliminate the problems experienced in accessing health services. There is something for all of us in initiatives to promote the awareness that children with disabilities have equal rights with everyone, to protect them, to allow them to be intertwined with society, to improve their quality of life, to contribute to a happy, healthy, peaceful, hopeful psychology and to support their independence.

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Chapter 8

SIMULATION IN NURSING EDUCATION

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Changes in the production, transfer and use of information with changing and developing technology have led to rapid developments in the education system, leading to the emergence of new approaches (Karadağ & Uçan 2006; Genç & Eryaman 2007). With the integration of developing technology into education, the creation of innovative educational environments has accelerated. Training programs covering cognitive, affective and psychomotor fields, which contain innovative practices, should be prepared for the training of qualified nurses in the nursing profession, which has a complex structure (Karaöz, 2003; Göriş, Bilgi ve Bayındır 2014; Korhan, Tokem, Yılmaz ve Dilemek 2016).

According to the World Health Organization (WHO), the purpose of nursing education; To raise individuals who use an evidence-based approach in their practice, have clinical decision making, critical thinking and problem-solving skills, can use and manage their resources safely and effectively, cooperate with other health disciplines, and have leadership and continuous professional development (World Health Organization (WHO), 2009).

During clinical practices, which are an indispensable part of nursing education; Insufficiency of application areas, students not feeling ready, fear of harming the patient and making mistakes increase students' anxiety levels. The fear and anxiety experienced by students when they encounter real patients prevent students from reflecting their knowledge and skills to real patient care. (McNett, 2012; Ross, 2012; Öztürk & Dinç 2014). Also, the increase in the number of students per patient in clinical practice areas in recent years leads to a decrease in student exposure times and the frequency of seeing and practising skills. For this reason, the use of skill laboratories is critical for nursing students to improve their clinical skills (Houghton, Casey, Shaw and Murhpy, 2012).

Within the scope of nursing education, realistic environments are prepared in clinical skill laboratories, and it is aimed to train qualified nurses with quality education (Houghton, Casey, Shaw and Murhpy 2012). While the laboratory environment provides competence in skills using learning through application and learning gains with feedback, it allows experiencing rare clinical situations and repeating skills. Considering the complex hospital environments, students who are educated in simulated environments will not have difficulty transferring the theoretically learned knowledge to the clinical situation when they work (Cant & Cooper 2010). Otherwise, learning at the desired level is prevented, students are not able to show their performance adequately, and they have problems transferring their knowledge and skills to the real clinical environments in which they interact with the patient (Sarmasoğlu, Dinç & Elçin, 2016). For this reason, the use of new teaching methods is emphasized within the scope of cur-

rent teaching methods and opportunities provided by developing technology in skill development laboratories (Akbaş, 2010; Cant & Cooper 2010; Houghton, Casey, Shaw and Murhpy 2012).

One of the most effective education methods in gaining cognitive and psychomotor behaviours to students is interactive methods that enable the student to participate in the learning process actively. The simulation that takes place in these methods is useful in developing students' cognitive, psychomotor and attitudinal knowledge and skills by providing real-life situations and a realistic learning environment they experience (Cant & Cooper 2010; Kapucu & Bulut 2011). In recent years, simulation has gained importance in nursing education due to the increase in sensitivity in patient safety, despite the increasing number of students, the inadequate number of educators, and the ability to re-apply skills without quickly reaching and harming (Edeer & Sarıkaya 2015).

The World Health Organization also published the gold standards in professional nursing education and recommended that nursing schools use simulation, which is an innovative teaching method. (WHO, 2009). The American Association of Colleges of Nursing = AACN and the United States (USA) National Council of State Boards of Nursing = NCSBN report that it is useful to use simulation and online learning methods in nursing education (AACN 2005; NCSBN, 2005). ABD Ulusal Hemşirelik Birliği de (National League for Nursing=NLN) bildirdiği hemşire eğiticilerin temel yeterliliklerinde, öğrencilerin öğrenmesini kolaylaştırmak ve öğrenme sürecini desteklemek için bilişim teknolojilerinin (bilgisayar programları, simülasyonlar, web v.b.) kullanılmasını önermektedir (NLN, 2005).

SIMULATION TYPES

In the health education system, many different types of simulations bring knowledge and skills with the integration of technology into education, which increases the similarity from real to simple situations. When the simulation types are examined; A wide range of educational materials have been developed with the effect of rapidly changing and evolving technology (Cant & Cooper 2010; Edeer & Sarıkaya 2015).

Models and Mannequins with Low Technological Properties

These are static models consisting of models and mannequins with low technological features designed to teach necessary psychomotor skills and procedures. In models representing certain anatomical parts of the human body, the real rate is flat compared to the others, and there is no vitality. It is a simulation method that has a lower cost than others. (Maran & Glavin 2003; Bradley, 2006; Nestel & Bearman 2014; Seropian, Brown, Gavilanes and Driggers 2004).

Necessary skills such as vascular access, bladder catheterization in nursing education are provided with models and mannequins with low-level features. Houghton and their friends reported that their level of education would increase when students overcome their first experience anxiety. Therefore, in clinical laboratories, the importance of students getting over the shock of reality at the first encounter with the hospital environment has been reported (Houghton, Casey, Shaw and Murhpy, 2012). Standard mannequins are sufficient to support students in this regard.

Computer-Aided Simulation

Computer-aided simulations (screen-based computer simulators) are designed to model human physiology, specific tasks or environments with various aspects through various computer software. These simulations allow the student to make clinical decisions about the situation and to observe the results of the actions. Computer-aided education programs and web-based programs can be counted in this group. This low-cost method has the feature of being used individually or in groups. These types of simulations provide the opportunity for the student to access, reuse or repeat as much as she/ he wants, by removing the distance between the student's learning environment (Hughes, 2008; Bradley, 2006; Zıv, Small and Wolpe 2000; Maran and Glavin 2003; Ziv, Wolpe, Small and Glick 2006; Decker, Sportsman, Puetz, & Billings, 2008; Gaba, 2007; Byrne, Heavey & Byrne P. J.; 2010). It is preferred that computer-aided simulation is easy to use, it also creates less stress for the student and the educator, and provides a learning experience without harm (Seropian, Brown, Gavilanes and Driggers, 2004). For these reasons, the use of computer-aided simulations has become widespread (Zıv, Small and Wolpe, 2000). However, it is not used much in skill training with intensive psychomotor steps. It is used in web-based, theoretical and animated pieces of practice that are supported by video display of real situations. Öztürk and Dinç reported that web-based education had positive effects on the urinary catheterization skills of nursing students, and its positive impact increased for both knowledge and expertise when it supplements classroom instruction. Based on these results, they suggest the use of web-based education as a supplement to traditional classroom instruction for nursing education (Öztürk & Dinç, 2014).

Standard Patient

Standard patients are individuals who have been trained on this subject, with their voluntary participation in practices and portraying the patient by the steps planned to be taught. In nursing and medical education, the standard patient is frequently used in the development of students' communication skills, history taking and physical examination skills. Standard patients; they exhibit predictable behaviour, are always available by the

needs of training programs and can be trained to simulate disease findings and all complications by the chosen scenario (Issenberg et al. 1999). The most important advantage of education integrated with the standard patient method is that it offers students the opportunity to work with people who act as real patients in a laboratory environment similar to the actual clinical setting (Turan, Öner and Elçin 2010). The most crucial benefit of standard patients compared to real patients is that they present the same problem consistently for all students by the specified place and time (Barrows, 1993). Andrea & Kotowski reported that using standard patient in an undergraduate health assessment class can enhance students' ability to communicate and interact with patients in gathering a health history, thereby strengthening patient care. (Andrea & Kotowski P, 2017).

Simulations Used in Learning Complex Functions

Simulations used in learning complex functions (complex task trainers); are electronic systems that simulate the mechanical effect and physiological response, which is the response to touch (haptic), taken by the sensors in the simulation in the virtual reality environment. This method is especially; It provides an active learning environment to the student in cases where the student is able to identify the patient and the trainer cannot observe clearly. For example, when the student does a pelvic exam, it is difficult to determine if this is done correctly by the instructor. This difficulty was overcome by the sensor placed inside the pelvic model in touch system technology. The tactile pressure created by the student during the pelvic examination is taken with the sensor and provides feedback about this area. As physical interaction occurs in a virtual environment, coach simulations are combined with models or models with low technological features in learning complex functions (Hughes, 2008; Zıv, Small and Wolpe, 2000; Seropian, Brown, Gavilanes and Driggers, 2004; Ziv, Wolpe, Small and Glick 2006; Kneebone, Scott, Darzi, & Horrocks, 2004).

Integrated (Integrated) Simulation

These simulators are a combination of a mannequin or part of the body with computer technology and thus provide students with a more realistic learning experience by incorporating advanced technologies such as pulse rate, blood pressure, respiratory movements, and transferring physiological signs and symptoms to the monitor (Maran and Glavin, 2003; Bradley 2006). These integrated simulators are high-fidelity simulators. High-fidelity patient simulators are one of the latest technologies and one of the simulation methods used in nursing education. These high-fidelity mannequins can give physiological responses such as breathing, pulse, heart sounds, pupil movements. Further models can communicate with students (Zıv, Small and Wolpe 2000, Maran and Glavin 2003; Bradley 2006).

High-fidelity simulators, new technology in nursing skill labs, have been used to expand course materials, focus on active learning and evaluate students' skill performance (Hodge, Martin, Tavernier, Perea-Ryan and Alcala-Van Houten 2008, Swanson et al. 2011). Swanson et al. effects being assessed of three teaching strategies on the outcomes of performance and retention performance of intervention activities, student satisfaction and self-confidence, and educational practice preferences in their study. They reported that students' scores were significantly higher in retention performance than in first performance. There was a significant interaction effect for time and teaching strategy. Nursing education needs to focus on the use of high-fidelity simulation as a teaching strategy (Swanson et al. 2011).

THE BENEFITS OF SIMULATION BASED TRAINING

Simulation, which is one of the active learning methods, which is becoming more widespread today, providing a link between theory and clinical practice, development of psychomotor skills, development of decision-making and critical thinking skills, has many benefits for educators, students and patients.

These benefits provided by simulation-based training are given below:

Benefits of Simulation-Based Education to Students

• It offers a student-centred approach throughout the education process (Jeffries, 2005).

• It allows students to try their skill applications over and over again.

• It will enable students to evaluate both their peers and themselves (Weller, 2004).

• Allows students to experience an equal number of patients.

• It offers the opportunity to learn in a supportive, reliable and lifelike environment.

• It helps students gain experience by learning from their mistakes (Ricketts, 2011).

• Motivates students by focusing on their performances and encourages them to learn.

• It increases students' self-confidence, reduces stress, improves decision making, teamwork, leadership and communication skills.

• Improves students' cognitive and psychomotor skills (Ballard et al. 2012; Ross, 2012).

• It provides a link between theoretical knowledge and clinical skill applications.

• It helps students feel like a nurse during applications, thus motivating them to practice skills (Berragan, 2011).

• It provides an opportunity for all students to experience one-on-one with exceptional cases that are difficult to encounter.

• It offers students the opportunity to make critical decisions about the application, evaluation, reapplication, problem-solving, and findings (Göriş et al. 2014).

• It ensures that every student receives equal and standard education regarding the same clinical situation and skill. This is important for equal opportunities in education.

Benefits of Simulation-Based Education to Patients

• Prevents students from being harmed during the student's treatment and care processes.

• It contributes to the provision of higher quality care in health care (Şendir & Doğan, 2015).

• Reduces practices that can harm patients' rights.

• Prevents patients from being exposed to repeated attempts by students (Waldner and Olson, 2007).

• It is patient-centred as well as student-centred, it helps to minimize ethical concerns as it reduces the skill practices to be applied to the real patient (Maran and Glavin, 2003).

Benefits of Simulation-Based Education to Educators

• Provides an interactive learning environment and increases the pleasure of education (Jeffries, 2005).

• It provides skill practices in a safe, risk-free learning environment.

• The degree of difficulty of the learning environment can be increased or decreased gradually in line with the educational objectives.

• It allows students to evaluate their performance in a standard and objective manner (Weller, 2004; Şendir, 2013).

• It enables the trainer to use his time more effectively and efficiently.

• Educators will have the opportunity to be informed about the latest developments and implement the latest developments by actively participating in students' practices and evaluations (Ziv, Wolpe, Small and Glick, 2003).

Effects of Simulation-Based Education on Training Program

• It enables practical applications on the model outside the clinic.

• It can also be observed whether the student can achieve the expected performance for operations performed with a particular procedure.

• The application can be stopped at any time by the trainer for explanation or correction.

• It is possible for the trainers to participate and evaluate actively.

• Training time is shortened.

• It can be used for testing or certification purposes.

• In the training on a model, the standardization of the training is provided by using the same training guides for each student (Author, 2003).

Effects of Simulation-Based Education on Institution

• It provides prestige to the institution.

• It creates respect for patient autonomy.

• It increases the quality of the institution.

• Lack of skills helps to reduce malparktis cases (Author, 2003; Sunal, 2013).

LIMITATIONS OF SIMULATION BASED TRAINING

Besides the benefits of simulation education, it also has some limitations.

• Expensive equipment is required to use the simulation technique. School and clinical education budgets are limited to get this equipment (Jeffries, Bambini, Hensel, Moorman and Washburn, 2009).

• The student may experience anxiety while using the simulation technique he has just met, and this may affect the learning process of the student negatively (Rhodes and Curran, 2005).

• Educators must have received intensive training to apply the simulation to students effectively.

• No matter how realistic the simulation offers, it is not a substitute for learning in the clinical setting, but it supports clinical education (Pamela, 2010).

• The materials used in the simulation method should be maintained and checked regularly.

• For simulation to be used as an effective teaching technique, scenarios that reflect reality best must be created. Creating these scenarios creates a lot of workload for the trainer and may create a problem in terms of time (Rauen, 2004).

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Chapter 9

Mass Spectrometry in Clinical Diagnosis: Cancer Biomarkers

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Mass spectrometry (MS) appeared for the first time in 1913, when J.J. Thomson studied the movement of ion clouds in the electrical field. To date, it has made many improvements, but Thomson's work has always been fundamental. Mass Spectrometer has taken its present form with the studies of Alfred O.C. Nier, A. J. Dempster, Kenneth Bainbridge, Josef Mattauch and Walter Bleakney (Griffiths, 2008). Today, mass spectrometry is widely used in recognition, diagnosis and quantification studies. The development of ionization sources such as electrospray ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI) and combining it with liquid chromatography in the Mass Spectrometer have expanded its usage area. Fast, accurate and ultrahigh sensitivity at femtomolar level has found use in analytical quantification and structural analysis as well as clinical diagnosis. MS contributes to studies for correct and fast medical diagnostic tests, to reach the conclusion in a short time, to make clinical treatment decisions and to protect human health.

In clinical diagnosis, mass spectrometry is used in the discovery, development and validation of biomarkers.

Routine tests with spectroscopic and immunological methods in medical laboratories have an important place in establishing the clinical diagnosis and establishing the treatment protocol. Mass spectrometry gives results with better analytical specificity and precision than these methods used in clinical laboratories. Mass Spectrometry is used in clinical laboratories for toxicology tests, therapeutic drug monitoring (TDM) and newborn and drug screening tests. Mass spectrometry started the golden age with its use in biomarker discovery and detection in the clinic. Biomarkers are biological substances, disease-specific molecules in tissue or body fluids and are used to determine the diagnosis and treatment protocol.

Clinical necessity of biomarkers

Evaluation and interpretation of endocrine functions, cardiac markers and cancer markers play an important role in making clinical treatment and diagnosis decisions (Dolci A. et.al. 2006). Therefore, accurate and specific detection of biomarkers has become an essential requirement in the diagnosis and management of the treatment. In addition to the correct detection of biomarkers, the lowest detectability limit is also important in "cut-off". When screening, recognition, detection and screening tests are successful in the potential applications of biomarkers in the clinic, clinical measures can be taken by diagnosing the disease in the early phase. For example: With malignant tissue, metastasis is prevented by early diagnosis of cancerous cells.

In the light of this information, we can describe Cancer biomarker as a biological molecule that indicates the presence of cancer. These mole-

cules are markers found in tissue and body fluids that characterize cancer, are released from cancer cells or appear as the body's response. They are the molecules that will lead to the determination of the type of cancer in diagnosis, prognosis, the condition of the cancer patient without treatment or treatment, and which treatment is a good response to the patient as preventive (Davis J. 2017). Bence Jones Protein is used as the first cancer biomarker found for the diagnosis of multiple myeloma (Jones B. 1847).

Biomarker Definition

The definition of biomarker is considered as characteristic of the pharmacological responses of pathogenic processes or treatment methods, which are objectively measured, evaluated as indicative of the normal biological process (Atkinson A.J. 2001). As can be seen from its definition, biomarker studies require that many disciplines such as biology, pharmacology, pathology, medicine and analytical chemistry work together. Therefore, it is thought that the classification of biomarkers will facilitate understanding without getting lost in this wide area (Naylor S. 2003). The United States National Institude of Health (NIH) classified biomarkers into 3 types according to their clinical use and validation (Frank R, et al. 2003,).

Type 0: Biomarkers are biomarkers that occur by measuring the clinical history of the disease and the clinical history of the disease.

Type I: Markers resulting from an intervention (For example: after drug intake)

Type II: Biomarkers considered as surrogate determining endpoint

Type I biomarkers attract attention due to their potential pharmaceutical properties by pharmaceutical companies.

Classification of Biomarkers Biomarkers are divided into protein and metabolic (Crutchfield A.C. et. Al. 2016). Protein biomarkers make up a very important part of the Biomarker group. While some can be detected by routine analysis methods, some require precise and selective mass spectrometry. For example: Albumin as a nutritional biomarker, alanine aminotransferase (ALT) is used as a marker of liver function, and routine analysis can be easily detected.

Those who have undergone post translational modification of protein biomarkers can be examined as a separate group. Proteins are modified by reactions such as glycolysation, methylation, their analysis can be done by Mass spectrometry.

Metabolic biomarkers are biomarkers found on the effect of the disease on molecules in human metabolism.

Biomarker Discovery

The rate of recovery and survival for many diseases is highly dependent on early diagnosis and effective treatment. Early diagnosis enables clinical decision making and rapid treatment. Biological markers, namely chemical components that can be determined, are substances that change in response to the body's disease and are released from tissues, body fluids and organs. The purpose of the biomarker discovery is biological molecules that are illuminated specific to that disease in the early stages of diseases. Mass spectrometry is one of the most important devices for this. Targeted and untargeted biomarker analyzes are performed by mass spectrometry. In the discovery of mass spectrometric biomarkers, untargeted studies are conducted by comparing the samples of patients and healthy individuals. It is the search for a known protein or peptide in targeted studies.

Ideal Biomarker Properties

A biomarker must have some properties, the properties are listed below (Cooner WH. 1993, Kamel M. et al. 2016). If it meets these criteria at a high rate, its use as a biomarker will be effective in diagnos
• Must be produced in each patient for a specific type of cancer (clinical diagnostic limit)

• It should be 100 % accurate when it is not specific to the disease (clinical specificity, low false positive)

• Must be tissue or organ specific

• Amount should be proportional in the body according to tumor volume or disease course

• Small changes in tumor and disease course should be easily monitored

• The proportion of healthy volunteers and sick individuals should be different (low levels in healthy individuals)

• Must state metastasis precisely

• Quantification should be standardized, reproducible validated methods

• Should be low cost diagnostic method

• Biological material should be obtained by easy non-invasive methods (plasma and serum).

Sample Collection and Quality

The quality of the blood, plasma and tissue samples collected in the Biomerker analysis determines the quality of the biomarkers produced and

analyzed. Storage conditions of the samples are very important. Biomarker analysis and discovery are made with the data obtained from the analysis of these samples. In sample collection processes, sample collection time, the tube used and the coagulation agent inside, the injected needle from the sample, the storage of the samples taken and the digestion are important

and important parameters (Lista S. et. Al. 2013). In the analysis, the pool is analyzed by creating a pool for biomarkers that are below the detection limit and cannot be seen. However, in individual analyzes, there are data from personal characteristics. For this reason, it would be more meaningful to hide the samples one by one and create a pool from certain parts of the work. Analysis of all samples by creating a pool is not recommended. Proteins, which constitute the most common group among biomarkers, are analyzed by comparing them in patients and healthy individuals (Parkera C. Et. Al. 2014).

The purpose of finding a protein marker is to describe, in the simplest terms, a protein or protein panel that distinguishes patients affected by a particular disease from healthy individuals. Although this definition seems simple, it is very important. Biological fluids such as urea, serum, etc. can also be collected and analyzed routinely, meeting the criteria of the marker. Unfortunately, these biological fluids are extremely difficult to characterize even by the most advanced MS technologies. For example, physiological and analytical difficulties in discovering a tumor-specific protein biomarker in serum are very clear. Serum is a simpler biological sample than plasma, but it is not preferred for use in biomarker discovery. Since the coagulation process is different, it will not change the serum content (Randall S.A. et. al.,2012). It releases aberrant protein from tumor cells to the circulatory system. The blood collected from the venous vein and the serum or plasma sample is prepared from the 7.5 L circulatory system consisting of approximately 100 000 km veins, arteries and capillaries. The local concentration of the biomarker in the microenvironment of the tumor may be high, but there may be differences when it reaches the blood collection area by traveling thousands of kilometers along the vein. During this long journey, it can create different analytical effects, known or unknown, with the most visible reduction in concentration. As the biomarker has a very high concentration around the tumor, there may be large decreases with dilution as it travels through the circulatory system. In cases where the activity of PROTEAS in the blood is high, the digestion of biomarkers into different Fragments before collection can be seen. For this reason, biomarkers may exhibit different features at the points they enter into my circulatory system and collection points (Conrads T.P. et.al. 2006).

The complexity of protein sequences of biomarkers in serum and plas-

ma is the most important challenge. Although the number of proteins is not known exactly, it is estimated that there will be 10^5-10^6 in different types of blood proteome. In addition, the blood proteome is in the form of a permanent flux with proteins released from necrotic and apoptotic cells and materials exchanged from healthy cells (Veenstra TD. et.al. 2005).

Sample Preparation

Cancer is among the deadly diseases that come after heart diseases all over the world. Illumination of potential biomarkers with different techniques is important for the diagnosis and prognosis and treatment of the disease. The most important stage that finds wide area in cancer biomarker researches is seen as sample preparation stage. In the sample preparation phase, meaningful data is obtained in MS by making complex structures easier to process by reducing them. Separation of proteins with different separation techniques in biological samples will provide different and processable data (Albulescu R.et.al.2019). Proteins such as albumin and immunoglobulins, which are abundant in body fluids such as plasma and serum, make it difficult to analyze the proteins with biomarker potential and in small amounts. For this reason, it is important to eliminate or reduce the amount of protein structures that are abundant compared to the biological material studied. Electrophoretic separation has been used as the most powerful technique to perform this separation and elimination. For this, while 1D-SDS gel electrophoresis was used for the first time, now more effective 2D-PAGE electrophoresis methods are widely used. 2D gel electrophoresis, isoelectric focusing and subsequent techniques with MS have enabled effective separation. A more effective method developed in recent years for separation of protein type biomarkers is the analysis of proteins with low detection by sample-control analysis and control and sample mixture analysis simultaneously with the Two-dimensional Flourescence Difference gel electrophoresis (2D-DIGE) method. The technique created by combining the capillary electrophoresis system with MS is also an effective method for separating proteins.

Analysis Techniques

There is a need for good diagnosis and separation methods in biomarker analysis in complex body fluids such as urine, plasma and blood. For this, identification and characterization of proteins can be made by mass spectrometry following different separation techniques (Veenstra TD. et. al. 2005). Two-dimensional Polyacrylamide gel electrophoresis (2D-PAGE) and liquid chromatography (LC) are the most important separation techniques used. 150-235 different and single proteins can be found

from the urine and serum from 1400-3700 proteins by 2D-PAGE method and Mass spectrometry respectively (Pieper R. Et.al. 2004, 2003). While liquid chromatography-mass spectrometry (LC-MS) can be found in 1500-2000 protein serum and plasma, 100-250 protein can be detected in urine (Shen Y. Et.al. 2004, Spahr CS. Et.a.2001). Biomarker searches can be made by analyzing protein from tissues (Palmer T. et.al. 2005). Cell level searches can also be made (Crockett DK. et.al. 2005).

As a result of MS analysis, extensive information about identification, localization, interaction, abundance and post translational modification of protein based biomarkers is obtained (Kall L. Et. All. 2011). MS is very effective in protein characterization. Post-PTM analyzes can be done effectively even if the samples become complex. Nano or capillary C18 opposite phase columns are used for separation in chromatographic systems according to the complex structures of proteins. Ionization techniques are also important in the analysis of proteins in mass spectrometry. Electrospray ionization (ESI) technique and Matrix asissted Laser Desorption (MALDI) techniques are used as effective ionization techniques (Castellino S. et. al. 2011).

It is an effective technique in the detection and analysis of biomarkers with the detection of the differences in patients and normal individuals by examining the spectra formed by ESI technique. ESI technique is a modern ionization technique that provides great advantage. In this ionization technique, it is a technique that allows large molecule-weighted, non-volatile molecules to ionize without re-denaturing by preserving their covalent bonds and receptor ligand bonds (Wilm M. 2011). In the atmospheric pressure technique, which is one of the soft ionization techniques, there is little or no fragmentation in the molecule. It is a technique that creates droplets and ionization by applying 2-5 kV potential at low flow rate (10-100 µm) with 1-2% electrolyte (acetic acid, ammonium format, formic acid etc.) added to a polar solvent in ESI technique. The droplets formed (less than 10 µm in diameter) contain solvent and analyte molecules. By evaporation of the solvent with heated inert gas, ionization is observed in the analyte (Liuni P. 2011). MALDI is another technique used other than ESI in cancer biomarker discovery and analysis. The MALDI analyte molecule is recrystallized with matrix material and analyzed. Here, the matrix molecule is small organic molecules (sinapinic acid, dihydroxy benzoic acid, etc.) that facilitate ionization, and they can be added up to 100-100,000 times as much. The laser beam is sent to the recrystallized sample to form an ion in the matrix. Generally, identification of biomolecules with minimum ionization in large molecules can be made easily. UV N2 laser is used in MALDI devices. According to ESI in MALDI, single charged molecules are seen in ionization. This facilitates data evaluation (Figure 1).

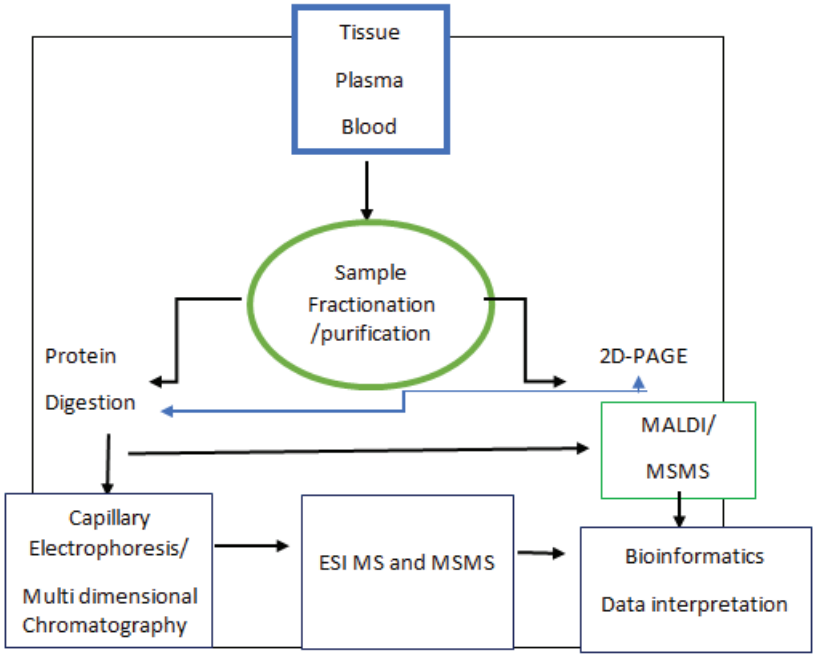

Figure 1: Basic analytical methods in biomarker discovery and analysis

Plasma biomarkers are preferred for early diagnosis in the screening of the general population since they are obtained by easier methods. In contrast, tissue proteins can be obtained using the invasive method and are not preferred due to the difficulties in their analysis. There are analytical difficulties due to high abundant proteins in biomarker analysis from plasma or tissues. Nearly 20 abundant proteins make up 99% of the plasma. (Anderson NL, 2002). For this reason, it is very difficult to analyze in addition to proteins with low amount of rare proteins that are released into the systemic circulation that are released or excreted by tumor cells. The most known albumin in plasma is 50 mg / mL. The amount of proteins to be used as cancer biomarkers is 10 million times less than this amount, at the nanogram level. Detection of such a small amount of molecules with MS is almost impossible. Therefore, sample preparation and enrichment step is important before mass spectrometric analysis. Since the biomarkers of protein structure are high in tumor tissues and proximal fluids (urine, saliva, tumor secretion fluids, etc.), they are easy to detect by mass spectrometry.

There are two ways in protein biomarker studies.

1. Investigation of whether candidate protein is present in plasma

2. To investigate whether the plasma level of candidate protein increases or not by comparing it with healthy individuals.

In protein analysis, a known peptide or protein is analyzed in search of targeted biomarkers. In this case, the m / z ratio of the peptide structure of the protein sought is given for each peptide (after trypsin digestion), and the MS device can only scan these molecules (Multiple reaction Monitoring: MRM, Selected Ion Monitoring: SIM, Selected Reaction Monitoring: SRM). analysis is done. In this way, we can find low protein and peptides in targeted MS analyzes by Mass Spectrometry (Keshishian H. Et.al. 2007). Another way is to pool the plasmas from more than one patient to analyze low protein structures and reach concentrations at the detection limits.

MRM is a technique performed in triple quadropole mass spectrometers. In this technique, precursor ion is selected first, which is the molecular weight of the protein or peptide of interest, or the molecular weight of a large part of it. This selected m / z ion takes place in the first quadropol. Then, in the 2nd Quadropol, these selected ion fragments are separated by a heated inert gas and this ion is called product ion. 3. This ion is used in the quadrup for selective recognition and quantification. These fragmentation and election events occur within milliseconds (Boja ES et.al. 2012, Makavita S., Diamantis EP 2010). In these studies, parameters such as collision energy and mass related temperature, gas flow should be determined for each ion and applied in a fixed form (Boja ES.et.al. 2011). It is an effective way to perform verification of proteins analyzed in MRM mode by analysis of isotope-labeled C13 / N15 protein and peptides. (Tan HT, et. Al. 2012, Picotti P. Et. Al. 2010). According to ELISA method, cheaper analyzes are carried out in MRM Tandem MS analyzes since antibody is not required.

The most important problem in the analysis of protein and peptides in MS systems is ionization problem. If ionization does not occur, proteins cannot be detected. Ion sources are developed and solutions are sought for this problem. ESI and MALDI are solutions to this problem up to a point.

Biomarker development consists of many stages. These stages are grouped as 1. Discovery, 2. Qualification, 3. Verification, 4. Method optimization, 5. Validation, 6. Preparation and clinical evaluation respectively (Smith RD 2012).

In biomarker analysis, proteins are digested into peptide parts by digesting with trypsin to divide large molecules into small pieces in order to facilitate analysis by mass spectrometry. In this way, they can be detected more effectively with MS and their structures can be illuminated more easily. It is appropriate to start the analysis by taking a larger sample in the analysis of low amounts of proteins. In chromatographic separation, 30-100 fractions can be obtained with a separation, such as approximately one hour. Their

analysis can take days or even weeks. This is a challenging step in biomarker discovery and analysis. When working in MRM and SRM modes in mass spectrometry, the use of hybrid shapes "quadropole-orbitrap" or "quadropole-TOF-MS" has been developed and targeted systems have been created (Eliuk S, et. Al. 2015, Makarov A. et.al. 2006). Tandem MS systems will effectively display multiple peptide fragments with discrimination and accuracy. Biomarker analysis with mass spectrometric methods starts from the patient selection, and the diagnosis is made by evaluating the data after sample collection, preparation and analysis (Figure 2).

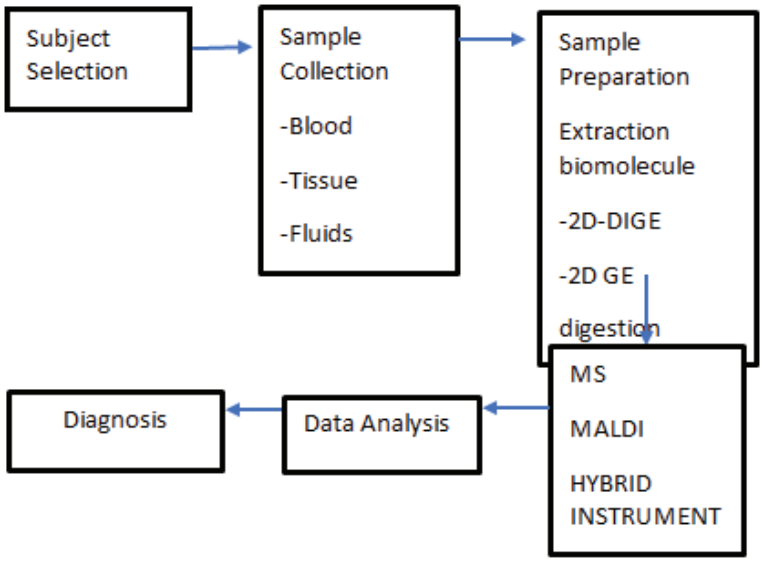

Figure 2: Schematic overview of the workflow

There are approximately 23 biomarkers approved by the FDA in 2014. Some biomarkers used for diagnosis, treatment and monitoring in the clinic are shown in Table I. (Rifai N. Et. Al. 2006).

Table I: Cancer Biomarkers uses in Clinical practice

Cancer Biomarker	**Cancer type**	**Application**
Prostate spesific antigen (PSA)	Prostate	Screening, diagnosis
Carbohydrate antigen125 (CA125)	Ovarian	Diagnosis, prognosis, monitoring
HER2	Breast	Monitoring Therapy
Alpha fetoprotein	Hepatocellular carcinoma	Diagnosis,monitoring
CA 19-9	Pancreatic	Monitoring Theraphy
Carsinoembryonic antigen (CEA)	Colorectal/hepatic	Monitoring Therapy, diagnosis

Biomarker verification and validation The enzyme-linked immunosorbent assay (ELISA) is a commonly used analytical biochemistry assay, used in target protein or peptide analysis in biomarker verification and validation. The development of the ELISA method is very expensive and has limited capacity as well as time consuming processes such as 1-2 years. Its selectivity is low with cross reactions and therefore its validity must be verified. Mass Spectrometry was chosen as the "method of the year" by Nature Methods magazine in 2012 (Editors Nature Methods 2013, Evanko D. 2012) as a suitable method for many biomarker analysis waiting to be verified due to its higher selectivity (SRM and MRM modes).

There are still points in protein spectrometry and mass analysis that need to be improved, especially in selectivity and precision. However, mass spectrometry is one of the most effective methods for biomarker discovery and use in diagnosis.

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Chapter 10

CONTROLLED HYPOTENSION IN ANESTHESIA

‘’Hypotensive Anesthesia”

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INTRODUCTION:

The concept of controlled hypotension was first proposed by Cushing in 1971 and introduced into clinical practice by Gardner in 1946. In 1947, it was widely used by Grifitt in England to reduce bleeding in spinal surgery. Controlled hypotension is defined as a reduction of the systolic blood pressure to 80-90 mmHg, a reduction of mean arterial pressure (MAP) to 50-65 mmHg in patients without hypertension, or a 30% reduction of baseline MAP in patients with hypertension. Controlled hypotension is induced to provide a bloodless surgical field by reducing bleeding and the need for blood transfusion, which is frequently used in various neurosurgical procedures such as oral and maxillofacial surgery, endoscopic sinus or middle ear microsurgery, spinal surgery and aneurysm, major orthopedic surgery (knee or hip replacement, spinal), prostatectomy, as well as surgical procedures with high bleeding potential such as cardiovascular surgery and liver transplant surgery. The ideal hypotensive agents must be easy to administer, have a short onset time, an effect that disappears quickly when administration is discontinued, a rapid elimination without toxic metabolites, negligible effects on vital organs, and a predictable and dose-dependent effect (1-3).

I. AIM OF CONTROLLED HYPOTENSION:

Controlled hypotension aims to reduce bleeding, improve surgical field condition, and reduce the need for blood transfusion. In neurosurgical operations, it is also aimed to reduce cerebral blood flow, cerebral blood volume, intracranial pressure, and CSF (cerebrospinal fluid) production. In normotensive patients, arterial pressure must be maintained at 80-90 mmHg or mean arterial pressure (MAP) at 50-65 mmHg. It is defined as a 30% decrease in basal MAP in patients with hypertension.

Controlled hypotension is used in neurosurgical operations, major orthopedic surgery, middle-inner ear surgery, cancer surgery, portocaval shunts and vascular surgeries. Reduced bleeding stems from reduced blood pressure rather than cardiac output. Hypotension does not always result in similar reduced bleeding in patients with operational bleeding, which is due to differences in position and ventilation affecting venous return (4,5)

Although invasive blood pressure is monitored during controlled hypotension, what actually needs to be monitored is the amount of bleeding in the surgical field as less aggressive hypotension may be sufficient to provide adequate bleeding control (6).

II. Effects of Hypotension on Organ Blood Flow and Functions

A) Central Nervous System: Cerebral blood flow is regulated and

remains constant over a mean arterial pressure (MAP) range of 60-130 mmHg. Autoregulation is impaired when MAP falls below 60 mmHg. Changes in systemic blood pressure are directly reflected on the cerebral blood flow. Cerebral perfusion pressure (CPP), which is defined as the difference between systemic blood pressure and intracranial pressure, normally ranges between 80-100 mmHg. MAP below 50 mmHg may result in cerebral hypoxia due to insufficient oxygen in cerebral blood flow (7-9)

B) Cardiovascular System: Coronary blood flow is well regulated during controlled hypotension and ischemia is rare. However, ischemia may develop in case of low diastolic pressure, especially when combined with tachycardia or coronary artery disease (10).

C) Effects on Respiratory System: Physiological dead space does not increase as long as the cardiac output is maintained. However, dead space and shunts increase with decreased cardiac output due to the effect of position and/or high airway pressure. In such case, partial carbon dioxide pressure ($PaCO_2$) may increase while partial oxygen pressure (PaO_2) decreases. Therefore, regulation of respiration is required (11).

D) Effects on the Urinary System: Renal blood flow is preserved and no significant dilation associated with hypotensive drugs occurs since resting tone in the arterioles is already low. Renal failure following hypotension results from severe reflex arteriolar spasm and is mostly caused by hypovolemic hypotension. Controlled hypotension dropping systolic blood pressure below 50-75 mmHg reduces glomerular filtration, the excretion of which may prolong the effects of kidney-bound drugs (12).

E) Effects on Hepatic System: Hepatic perfusion changes may occur during hypotensive anesthesia since pressure-flow autoregulation is nonexistent in the portal circulation and limited to the hepatic arteries. Extrinsic regulation of hepatic blood flow is maintained by alpha-1 vasoconstriction. Baroreceptor reflex, surgical stress and vasopressors reduce hepatic blood flow. Unless hypotension is very severe, hepatic blood flow is well preserved with no significant change in its functions (10)

Suttner et al. investigated the effects of hypotensive anesthesia on liver functions. They found a significant increase in alpha-glutathione-s-transferase level compared to basal values, which is one of the liver enzyme markers examined 2 hours postoperatively. This suggests that hypotension causes a temporary disruption in hepatocellular integrity (13).

F) Eye: Ocular blood flow and intraocular pressure decrease in parallel with the mean arterial pressure, possibly resulting in blurred vision in the postoperative period (6,14)

III. CONTROLLED HYPOTENSION TECHNIQUES

A. Physiological Techniques

Parameters such as body position, hemodynamic ventilation effects, heart rate and circulating blood volume play a role in reducing blood pressure and proper adjustment of these physiological parameters ensures that hypotensive drugs with side effects and toxic effects are used in smaller doses.

Position: Blood flow in the surgical area can be reduced by keeping the surgical site above the heart level. This was first described by Enderby and named postural ischemia meaning that every 2.5 cm above the heart level causes a drop of 2 mmHg in blood pressure (15).

Controlled expiration and PEEP (positive end expiratory pressure): Positive pressure ventilation used during general anesthesia increases intrathoracic pressure and reduces venous return. With reduced venous return, reflex venous vasoconstriction and tachycardia prevent cardiac output from being adversely affected. However, drugs such as ganglion blockers and beta-blockers prevent this effect and reduce blood pressure. Positive pressure ventilation and PEEP also help to reduce the dosage of these drugs (16).

B. Pharmacological Techniques

Pharmacological methods act mainly through sympathetic blockage by affecting different systems, starting from the central nervous system. Although the reduction of bleeding by vasodilators may seem contradictory, what is actually significant here is that it reduces MAP. Thus, local blood flow is also reduced. Local mean blood pressure should be around 60-70 mmHg and venous drainage should not be blocked.

An ideal hypotensive agent should have the following properties: easy to administer, short onset time, quick disappearance of its effect by the time the administration is discontinued, rapid elimination without any toxic metabolite residues, and no adverse effects on vital organs while being dose-dependent with predictable effects (16).Unfortunately, there has been no ideal agent found to date.

1. Inhalational anesthetics: halothane, isoflurane, sevoflurane, desflurane

Inhalational anesthetics directly depress arteriolar vasodilation and vasomotor centers, resulting in hypotension. Halothane, enflurane, sevoflurane and isoflurane can be used alone or in combination with other hypotensive drugs. Isoflurane is preferred more frequently as it has no direct

depressant effect on the myocardium and peripheral vasodilation can be easily corrected. Although it may increase intracranial pressure, this effect is much less potent compared to halothane and enflurane (14).

2. Ganglion blockers: trimethafan, pentolinium

They are not widely preferred in current medical practice. Trimethaphan and pentolinium dilate the resistance and capacitance vessels by blocking the sympathetic ganglia. However, they may also affect parasympathetic ganglia and result in tachycardia (17).

3. Direct-acting vasodilators: sodium nitroprusside, nitroglycerin

Sodium nitroprusside relaxes arteriolar smooth muscles by interacting with the sulfhydryl group, inhibiting calcium entry into the cell and its intracellular activation. Thus, it relaxes both capillary resistance and postcapillary capacitance vessels, reducing peripheral resistance and venous return while lowering blood pressure. It is one of the preferred hypotensive agents due to its short onset time, quick disappearance of effects and easy control. It enzymatically decomposes to nitric and hydrocyanic acid in erythrocytes and plasma. Hydrocyanic acid is converted into thiocyanate by combining with thiosulfate in the liver and excreted through urine. Excessive dosage can release free cyanide. Cyanide and thiocyanide intoxication may cause reactions from metabolic acidosis and toxicity to death. Sodium nitroprusside may result in rebound hypertension and reflex tachycardia. This can be prevented by gradual discontinuation of the infusion and the use of antihypertensives such as esmolol and captopril. Their area of usage is limited as they may increase intracranial pressure due to vasodilation and aggravate bleeding due to arrhythmias, tachycardia, increased pulmonary shunts, and inhibition of platelet aggregation (18).

Nitroglycerin dilates the main capacitance veins with its direct effect on vascular smooth muscles. On the other hand, its effect on the resistance vessels is less significant. Therefore, it decreases systolic arterial pressure (SAP) more (17). Limited decrease in diastolic arterial pressure (DAP) ensures the preservation of coronary and cerebral perfusion. However, it increases intracranial pressure more than sodium nitroprusside. Its effect is slower and weaker. Blood pressure returns to normal 10-20 minutes after discontinuation of the infusion (17,14)

4. Arteriolar vasodilators: adenosine

It acts on the adenosine receptors on the vascular wall and AV node. It has a very short duration of action and decreases the systemic vascular resistance.

5. Beta-blockers: propranolol, esmolol, labetalol

Beta-blockers may cause bradycardia. Atropine is used to treat brady-

cardia associated with beta-blockers (19,20)

Esmolol is a beta-1 blocker with a very short duration of action.

Labetalol is an alpha-beta-adrenergic antagonist that causes hypotension by reducing cardiac output and systemic vascular resistance.

6. Alpha 2 agonists: clonidine and dexmedetomidine

7. Opioids: fentanyl, remifentanil

8. Prostaglandin E1 and magnesium sulfate

They cause moderate hypotension, does not affect cerebral blood flow and carbon dioxide reactivity, and has limited areas of usage. They are mostly preferred in cerebrospinal surgery and obstetrics (17,14)

C. Controlled Hypotension Indications

1. Aneurysm, arteriovenous malformation and tumor surgery, laminectomy procedures in neurosurgery

2. Reconstructive head and neck surgery in plastic surgery, rhinoplasty and microvascular surgery

3. Septoplasty, rhinoplasty, functional endoscopic sinus surgery, middle ear surgery, laryngectomy, parathyroidectomy operations in otorhinolaryngology

4. Aortic coarctation in vascular surgery

5. Hip replacement and scoliosis surgery in orthopedics

6. Regulation of systemic hypertension following coronary artery bypass grafting in cardiac surgery, which forces anastomotic lines and causes ischemia, and regulation of pulmonary hypertension that may cause pulmonary edema

7. Liver transplantations

8. Prevention of excessive blood pressure spikes during pheochromocytoma surgery

9. Controlled hypotension is preferred in surgical interventions for patients who fail to find suitable blood and/or do not want transfusion (6,21).

D. Controlled Hypotension Contraindications

1. Severe cardiac disease and carotid artery stenosis

2. Myocardial ischemia

3. Kidney and parenchymal liver injury

4. Degenerative diseases of the central nervous system

5. Subarachnoid hemorrhage accompanied by vascular spasm

6. Peripheral vascular disease accompanied by claudication

7. Pulmonary diseases

8. Glucagonoma

9. Addison's disease

10. Pregnancy

11. Uncontrolled hypertension

12. Hypovolemia

13. Severe anemia

14. Uncontrolled glaucoma

15. Technical insufficiency (1,22)

Although regulated hypertension is not a definite contraindication, it should be taken into consideration that the cardiovascular control mechanisms of these patients do not work regularly and that they are more sensitive to antiadrenergic and vasodilator drugs compared to normotensive individuals (6).

E. Controlled Hypotension Complications

Review of published mortality reports on controlled hypotension demonstrates complication rates between 0.01% and 0.007%. Considering the mortality rate of general anesthesia of 0.01%, it can be said that the hypotensive anesthesia techniques do not pose a negative impact on mortality.

Complications can be minimized by maintaining oxygen concentration in the inhaled gas mixture below 50% during hypotension, preserving radial pulse, ensuring favorable capillary circulation in the nail beds, monitoring blood pressure frequently and closely, and appropriate patient selection. Complications increase with prolonged hypotensive process in controlled hypotension. Hypotension that does not exceed 1-1.5 hours with systolic arterial pressure maintained above 80 mmHg is well tolerated. However, permanent ischemic damage may occur with decreased cerebral and myocardial blood flow in cases of prolonged hypotension. These drugs may sometimes cause unwanted side effects with increased doses that are administered to induce the desired level of hypotension. Uncontrolled low blood pressure may cause cerebral thrombosis, hemiplegia (disrupting spinal cord perfusion), blindness, acute tubular necrosis, massive hepatic necrosis, myocardial infarction, or cardiac arrest (22,23)

Despite the risk of tissue hypoperfusion and hypoxia, no specific complication was found in cases with prolonged controlled hypotension (MAP <50 mmHg). The most common factors for morbidity include bleeding due to unsuccessful bleeding control, prolonged anesthesia recovery, cerebral and coronary artery thrombosis, postoperative oliguria/anuria and cerebral dysfunction. The risk of complications increases in patients undergoing controlled hypotension if they have anemia (6,23)

Most clinical trials on controlled hypotension aim to determine the ideal pharmacological agent. TIVA (Total Intravenous Anesthesia) has been recently preferred over inhalational anesthesia to provide controlled hypotension as it enables rapid recovery and minor surgical field bleeding in patients, as shown by available studies in which intravenous anesthesics along with additional antihypertensive drugs are used, as well as in comparison with total intravenous anesthesia and inhalation anesthesia. (24-26)

F. Total Intravenous Anesthesia for Controlled Hypotension

The use of intravenous anesthetics is getting more popular in surgical procedures, considering the toxic effects of inhalational anesthetics, increased risk with repetitive applications, and ambient air pollution negatively affecting employees. This can be considered as an example of balanced anesthesia in which the hypnotic effect is provided by infusion of the intravenous agent (1). Many different intravenous compounds can be used in different combinations for TIVA. The mixture of an opioid with a hypnotic agent is the most common combination (27). The aim of TIVA is to provide a certain plasma level by balancing the infusion and elimination rate. Propofol has suitable pharmacokinetics for TIVA (27).

Compared economically, TIVA is a more expensive technique than inhalational anesthesia, but TIVA, which is administered with propofol, has advantages such as fast return of cognitive functions and rapid recovery, with a relatively rare incidence of nausea-vomiting (28, 29). There has been an ongoing increase in the number of studies in which shorter recovery and bloodless surgical field are observed in patients administered TIVA, especially in otolaryngology (24, 30, 31).

In conclusion, controlled hypotension is an effective method in reducing blood loss and improving surgical field condition. It is frequently applied in many surgical operations to reduce bleeding in the surgical field. Threshold of mean arterial pressure is 50-55 mmHg in young healthy patients. Although it is difficult to determine the safe threshold in elderly comorbid cases, maintenance of mean arterial pressure at 60 mmHg can be accepted as a safe limit. It may be wise to induce controlled hypotension more frequently during anesthesia to reduce the need for transfusion.

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Chapter 11

RADIOTHERAPY FOR NONMALIGNANT DISEASES

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Introduction

Radiation therapy, a clinical modality for treatment of patients with malignant neoplasias is also utilized in treatment algorithm of some benign diseases (1). Beam quality, prescribed dose, overall treatment time, organs at risk in or adjacent to treatment field should be evaluated sensitively. Additionally, treating children should be avoided unless referring for a very exceptional cases with a considerable and rationalized risk benefit ratio. Benign diseases are generally localized lesions with low progression, peripheral invasion or distant metastasis potential. Pathologically, includes well differentiated, nonmalignant cells with no need of any treatment. However, some of these benign diseases can lead clinically discomforting consequences such as huge mass effect, pain, mass related infirmative symptoms, inconvenient secretory functions etc. (2-4). Therefore, radiotherapy has been utilized since first decades of 20th century for benign lesions such as acne, body hair, scalp ringworm, enlarged tonsils, enlarged thymus, enlarged neck lymph nodes (5). Radiation therapy was used in aforementioned conditions and many others due to lack of medical and surgical effective therapies. However, growing mindfulness of late radiation sequelae especially radiation carcinogenesis induced a decline in radiotherapy as a choice in treatment strategy for benign diseases despite modern radiotherapy devices and techniques, planning with higher consideration of radiobiology.

Radiobiologic Effects of Radiotherapy on Benign Diseases

Radiobiologic effects of radiation is thought to be a result of complicated multicellular interactions targeting various cell types in tissues (6,7). Development of many benign lesions can not be explained with a clear cause or mechanism but some others like keloids or heterotopic ossification are known to be stimulated by trauma. Underlying mechanism is proposed as local inflammation leading to a stimulation of growth factors and cellular proliferation resulting a repair forming a new aberrant tissue. Radiotherapy breaks this chain via inhibiting cell proliferation and differentiation without causing cell death. Vascular endothelial cells show a rapid response to radiation damage via up-regulating the cytokine-mediated reactions within the cell which are mainly responsible for inflammatory changes. Endothelial cell damage with higher single or total doses can cause sclerosis and obliteration of blood vessels. Therefore, in hemangiomas or arteriovenous malformations, high radiation doses may be used to induce occlusion of pathologic vessels. Besides cell killing may also be play a part of the treatment aim in the management of benign meningiomas, pituitary adenomas, or neuromas where higher, doses of radiation may be required. Low-dose irradiation such as <12 Gy total dose with 0.3-1.0 Gy or less per fraction

2-3 times a week (LD-EBRT), is shown to have anti-inflammatory effects on the capillary endothelial cells and mononuclear cells (8). Previous reports proposed several anti-inflammatory effects:

a) Changes in expression of cytokines and adhesion molecules on the activated leukocytes, endothelial cells leading to a capillary permeability modulation and allowing the migration of inflammatory cells (lymphocytes, macrophages, monocytes) into interstitial space. These inflammatory cells express cytokines or necrosis factors which influence the cascade and enzymes of inflammatory reaction and they are also radiosensitive (9).

b) LD-EBRT between 0.3 and 1.25 Gy is shown to significantly reduce nitric oxide (NO) production in activated macrophages. This modulation in NO production, oxidative break out in activated macrophages, and consequential reduction in release of reactive oxygen species (ROS) results in antiinflammatory and analgesic effects (10,11).

c) LD-EBRT induce apoptosis in peripheral blood mononuclear cells (PBMC). The peak of apoptosis is seen after a single dose between 0.3 and 0.7 Gy and higher doses (up to 3 Gy) was not more effective (12).

d) LD-EBRT (0.1 - 0.5 Gy) significantly reduces adhesion of PBMC to endothelial cells which is the intiating event of inflammatory invasion in tissue (13). Mechanism underlying the reduced adhesion is attributed to high mRNA expression and protein secretion of transforming growth factor β1 (TGF-β1) in irradiated endothelial cells after 0.5 Gy (14). All these mechanisms seem to be dose dependent showing a maximum effect between 0.3 and 0.7 Gy (15).

In an attempt to form a consensus, the German Working Group on Radiotherapy of Benign Diseases published DEGRO practical guidelines for radiotherapy of nonmalignant disorders consisting of 4 parts.

These guidelines published as;

1- Part I: Physical principles, radiobiological mechanisms and radiogenic risk. This work evaluates the basic principles of radiation physics and treatment delivery, evaluation of putative underlying radiobiological mechanisms, and the assessment of genetic and cancer risk following low-dose irradiation (16).

2- Part II: Painful degenerative skeletal disorders. This part summarizes recommendations for the treatment of benign painful degenerative skeletal disorders with low-dose radiotherapy such as enthesiopathies and painful arthrosis. It is concluded that low-dose radiotherapy is effective in the majority of painful degenerative skeletal disorders. Suggested dose scheme is single fraction doses of 0.5–1.0 Gy and total doses of 3.0–6.0 Gy in 2–3 fractions per week (17).

3- Part III: Hyperproliferative disorders. This report aimed to form a consensus on treatment decision, dose prescription, and RT technique for several hyperproliferative disorders including Morbus Dupuytren (MD)/ Morbus Ledderhose (ML), keloids, Peyronie's disease (induratio penis plastica, IPP), desmoid tumors, pigmented villonodular synovitis (PVNS), symptomatic vertebral hemangiomas (sVH), and Gorham–Stout syndrome (GSS). RT can be recommended in treatment strategy of these disorders as a primary treatment approach or an effective supplementary modality (18).

4- Part IV: Symptomatic functional disorders. This paper evaluates the role of low-dose radiotherapy in the treatment of functional disorders in cases of heterotopic ossification (HO) and Graves orbitopathy (GO). Low-dose radiotherapy for these functional diseases has proven to be an effective prophylactic and therapeutic option. Single-fraction of 7–8 Gy or five fractions of 3.5 Gy for HO, single-fraction of 0.3–2.0 Gy, and total doses of 2.4– 20 Gy for GO are recommended (19).

Benign Neoplasms of the Brain, Head and Neck

Benign disorders in central nervous system (CNS) and neck can cause severe, life-threatening symptoms due to pressure and mass effect on critical structures.

Meningioma:

Meningiomas are the most common tumors of the CNS with a peak incidence in the seventh decade. It arises from the arachonoid meninges of the brain and grow at a slow rate (20). The majority of meningiomas are benign and classified as grade I by the World Health Organization (WHO) (21). WHO grade II meningiomas have a higher risk of local recurrence, and WHO grade III/malignant meningiomas (anaplastic, rhabdoid, papillary) are rare. Patients mostly present with headache and a homogeneously and intensely enhancing extra-axial mass on computed tomography (CT) or magnetic resonance imaging (MRI).

Nonradiotherapeutic approaches: Active Surveillance is considered appropriate for asymptomatic patients with small meningiomas. A large retrospective series from Japan which demonstrated that the majority of patients do not require intervention in the short-term confirmed the safety and rationalized this approach (22). Surgical resection is the mainframe of the treatment while it ensures both symptom relief and pathologic diagnosis. The primary goal of surgery is maximal safe resection which is primarily attempted in appropriate locations (23). Local relapse rate after gross total resection (GTR) is 10% where it can rise to 40% for patients with incomplete resection (24). Reported average interval to recurrence after surgery is 4 years (25,26) depending on extent of resection and degree

of dural involvement. As meningiomas can be highly vascularized tumors, preoperative embolization is used to decrease blood loss and improve the extent of resection (27).

Radiotherapy: In Grade I meningiomas radiosurgery can be used as primary therapy for tumors in inconvenient locations for complete resection (e.g., optic nerve, cavernous sinus), for medically inoperable patients or adjuvant for growing remnants. Grade II and III meningiomas have high recurrence rates, up to, 30–40% and 50–80% at 5 years respectively, where it is 10% for grade I. Therefore, adjuvant radiation therapy of the tumor zone might be beneficial even after gross total resection (28-30). RT can be applied using several techniques such as conventionally fractionated three-dimensional conformal radiotherapy (3DRT), conventionally fractionated intensity-modulated radiation therapy (IMRT), frame-based or linear accelerator–based fractionated stereotactic radiotherapy (FSRT), stereotactic radiosurgery (SRS), or protons and heavy ions. Registration of the MRI sequences showing the gross tumor volume (GTV) most accurately with the treatment planning CT scan should be done for optimal treatment plan and delivery. For FSRT or SRS, it is important to distinguish residual tumor from postoperative change which makes the assistance of neuroradiologist and neurosurgeon essential for GTV delineation. Clinical target volume (CTV), constructed by adding a symmetric margin (0 to 1 cm for WHO grade I; 1 to 2 cm for WHO grade II/III) around the GTV is adequate for 3DRT or IMRT treatments. An additional 3 to 5 mm is recommended to form planning target volume (PTV). For sure these margins may be individualized for institutions according to availabilities. Suggested dose for benign meningiomas is 50 to 54 Gy in 1.8- to 2-Gy daily fractions based on previous retrospective data reporting inferior local control with doses of <52 Gy. For WHO grade II/III tumors 59.4 to 63 Gy dose should be given. Reported 5-year local control rates are approximately 89% to 98% (31-34). Despite the contaversy on the benefits of adjuvant radiotherapy for WHO grade II meningioma a survival benefit for adjuvant radiotherapy in patients with subtotal resection is demonstrated by an analysis of National Cancer Database (NCDB) (35). Dose for frame-based SRS is 12 to 16 Gy prescribed to the 50% isodose line (IDL) and for a frameless robotic radiosurgery platform it is 14 to 18 Gy prescribed to the 80% IDL depending on tumor size and proximity to critical structures. In perioptic tumor locations 24 to 30 Gy in 3 to 5 fractions (to the 80% IDL) can be a reasonable option with high tumor control rates and visual preservation (36). FSRT should be used for optic nerve sheath meningiomas because of the high rate of preservation of visual acuity (37). Reported 5-year local control rates with SRS are 98% to 100 (38-39). Coverage of the dural tail in target volume is shown to improve disease-free survival (40). High mitotic rate, nuclear atypia, spontaneous necrosis and WHO grade III pathology are suggested

as additional adverse features by Ferraro et al. (41). Protons and heavy ions are other becoming modalities because of their unique physical properties leading highly sufficient local control rates (42-43).

Pituitary Adenoma:

Pituitary adenomas are secretory in approximately 65-70% of the cases. Most common subtypes are prolactinomas and growth hormone (GH)–secreting adenomas. They are classified as picoadenoma, microadenoma and macroadenoma according to size with <0.3 cm, <1 cm, and >1cm respectively. Clinical presentation can vary according to functionality and secreted hormones. Workup should include history and detailed physical examination, laboratory analysis of pituitary hormone levels, contrast-enhanced MRI with thin slices through the pituitary and tissue sampling for differential diagnosis (44).

Nonradiotherapeutic Approach: Surgery provides immediate compressive symptom relief and decrease in hormone secretion. Local control rates are reported to be 50-80% after surgery alone irregardless of functioning or not (45). Postsurgical elevated hormones necessitate adjuvant pharmacotherapy and/or radiation therapy. Medical treatment includes agents such as bromocriptine and cabergoline, octreotide and ketoconazole although the results are not satisfying in terms of cure.

Radiotherapy: RT is used as a primary modality for medically inoperable patients or as an adjunct to surgery in recurrence, postsurgical persistant elevated hormone levels and postsurgical residual disease. Tumor growth control is satisfactory especially for nonfunctioning adenomas (46), however, endocrine response of functioning adenomas may take years (47). Pharmacologic therapy should be discontinued 1 to 2 months prior to RT based on reported reduced radiosensitivity when given concurrently (48). 3DRT, IMRT, single-fraction SRS, SBRT and FSRT are possible options of RT. Postoperative MRI should be used for GTV delineation (preoperative GTV in the case of GTR). PTV is obtained by adding 3 to 5 mm to gross disease and subclinical extent of the tumor. Nonfunctional adenomas are generally given a dose of 45 to 50.4 Gy in 1.8- to 2.0-Gy daily fractions. A dose range of 50.4 to 54 Gy are recommended for secretory adenomas while they are thought to be more locally aggressive and less radioresponsive. SRS also has become a reasonable novel option for pituitary adenomas. FSRT preferred over SRS for lesions >3 cm or lesions near critical structures such as optic chiasm. Recent retrospective studies reported a local control rates of 92-100% for nonfunctional adenomas with 14 to 25 Gy doses in a single fraction at the edge of the tumor (49). Commonly used single fraction doses are 16 to 20 Gy for nonfunctional adenomas and 20 to 25 Gy for functional adenomas with robotic radiosurgery platform.

Craniopharyngioma:

Craniopharyngiomas make up 6-10% of pediatric CNS tumors (50). These epithelial benign tumors arise from remnants of Rathke pouch and mostly located in the suprasellar region abutting the hypothalamus and third ventricle. Histologically, they are investigated in two subtypes: adamantinomatous and squamous. Patients present with intracranial mass effect and endocrine dysfunction which is mostly GH deficiency.

Nonradiotherapeutic approach: Complete surgical resection is not allways achieved while GTR may cause high rates of neurologic sequelae. Therefore, maximal safe resection is mostly preferred surgical approach. Intracavitary Bleomycin injection into the cyst decreases the rate of recurrence (51). Another intralesional approach is a beta emitter radioactive isotope injection particularly for tumors without large solid component (52).

Radiotherapy: EBRT is mostly treatment of choice in adjuvant setting for patients with residual disease or who are at high risk for progression (53). In children <3 years old, RT may be deferred even after STR while it is reported that local control and overall survival rates are similar with "salvage" RT at the time of relapse (54). EBRT can be given via 3DCRT, IMRT, FSRT and proton therapy. GTV delineated as the postoperative residual tumor volume, including the cyst wall seen in the postoperative MRI images. The CTV includes the GTV and subclinical disease. At least 54 Gy with 1.8-Gy fractions is prescribed to PTV formed by adding set up margins to CTV. Regrowth of cysts during fractionated radiotherapy has been previously reported. Therefore, for large cysts or those that demonstrate growth during RT weekly reimaging or monitoring with cone beam CT in IGRT devices is recommended (55). Niranjan et al evaluated 51 SRS procedures they performed in UPMC. Median tumor volume was 1.0 cm^3 (0.07–8.0) and median tumor margin prescription dose was 13.0 Gy (9–20). After 62.2 months (12– 232) follow up overall survival was 97.1% at 5 years. The 3- and 5-year progressionfree survivals were 91.6% each. Part of the tumor adjascent to optic chiasm is exposed to lower doses (9–10 Gy). Dose fall off to the optic chiasm is kept below 8 Gy (56).

Vestibular Schwannoma (Acoustic Neuroma):

Acoustic neuromas (AN) are derived from vestibulocochlear nerve. Presenting symptoms are sensorineural hearing loss, tinnitus, and vertigo. In the initial workup detailed physical examination of CN V and CN VII and audiometry must be done. Contrast enhanced MRI with thin slices through the internal auditory canal should be added (44).

Nonradiotherapeutic approaches: Observation is reasonable for patients age >60 with multiple comorbidities, small tumor size and risk of

hearing loss. Surveillance consists of MRI and audiometry every 6 to 12 months. Treatment is initiated in case of rapid and significant growth or symptom burst (57). Surgery is the primary treatment especially for large, symptomatic lesions. A complete resection is available for most patients. Recurrence rate is approximately 15% in case of subtotal resection (57).

Radiotherapy: SRS or FSRT are preferred forms of RT for the primary treatment due to higher rates of hearing and facial nerve preservation compared with surgery. SRS doses given with frame-based platforms are 12-13 Gy prescribed to the 50% IDL. In a study by Flickinger et al. 98.6% local control with 70.3% and 4.4% rate of hearing preservation and trigeminal neuropathy respectively (58). FSRT dose prescriptions are 5 Gy X 5 fractions, 3 Gy X 10 fractions, and 50-55 Gy in 25-30 fractions. In a proton treatment series from MGH, 88 patients were treated to median 12 (10-18) GyE dose in a single fraction prescribed to IDL of 70%. In the same study 2 and 5-years tumor control rates were 95.3% and 93.6% respectively. Univariate analysis in the very study revealed that prescribed dose ($P = 0.005$), maximum dose ($P = 0.006$), and the inhomogeneity coefficient ($P = 0.03$) were significantly associated with risk of long-term facial neuropathy (59).

Chordoma:

Chordomas are slowly growing but locally aggressive tumors derived from embryonal notochord remnants. They occur in the skull base (35%), vertebral column (15%), or sacral regions (50%) (60). Workup should include contrast enhanced MRI and a biopsy to distinguish chordoma from chondrosarcoma or any other pathologies. Complete surgical resection is the mainstay of treatment but often not possible due to unsuitable location. Even a negative margin is achieved surgically, reported recurrence rates are around 50% (61).

Radiotherapy: Adjuvant radiation therapy is indicated to reduce recurrence rates for all skull-based chordomas based on the retrospective data reporting inferior 5- and 10-year overall survival rates with salvage RT compared to adjuvant RT (62). Conventionally fractionated EBRT, IMRT, charged particle therapy (protons, carbon ions) and FSRT are possible RT techniques for treatment of skull-based chordomas. Preoperative and/or postoperative MRI should be used for delineating GTV appropriately. Margins for CTV and PTV should be 1-2 cm with an additional 3-5 mm for set up uncertainties respectively. Recommended dose prescription is at least 60 Gy with 1.8-2.0 Gy daily fractions. It is possible to prescribe higher doses more safely with proton-based therapy. Recent papers found local control rates of 81% at 3 years and 70-77.2%, at 5 years, overall survival 81.1% and disease-free survival as 50.3% with 60 to 70 GyE proton therapy (63,64).

Glomus Tumor/Chemodectoma/Paraganglioma:

Glomus tumors are benign tumors derived from embryonic neural crest. They are seen near carotid bifurcation (carotid body tumor), the jugular bulb (glomus jugulare) or the middle ear (glomus tympanicum). The peak age is in the fifth decade of life. Symptoms can be variable such as headache, cranial nerve dysfunction, dysphagia, tinnitus, vertigo or pulsating neck masses. In case of hypertension urine and serum metanephrines should be evaluated. If multiple tumors are suspected, imaging with metaiodobenzylguanidine (MIBG) may be useful. Embolization must be done in carotid region before resection. In inappropriate locations neurosurgical intervention is often deferred to avoid stroke and cranial nerve injury (44).

Radiotherapy: RT can be used as primary treatment for locations which are not suitable for surgery, adjuvant treatment after STR or as salvage therapy in case of postsurgical recurrence. Comparison of RT with surgery in retrospective analysis revealed similar outcomes and local control with less morbidity in favor of RT (65). RT can be performed using conventionally fractionated 3DCRT/IMRT, SRS, or FSRT. For clinical and set-up margin 1-1.5 cm is added to GTV determined via diagnostic MRI. RT is given as totally 45-55 Gy with 1.8-2 Gy daily fractions. Local control rates are reported to be >90% (66). Treatment with frame-based platform to doses of 12.5 to 20 Gy prescribed to the 50% IDL and with LINAC-based SRS to doses of 15 - 25 Gy provides 90-100% local control (67,68). On the basis of a confirming consequential metaanalysis, SRS is suggested as primary treatment of glomus jugulare tumors (69).

Juvenile Nasopharyngeal Angiofibroma:

Juvenile nasopharyngeal angiofibroma (JNA) is a benign, vascularized tumor originating from the first branchial arch artery. Initial symptoms are recurrent epistaxis and impaired nasal breathing. Progressive local extension may lead to facial swelling, visual problems, cranial nerve deficits and headaches. As biopsy may cause massive bleeding, JNA is often diagnosed via CT or MRI (70). Surgery with embolization is the treatment of choice with local control rates of almost 100% for cases without intracranial extension.

Radiotherapy: RT is the primary modality for medically inoperable patients and tumors with intracranial extension or recurrent tumors. To avoid radiation damage to adjacent critical structures, fractionated IMRT is the preferred technique. The recommended dose to PTV is 30-50 Gy with 2-3 Gy daily fractions with reported local control rates around 85-100% (71,72). After RT, JNA remission is slow, and late recurrences may occur.

Langerhans Cell Histiocytosis (Histiocytosis X):

Langerhans cell histiocytosis (LCH) results from accumulation and/

or proliferation of Langerhans cells which is a myeloid dendritic cell expressing the same antigens with Langerhans skin cell (73). Patients are stratified according to extent of disease: single-system disease at a single site, single-system disease involving multiple sites or multisystem disease. Skeletal system is the most common site in children. Bony lesions are predominantly lytic and the skull is the most frequent location. Cutaneous involvement resembles a seborrheic dermatitis. Pulmonary involvement is more typically seen in adults. LCH work up should include CBC, a skeletal survey with or without bone scan, cranial MRI in case of suspicious brain involvement, and thorax CT to evaluate pulmonary involvement.

Nonradiotherapetic Approach: For patients with skeletal system involvement only, curettage, excision or intralesional steroid injection are reasonable options with response rates of 70-90% (74). Systemic therapy including corticosteroids or chemotherapy such as vinblastine +/- etoposide is needed in patients with single-system multifocal bone disease, multisystem symptomatic disease or organ dysfunction. In case of only skin disease, topical nitrogen mustard and methotrexate are effective options (75).

Radiotherapy: Radiation therapy to bony sites is indicated in case of relapse, persistent disease, pain and mass effects on critical structures. 3DCRT is the recommended technique. Treatment field for bone should encompass the abnormality in imaging with a small margin. Although higher doses can be used for adults, total of 5-10 Gy with 1.5-2 Gy daily fractions is suggested for children. A 77% complete remission and 80–88% long term control was reported with a median total dose of 8-15 Gy (76, 77). The recommended prescribed dose is 15 Gy in 1.5 Gy daily fractions for Diabetes Insipitus (DI) to a target volume encompassing the pituitary region. In a report from the Mayo Clinic, doses of >15 Gy is recommended for higher response (78).

Vascular Disorders

Vascular disorders include subtopics such as hemangiomas, arteriovenous malformations (AVMs) and cavernous hemangiomas. Radiosurgery has become a common treatment option for AVMs.

Arteriovenous Malformations:

AVMs are congenital vascular abnormalities composed of conglomerations of arteries and veins with a tangle of vessels connected to several fistulas so called nidus and has a high risk of rupture. The prevalence is low and peak incidence is at 20 and 40 years. Bleeding is the presenting symptom in 50% of patients with a death risk of about 10%. Gold standard of diagnostic imaging is angiography, which allows for full grading of

the AVM providing to predict hemorrhage risk. Complementary imaging tools such as MRI, MR angiography, and CT may also be used. Treatment should aim to completely obliterate the nidus. Complete surgical excision has the risk of intraoperative bleeding, ischemic cerebrovascular accident, infection, and death although it may provide immediate cure. Endovascular embolization may decrease the risk of intraoperative bleeding or decrease the size of the nidus for radiotherapy.

Radiotherapy: SRS is the preferred modality for AVMs leading to sclerosis and prevents hemorrhage with a single fraction of high-dose irradiation to a small volume. It makes a safe and effective treatment available for lesions <3 cm, in deep locations of the brain. Latent period for response ranges from 1-4 years after SRS which means a continuous but reducing risk of bleeding (79). Complete obliteration rate with 15-30 Gy minimum doses in the periphery of the target is reported to be 71- 89% within two years (80,81).

Functional Disorders

Trigeminal Neuralgia (Tic Douloureux):

TN is currently classified into type I TN and type II TN, which is based on pain characteristics (82). In the classic form type I TN, there are sudden shocks of sharp, lancinating facial pain lasting between a few seconds to minutes with pain-free intervals which responds to decompression better than Type II and have longer response duration. Patients with type II TN suffer from constant burning, aching or throbbing pain with low intensity. Cranial MRI is warranted to rule out any structural causes of clinical symptoms (83).

Nonradiotherapeutic Approach: Carbamazepine is the first and most common utilized agent for medical treatment. Various agents such as lamotrigine, neurontin, pimozide, tizanidine and topiramate are also advocated to treat carbamazepine-refractory patients. In medically refractory diseases, microvascular decompression, radiofrequency ablation, glycerol injection or balloon compression are other alternative invasive options for symptomatic relief.

Radiotherapy: SRS is a minimally invasive procedure used in treatment of TN with successful results. The target for SRS varies along the track of the trigeminal nerve seen in contrast enhanced MRI. Potential targets proposed to date are the anatomical emergence of the trigeminal nerve from the pons—proximal dorsal root entry zone (DREZ), the prepontine cistern, anterior of it, the retrogasserian zone and the semilunar ganglion (84-86). Typical doses for frame-based radiosurgery platform are 70-90 Gy, prescribed to an isodose 50-100% based on mainly trials originating

from Kondziolka et al. prospectively randomizing patients to 60-65 Gy or 70-90 Gy SRS and proving higher pain relief in the high-dose arm (87). The median time to response varies from 2-4 months leading to higher response rates with higher doses (79, 83, 88). Adler et al. (89) reported 85% complete response, 96% excellent or good outcomes with a mean marginal prescription dose of 58.3 Gy and mean maximal dose of 73.5 Gy to a 6-mm segment of the trigeminal nerve in 46 patients treated via frameless robotic radiosurgery platform.

Diseases of The Eye and Orbit

Pterygium:

Pterygium is a chronic fibrovascular and degenerative process arising from conjunctival–corneal border extending to the cornea. Symptoms may include irritation of the eye impairment of vision despite most of the cases are asymptomatic. Recurrence rate after surgery alone is about 20-30%. Therefore, several adjunctive approaches such as sliding conjunctival flap; rotational conjunctival autograft; free conjunctival or limbal autograft are needed to improve local outcome (44).

Radiotherapy: Treatment of pterygium with Beta-irradiation using strontium (Sr90) plaques is a mould brachytherapy improving local control especially if administered immediately after surgery. Early postoperative (within 24 h from surgical excision) radiation is effective and safe with improving local control rates compared to excision alone (90). However, the fractionation scheme is a matter of controversy. Doses reported in previous studies ranged from a single large dose at 20–30 Gy to 60 Gy/six fractions/6 weeks, and 30–35 Gy with 3 fractions/2–3 weeks is the most frequently used schedule. Local recurrence rates are 5-12% (90, 91). Nakamatsu et al. (92) found nonsignificantly higher 2-year local control with 30 Gy/3 fractions (85% vs. 75%) compared to 40 Gy/4 fractions.

Choroidal Hemangioma:

Choroidal hemangiomas (CH) are rare vascular tumors that arise from the choroid. Patients may present with a visual disturbance and retinal detachment; macular edema or retinal pigment changes can be detected on fundoscopic exam (93).

Radiation Therapy: RT is indicated to treat lesions near the macula and papilla and given with conventional 3DCRT, proton beam therapy or brachytherapy. Data regarding radiosurgery in CH are scarce (94). Recommended dose prescriptions for 3DCRT are 18-20 Gy for localized CH and 30 Gy with 1.8-2 Gy for diffuse CH (95). Generally used proton radiotherapy fractionation scheme is median 20 GyE in 4 fractions. A 100% visual improvement with proton beam therapy is demonstrated by Levy-Gabriel

et al. (96). Brachytherapy with cobalt 60, iodine 125 or ruthenium 106 plaques to a total dose of 25-50 Gy has been advocated for circumscribed lesions (93, 97).

Age-Related Macular Degeneration:

Age-related macular degeneration (AMD) is one of the major causes of blindness. EBRT with photons, protons or brachytherapy have been used to treat macular degeneration. A Cochrane meta-analysis in 2010 was not able to provide satisfying evidence that radiotherapy is an effective treatment for AMD (98).

Graves Ophthalmopathy:

Graves ophthalmopathy (GO), is an autoimmune disorder affecting the orbital muscles. Smoking is the greatest risk factor which also predicts poorer response (99). Clinical features include proptosis, photophobia, upper eyelid retraction, periorbital edema, conjunctival erythema and visual impairment. Enlargement of the extraocular muscles and fatty infiltration is demonstrated in 70-80% of cases via CT or MRI images (99). The most commonly involved muscles are inferior and medial rectus muscles (100). Treatment options include glucocorticoids, orbital radiotherapy, and surgery. Patients should become euthyroid before initiation of treatment (44).

Non radiotherapeutic Approach: Glucocorticoids are mainstay of GO treatment, however, must be given for long periods and have many side effects. In case vision is impaired by optic neuropathy, immediate high-dose steroid (IV or oral) should be applied (101). Urgent surgical decompression is indicated for patients who failed after medical treatment, rapidly progressive optic neuropathy, severe proptosis or cases with exposure keratopathy not relieved by local measures (102).

Radiotherapy: As lymphocytes and fibroblasts are known to be radiosensitive, retrobulbar irradiation is a reasonable treatment modality (44). Donaldson et al. (103), reported not only clinical regression, improvement in functional deficits and cosmesis but also inhibition of side effects with RT. General recommendation is 3DCRT to a total dose of 20 Gy with 2 Gy daily fractions over 2 weeks targeting both orbits, including the entire extraocular muscles with 2 opposed lateral fields isocenter placed a few millimeters posterior to the lenses using a beam-split technique (44, 104). A retrospective study reported 96% overall response rate and 98% patient satisfaction. Clinical improvements were mostly found in soft tissue changes (89%), extraocular muscle dysfunction (85%) and corneal abnormalities (96%) (105). Matthiesen et al. (106), observed 97% stabilization of disease and 84% symptomatic improvement.

Reactive Lymphoid Hyperplasia/Orbital Pseudotumor:

This is a benign inflammatory condition that affects the soft tissue of one or both orbits (100) and accounts for about 10% of all orbital tumors (107). Although it is not clearly defined yet, orbital pseudotumor (OP) is thought to be fibroproliferative, infectious, or an autoimmune disorder (44). Clinical presentation usually occurs in the 4th to 6th decades with proptosis, palpable mass, eyelid swelling, visual problems, periorbital edema, retrobulbar pain and extraocular muscle dysfunction (100). OP diagnosed typically with clinical presentation, imaging and pathology. Radiographically, enlarged extraocular muscles, optic nerve thickening, and enhancement showing infiltration in the retrobulbar adipose tissue with enhancement after administration of iodinated contrast or gadolinium can be observed (108). After treatment patients should be closely monitored due to reported subsequent progression to systemic lymphoma rate as high as 30% (109).

Nonradiotherapeutic Approach: Corticosteroids are the major treatment of choice with response rates of 92% for optic neuropathy and overall response rate of 78% (100). However, long-term control can be achieved in only 33% of patients with a single course of steroids (110). Various chemotherapeutic agents such as methotrexate, cyclophosphamide, and 6-mercaptopurine (111) are also used. Surgery may be used only for easily accessible lesions and relapses are common after surgery (112).

Radiation Therapy: Low-dose orbital RT for OP has been investigated by numerous studies reporting variable outcomes (109, 113). RT is suggested for refractory diseases after steroid treatment, in cases with contraindications to corticosteroids, lesions not amenable to other treatments or postsurgical recurrences (112). Treatment with 3DCRT should be aimed to a target delineated with the diagnostic MR. Unilateral treatment can be given with a single lateral field or an anterior and lateral field. Bilateral cases are planned similar to GO. Typically, 20 Gy in 10 fractions over 2-weeks is prescribed sparing the lens (100, 109, 114). Reported rate of response in terms of clinical improvement and/or tapering of corticosteroid is around 81- 87.5% with 20 – 30.6 Gy causing no significant adverse effects (113, 115).

Benign Diseases of Soft Tissue and Bones

Osteoarthritis: Osteoarthritis (OA) is a chronically debilitating musculoskeletal disorder predominantly affecting older people. It presents with pain due to inflammatory reaction in joint surface and joint capsule lining resulting from anatomical changes in the joint such as cartilage destruction, bone modification and abnormalities in capsule and synovia (116).

Nonradiotherapeutic Approach: The preliminary aim for treating early OA is to reduce joint pain, stiffness, maintaining and improving the functional capacity of the affected joint(s) (117). Oral analgesic and anti-inflammatory medications, nonsteroidal anti-inflammatory drugs (NSAID) or weak opioids in selected patients should be added to treatment (118). In patients with severe OA or who have not responded to noninvasive therapies surgery may be considered. Total or partial joint replacement is mostly utilized surgical methods for knee, hip and shoulder. (119). Minimally invasive surgical procedures such as arthroscopy or arthroscopic surgery provides not only to examine interior of joint but also allows for treatment resulting in pain relief and functional improvement for a short time (120).

Radiotherapy: In patients who are not surgical candidates or in case of pharmacotherapy failure, low-dose RT may be considered. RT can provide pain relief and functional improvement (44) A 50-75% long-term pain relief and functional amelioration is reported in several previous single-institution studies. In a large German study with 4,544 patients from 188 institutions median total dose applied was 6 Gy (3-12 Gy) with a median single dose of 1 Gy (0.25-3 Gy). Most of the patients reported pain reduction for at least 3 months. However, a second course of RT was needed in 30% of patients due to inadequate pain response or early pain recurrence (121). Micke et al. (122), demonstrated good analgesic effect with low-dose RT causing minimal side effects using similar RT schema for gonarthrosis. Radiation may reduce pain and related dysfunction without any improvement in arthritis.

Gorham-Stout Syndrome:

Gorham-Stout disease (GSD), is a rare bone disorder characterized by progressive osteolysis and the proliferation of lymphatic vessels leading to destruction and resorption of the bone matrix. Common presenting symptoms are muscular weakness, limb tenderness, and pathologic fracture after minimal trauma cause disfigurement or functional disability of affected bones. Efficacy of systemic therapies such as zoledronic acid and interferon-α reported to be unsatisfactory (123). Heyd et al. (124), summarized evidence from 38 articles and concluded that conventionally fractionated external beam RT to a total dose of 36-45 Gy may prevent disease progression in 75- 80% of cases.

Pigmented Villonodular Synovitis:

Pigmented villonodular synovitis (PVNS) or tenosynovial giant cell tumor is a rare, proliferative lesion of synovial tissue that is almost always monoarticular frequently involving. knee and foot. It has local invasiveness and if left untreated, PVNS can progress resulting in irreversible joint destruction (125,126). Pathologically macrophage, multinuclear giant cell

and hemosiderin accumulation is observed (127). Two forms of this disease are defined: localized and diffuse which are histologically similar but completely different in biological behavior, treatment strategy and prognosis (125). Diffuse PVNS have a high rate of local recurrence about 30–45 % due to restrictions of complete resection and the treatment should be individualized (18, 125, 126). Surgical resection with either synovectomy or joint replacement is the preferred treatment of particularly localized PVNS (128). Satisfactory results are achieved in intra-articular masses with advanced surgical techniques and arthroscopic resection (129).

EBRT or brachytherapy after synovectomy has been studied in by several authors resulting in a contraversy due to the possible toxicity, and variable outcome (128, 130,131). Therefore, common accepted approach is using 36-50 Gy RT in cases with diffuse disease, bulky disease resulting in bone destruction, or in multiple recurrences after resection (132). Pre- and postoperative MRI is needed to delineate target volume accurately and the final RT dose should be tailored to the amount of residual disease (18, 125).

Vertebral Hemangiomas:

Hemangiomas are benign proliferations of blood vessels. Lesions involving the vertebral body are associated with pain and may lead to severe neurologic symptoms in case of progression. In symptomatic cases surgical resection, transarterial embolization, vertebroplasty, or intralesional injections can be used (133). Surgical decompression is usually difficult due to risk of hemorrhage; therefore, postoperative irradiation is recommended. Primary radiation therapy reduces pain in vertebral hemangiomas also when administered alone (134). In a German multicenter trial, complete or partial pain relief is seen 90% of patients. Doses ≥34 Gy, generally 36-40 Gy with 2 Gy per fraction is recommended (135).

Diseases of Connective Tissue and Skin

Desmoid Tumors:

Desmoid tumors are low-grade, locally invasive, nonmetastasizing tumors of connective tissue that arise from muscle fascias, aponeuroses, tendons, and scar tissue. Mutations in the APC genes result in desmoid tumors in 10-20% of patients with familial adenomatous polyposis (FAP) and Gardner syndrome (136). Diagnostic MRI helps to estimate size and environmental infiltration prior to incisional biopsy. Desmoid tumors have no known potential for metastasis or dedifferentiation, however, they are locally aggressive and have high rate of recurrence even after complete resection (44).

Nonradiotherapeutic Approach: For stable and asymptomatic cases observation is a reasonable approach. Complete surgical resection is the goal of treatment. Extra-abdominal desmoids are deeply infiltrative, non-

encapsulated and merge into the surrounding muscle, therefore, postsurgical local recurrence can occur in 10% to 100% even after complete resection (137). NSAIDs alone or in combination with tamoxifen, raloxifene, and progesterone, have been widely used for treatment of desmoid tumors (44, 138). A variety of palliative chemotherapeutic regimens and imatinib also have been investigated (139, 140). Kasper et al. (141), demonstrated a 2-year progression free rate of 45% with imatinib in patients not amenable to R0 resection.

Radiotherapy: When surgery is not recommended due to location or infiltration of vital structures radiation therapy is a reasonable option. Spear et al. (142), compared surgery-alone, radiation-alone and combination in a retrospective study and reported 5-year local control rates for surgery, radiation therapy, and combined modality as 69%, 93%, and 72%, respectively. Janssen et al. (143), found that RT reduced the risk of local recurrence in patients with microscopically positive resection margins. This effect was even more significant for recurrent tumors with positive margins. However, no significant benefit of adjuvant radiotherapy was observed for patients with negative margins. In general, radiation dose of 50-60 Gy in 6-7 weeks with 1.8-2 Gy per fraction is adequate in a postsurgical supplementary intend, for inoperable or recurrent tumors 60-65 Gy should be administered (18). However, risk of increased posttreatment toxicity with RT doses >56 Gy particularly in young patients (≤30 years) must be considered (144). RT should be used with caution for intraabdominal tumors because of the dose and increased field size leading to increased risk of bowel injury (145).

Peyronie Disease:

Peyronie disease is a chronic inflammatory connective tissue disorder involving the penile tunica albuginea that results in tissue proliferation and the development of hard plaques, most commonly on the dorsal surface of the penis, which may cause a curvature and painful angulation of the erect penis. Typically, slow progression over several months is seen, with spontaneous remission rates of <15% (146). Oral agents discussed can stabilize the scarring process and may result in some reduction of deformity (147).

Radiotherapy: Treatment with radiation provide symptom relief particularly pain. Retrospective studies reported that benefit of RT is best in early stages of disease, while radioresponsive inflammatory cells and fibroblasts are still active. The recommended total dose is 10-20 Gy with 2-3 Gy daily fractions of using a direct ventral field (148).

Dupuytren Contracture:

Dupuytren contracture is a connective tissue disorder, also known as Morbus Dupuytren (MD) if palmar fascia in hands is affected or Morbus

Ledderhose (ML) if plantar fascia in feet is affected. Estimated incidence is 3-6% and increases after the age of 40. Any genetic predisposition or environmental factors which leads to local ischemia and free radical release consequently stimulates fibroblast proliferation and cytokine production. In the early stage, subcutaneous nodules appear. Cords develop as the disease progresses. Thickening of the fascia and progressive contracture causes curling of the fingers and toes.

Nonradiotherapeutic Approach: Excision of diseased cords and fascia considered the gold standard treatment for Dupuytren contracture (149). After open surgery, major complications and wound complications occurred in 15.7-22% of cases with a recurrence rate of 30-50% at 3 years even in case of excellent resection (44). During the early stage, steroids, allopurinol, nonsteroidals, vitamin E may provide response with short duration. Although Collagenase *Clostridium histolyticum* (CCH) injection has proven to be a useful minimally invasive technique for patients with later-stage DC, recurrence rates of 35% at 3 years of follow-up have been reported (150).

Radiotherapy: Radiotherapy is used in patients with <10-degree deformity and is considered standard of care to prevent progression. Low-dose radiotherapy inhibits fibroblast proliferation and differentiation into fibronectin. Patient-reported effective symptom relief with radiation is demonstrated in previous studies (151, 152). Betz et al. (151) found 87% patient-hand–reported disease progression-free survival at 13-year follow-up in patients with <5-degree deformity. Seegenschmiedt et al. (152) compared the safety and efficacy of two different dose regimens; 10 × 3 Gy via split course (5 × 3 Gy) repeated at intervals of 12 weeks (Group A); and, 7 × 3 Gy (21 Gy) delivered over 2 weeks (Group B). After a mean follow-up of 8 years, the extension deficit increased in 7% of the 21 Gy group, and 4% of the 30 Gy group. Acute toxicity was more pronounced in group B. Radiotherapy target volume should include palpable or detectable nodules and cords surrounded by a 3-5 mm margin; and appropriate immobilization and shielding of unaffected joints are required.

Keloids and Hypertrophic Scars:

Keloids are an excessive tissue proliferation after scars or skin injuries from any cause. Keloids and hypertrophic scars differ from each other by typical infiltrative growth pattern, causing local pain and inflammatory reactions, and sometimes long-term progression of keloids. Excessive production of fibrous tissue beyond the wound, becomes hyalinized and does not regress spontaneously is a keloid. Silicone bandages, pressure dressings, and cryosurgery have all been used to treat for keloids (44). Intralesional injections of corticosteroids, 5-FU, and verapamil directly into

keloid lesions provides symptom improvement. In patients treated with excision alone, recurrence rates range from 45-100%. Therefore, surgical treatment alone is not recommended (153), and typically combined with perioperative and postoperative injections to prevent fibroblast proliferation and consequential recurrence (154).

Radiation Treatment: Radiotherapy should be considered in cases of repetitive postoperative recurrences or in high-risk of recurrence such as marginal resection, large lesion and unfavorable location (155). RT can also be considered as the primary treatment when resection would result in functional impairment and in active proliferating disorders within about 6 months after the triggering trauma. As proliferating fibroblasts and mesenchymal / inflammatory cells are the target for RT, response of fully matured keloids is insufficient. Prophylactic RT within the first 24 hours is most effective and reduces the risk of recurrence to 15-25% in most series. Radiation therapy is usually given to a total dose of 10-15 Gy in 2-5 fractions in 1-2 weeks starting within 24 hours after excision (156). Generally, 10, 12, or 20 Gy delivered within 1 week over 2, 3, or 4 fractions, is effective and have good cosmetic results. The target volume is scar plus a 1-cm margin; lead shielding can be constructed to protect normal tissue (18, 157). Brachytherapy was found to have a lower recurrence rate compared to electron and x-ray therapy as postexcisional radiotherapy (158).

Keratoacanthoma:

Keratoacanthoma is a rapidly growing benign tumor that may be locally invasive. Complete excision with adequate margins is the treatment of choice. Radiation therapy is recommended for recurrences after surgery or expected poor cosmesis with surgery. The doses range from 35 Gy in 15 fractions to 56 Gy in 28 fractions (44).

Aneurysmal Bone Cysts:

These are benign expansile lesions forming cavities within the bone lined by proliferative fibroblasts, giant-cells, and trabecular bone consequently filling with blood. Treatment is primarily surgical. However, 30-60% recurrence rates are observed after surgery alone. Radiotherapy is reserved for lesions which are surgically inaccessible, difficult to obtain clear margin, continue to grow or repeatedly recur particularly in spine and pelvis lesions. Generally recommended doses are 12-32 Gy with 2 Gy per fraction over 2-3 weeks (159).

Heterotopic Ossification:

Heterotopic ossification (HO) is a common complication of hip arthroplasty, hip trauma, or acetabular fracture. Underlying mechanism is transforming of primitive mesenchymal cells in the surrounding soft tissues into

osteoblastic tissue consequentially forming a bone. HO also occurs around the femoral neck and adjacent to the greater trochanter. Hip stiffness is the primary symptom and the diagnosis is made radiographically (160).

Nonradiotherapeutic Treatment: Standard of care for HO is surgical excision followed by some additional interventions for prophylaxis. Effect of postoperative NSAIDs has been shown in a meta-analysis (161). Indomethacin is the most commonly used agent which is a prostaglandin synthase inhibitor that also suppresses mesenchymal cells. The limited data is available resulting in controversial results on benefit of selective cyclooxygenase-2 (COX-2) inhibitors (162,163). Bisphosphonates are other medical alternatives used in preventing HO if used at the appropriate time based on their effect of delaying mineralization of osteoid. However, the high cost of bisphosphonate compared to indomethacin interdicted the routine use (164).

Radiotherapeutic Options: EBRT has been used as an effective method for prevention of HO after total hip arthroplasty since 1970s. A single fraction of 700 or 800 cGy to the region at-risk is recommended to be delivered either within 4 hours preoperatively or within 72 hours postoperatively (19). Radiotherapy prior to surgery has advantages of improved patient comfort, ease of treatment management, and avoidance of possible postoperative complications associated with moving and positioning the patient (165). Typically, an AP-PA field cranial field border approximately 3 cm above the acetabulum and the caudal field border encompassing two-thirds of the proximal implant (19). A prospective study comparing radiation therapy and indomethacin found both approaches similarly effective in the prevention of HO (166). However, meta-analysis of seven randomized studies concluded that radiotherapy is more effective than NSAIDs (167). In terms of economic analysis radiation therapy is not cost-effective when compared to NSAIDs (168).

Heel Spur / Plantar Fasciitis:

Degenerative changes in the plantar fascia may cause “painful heel” often associated with a plantar heel spur or plantar fasciitis. Basic mechanism is degeneration of the fascia at the calcaneal insertion with chronic microtraumas caused by vertical compression (169). This leads to inflammatory tissue reactions and consequential thickening of the fascia associated with fluid collection (170). Bone formation “plantar heel spur” triggered by this inflammation is found in 11–16% of asymptomatic population (169). Suggested effective and safe fractionation schedule is 6 × 0.5 Gy twice weekly. Target volume definition and fractionation of LD-EBRT is still a matter of controversy. Lack of randomized studies comparing LD-EBRT with other conservative therapeutic approaches is another handicap (171).

Hormonal Disorders

Gynecomastia: Usually seen in patients receiving estrogens or flutamide, luteinizing hormone-releasing hormone (LHRH) agonists alone or in combination, patients undergoing orchiectomy Breast irradiation is not effective as a prophylactic modality if given after estrogen. Generally recommended dose is 9 Gy as a single dose or 3 times 4-5 Gy daily fractions (44). If radiation is planned with palliative intend for painful gynecomastia after diethylstilbestrol therapy recommended dose is 20 Gy in 5 fractions or 40 Gy in 20 fractions. Pain relief is obtained after two weeks to 14 months (175).

Radiation Induced Cancer Risk

The risk of the induction of secondary tumors was overestimated in the past (172). Trott and Kamprad attempted to estimate the risk of cancer induction with radiotherapy for benign diseases using the epidemiologic data from previous studies with long-term follow-up (173). Absolute lifetime risk of secondary malignancy is a result of a complicated mathematical model result including various modifying factors such as age at exposure and gender, individual inherent sensitivity, anatomic site, type of disease, doses of radiation, volume irradiated and treatment techniques (174). An important risk factor for radiogenic-induced cancer is the patient's age by the time the radiation exposure occurs. The risk is already reduced in the 3rd decade of the patient's life, it starts to decrease steadily from the age of 40 (176). To estimate the risk associated with much lower doses of LD-EBRT, mathematical models on the basis of epidemiological long-term observations of atomic bomb victims have been developed by the The International Commission on Radiological Protection (ICRP). ICRP estimates the incidence of cancer from ionizing radiation increases linearly and is about 5.5%/Sv. Thus, for a patient exposed to 20 mSv irradiation increases the lifetime cancer risk by about $0.020 \times 5.5/\text{Sv} = 0.1\%$.7 (1, 177, 178).

Overall, older adults treated with RT especially for benign diseases located in peripheral tissues, risk of RIC is very small and even reduces further with increasing age. Skin cancer is clearly a potential risk, but low and are likely to be treatable. The red bone marrow volume exposed to radiation should be minimum to minimize risk of leukaemia. Low- to intermediate-dose RT for non-malignant indications has a place in modern medicine based on considerable evidences for a range of specific indications. RT at these doses has few side effects and often provides good long-term control with consequential quality of life improvement (6, 179-181). Limitation and decreases in the use can be attributed to anxiety over increasing risk of a RIC. The risk is smaller in older age and for peripheral tissues. However, enhanced radiosensitivity and expected longevity must be taken into consideration for younger adults and particularly children (171).

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Chapter 12

THE IMPORTANCE OF AUTOPHAGY IN DIABETIC TESTICULAR INJURY

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Diabetes mellitus

Diabetes mellitus (DM) is an important health problem that is associated with many microvascular and macrovascular complications and involves hyperglycemia due to the problem in insulin secretion.(Verhulst, Loos, Gerdes, & Teeuw, 2019) Diabetes is divided into two classes as Type 1 and Type 2 diabetes. Type 1 diabetes is a chronic autoimmune disease in children and adults.(Rak & Bronkowska, 2018; Yi, Huang, & Zhou, 2016) At the same time, Type 1 diabetes involves damage to t cell-mediated pancreatic beta cells and requires exogenous insulin therapy. Approximately 5-10% of diabetes cases constitute Type 1 diabetes. In diabetes, type 1 diabetes accounts for 5-10% of cases.(Frese & Sandholzer, 2013) Type 2 diabetes is seen in adults and progressively and mainly develops insulin resistance, and is also a disease that causes abnormal insulin secretion. (Bajaj, 2018; Yi et al., 2016) The majority of diabetic cases consist of type 2 diabetes.(American Diabetes, 2010) DM is an important disease that causes damage to many organs and causes different diseases. DM has been shown to cause heart failure, Alzheimer's, diabetic retinopathy, diabetic nephropathy, liver damage, testicular injury and male infertility.(Hayden, 2019; Liang et al., 2013; Long et al., 2018; Music et al., 2015; Sinclair & Schwartz, 2019; Yap et al., 2019)

Testis and infertility

Testicle is one of the most important organs for reproductive in men. In addition to fetal sexual differentiation, testicles play a role in gamete production and secretion of many hormones.(Ilacqua, Francomano, & Aversa, 2018; Weinbauer, Luetjens, Simoni, & Nieschlag, 2010) Formation of gametes describes Spermatogenesis. Steroidogenesis is known as the production of steroid hormones. Spermatogenesis occurs in seminiferous tubules. Steroidogenesis occurs in the interstitial space between the seminiferous tubules.(Weinbauer et al., 2010) Adult healthy mammalian testicles are capable of producing millions of mature sperm daily.(Robb, Amann, & Killian, 1978)

Infertility is one of the important health probes that affect 10-15% of couples and prevents them from having babies. According to the data of the World Health Organization, 50-80 million people experience infertility problems.(Babakhanzadeh, Nazari, Ghasemifar, & Khodadadian, 2020) Male factors are said to account for 20-30% of cases in infertility.(Agarwal, Mulgund, Hamada, & Chyatte, 2015) Hormonal deficiencies, physical problems, sexually transmitted diseases, environment, lifestyle and genetic factors have an important place in male infertility.(Babakhanzadeh et al., 2020) It has been shown that it causes infertility in diabetes and Type 1 and Type 2 diabetes act by separate mechanisms. The proportion of diabetic

patients in sperm parameters was seen to be worse than a healthy person. Low ejaculate, mitochondrial damage and low sperm motility were observed in type 1 diabetes. In type 2 diabetes, it shows inflammation and decreased sperm viability and DNA fragmentation with increased oxidative stress. These show that diabetes will lead to infertility.(Condorelli, La Vignera, Mongioi, Alamo, & Calogero, 2018)

Diabetes and testis injury

Diabetes is stimulated by streptozotocin (STZ), a glucosamine-nitrosourea compound used clinically as a chemotherapeutic and antimicrobial agent in the treatment of pancreatic β cell carcinoma. STZ is selectively toxic to beta cells in the pancreas.(Gu, Lian, Sun, Gao, & Fu, 2018; Lenzen, 2008) STZ is used to create both type 1 and type 2 diabetes.(Wu & Yan, 2015) In STZ-induced diabetes, hyperglycemia, decreased body weight, increased apoptosis, increased reactive oxygen species, and lipid peroxidation and oxidative stress are noted in the testicular tissue. Reactive oxygen species stimulate apoptosis. (Long et al., 2015) In STZ-induced diabetic testicular damage, there is also a decrease in seminiferous tubule diameters, thickening in the basement membrane and spermatogenic series deterioration.(Sisman et al., 2014) In different studies, testosterone levels have been shown to decrease when diabetes is created.(Zha et al., 2018) Diabetes has been shown to reduce sperm amount and motility.(Afifi, Almaghrabi, & Kadasa, 2015) Pro-inflammatory cytokines play an important role in systemic inflammation in diabetic conditions. These proinflammatory cytokines increase in diabetic conditions.(Kong, Sudirman, Hsu, Su, & Kuo, 2019) At the same time, endoplasmic reticulum stress (ERs) increases in the diabetic testis. With this increase, it leads to leydig cell damage and inhibition in spermatogenesis. Increased ER stress causes apoptosis.(Du et al., 2018) It has also been noted that diabetes will also lead to erectile dysfunction.(Cui et al., 2018) In another study, autophagy was shown to be suppressed in diabetic mice and this suppression causes apoptosis.(Liu et al., 2019)

Autophagy

Autophagy is an intracellular lysosomal event to ensure cell death or survival through the breakdown of organelles and proteins so that cells can survive and function. It is an important process in the continuation of cellular homeostasis and meeting the energy needs.(Czaja et al., 2013) Autophagy was first found in yeasts. It occurs in the state of hunger and to reduce nutritional stress. Autophagy is generally 3 types, chaperone mediated autophagy, micro autophagy and macro autophagy. In general, autophagy also includes autophagosome formationThe following processes play a role in autophagy; de novo double membrane structure or formation

of fagoffor, elongation of lipid membrane and coating of intracellular load to form autophagosome. Later, autophagosome and lysosome combine to form the autolysosome. This provides components to break down and require energy to maintain cell homeostasis.(Hale, Ledbetter, Gawriluk, & Rucker, 2013) ATG4B separates the C-terminal 22 residues of the leading LC3 (proLC3), which produces microtubule-associated protein 1 light chain 3 (LC3-I). Cytoplasmic LC3-I is then conjugated by ATG3 with phosphatidylethanolamine (PE). Lipidized LC3 (LC3-II) selectively adds to the autophagosomal membrane. LC3-II, which is associated with the outer membrane, takes place until autophagosome is formed. LC3-II, associated with the inner membrane, disappears when autophagolysosome is degraded by lysosomal prostheses. LC3-II is known as an autophagy marker. (Fader, Sanchez, Mestre, & Colombo, 2009; Hale et al., 2013; Jager et al., 2004) Beclin 1 contains a BH3 domain, which is contributes to the continuation of autophagy.(Sinha & Levine, 2008) Beclin1 contributes to the positive regulation of autophagy, and takes part in the correct formation of autophagosome in case of starvation.(Wei et al., 2015) P62 is a selective receptor for autophagy.(L. Wang et al., 2019) P62 interact with LC3-II. They are broken down later in autophagosome with the effect of proteases. However, if there is a deterioration in autophagy, P62 accumulation will occur.(Ichimura & Komatsu, 2010)

Autophagy and Diabetic testicular damage

Autophagy plays an important role in protecting against testicular damage occurring in hyperglycemic or diabetic conditions.(Liu et al., 2019) Cellular autophagy is inhibited and disrupted in diabetic conditions. (Sato, Kataoka, Kimura, & Mukai, 2016) Autophagy being excessive or insufficient leads to germ cell degeneration.(Y. Wang et al., 2014) Autophagy has been shown to delay apoptosis in testicular leydig cells.(Y. Wang et al., 2014) In another study, it was observed that apoptosis increased with increasing testicular damage in diabetes. But when they applied metformin, they showed that apoptosis inhibition and autophagy increased. Thus, they showed that testicular damage was reduced.(Liu et al., 2019) It is also excessively present in Leydig cells with autophagy testosterone production. It has been shown that testosterone production will also be lost if autophagy is impaired.(Gao et al., 2018) In testicular damage caused by both diabetes and other agents, events such as decrease in sperm parameters, deterioration in seminiferous tubules and increased apoptosis have been observed.(Bashir, Shagirtha, Manoharan, & Miltonprabu, 2019; Corrêa, Costa, Ribas, Boaventura, & Chagas, 2019; Mardanshahi, Rezaei, Zare, Malekzadeh Shafaroudi, & Mohammadi, 2019) In another study, it was observed that autophagy decreased in the testis with high dietary fructose application.(El-Mehi & Faried, 2019) In another study in rat testicles, auto-

phagy has been shown to be involved in testicular damage caused by chemotherapy.(Cha et al., 2018) They found that autophagy increased by P62 with resvaratrol treatment in testicular injury in type 1 diabetic mice. They also found that inflammation decreased and antioxidant capacity increased over this pathway.(Zhao et al., 2018) Some of the important pathological changes in the testicles of diabetic rats have shown that endoplasmic reticulum stress increases, inflammation increases and autophagy decreases. However, when rapamycin, which is used as an autophagy activator, is applied, it has been found that autophagy increases and protects the testicular tissue.(Shi W, Guo Z, & R, 2019) Another study with STZ-induced diabetic testicular injury has shown that excessive autophagy occurs in the testicle, causing damage to germ cells. In this study, *Lycium barbarum* polysaccharide (LBP) reduced testicular damage by inhibiting the level of Beclin-1 and LC3-1 mRNA expressions, which play an important role in autophagy.(Shi et al., 2018) In diabetic studies, activation of the target of rapamycin (mTOR) pathway that inhibits autophagy (mammalian) damages germ cells and causes testicular damage. It has been reported that it plays a protective role in diabetic testicular injury due to autophagy activated by inhibition of the mTOR pathway in Type1 and Type2 diabetes. (Shi et al., 2017)

Conclusions

Autophagy has been shown to contribute significantly in the pathophysiology of diabetes. It has been stated that it may play an important role in sustaining the life of cells in testicular injury caused by diabetes, in cell death and in maintaining testicular function.(Liu et al., 2019) While excessive increase in autophagy appears to increase in cell damage, insufficient autophagy also has a negative effect on the survival of cells.(Y. Wang et al., 2014) It has been observed that increasing the level of autophagy at certain levels is important for cellular continuity and contributes to male fertility. For the benefit of autophagy, more studies on autophagy activators and inhibitors can minimize cellular damage. The discovery and testing of new medicines can shed light on the development of therapeutic approaches for damage caused by diabetes.

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Chapter 13

Oral Manifestations in Ellis-van Creveld Syndrome- A Rare Case Report and a Literature Review

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INTRODUCTION

Ellis-van Creveld syndrome (EvC) is a very rare congenital genetic disorder with autosomal recessive inheritance. It was first discovered in 1940 by Richard W.B. Ellis and Simon van Creveld. It is known as chondroectodermal dysplasia or mesoectodermal dysplasia. Chondroectodermal is the term used to describe the type of tissue involved in the disease, mainly consisting of skeleton, nails and long bones. Mesoectodermal dysplasia is another term used to emphasize the congenital heart defect that is associated with the disease in 60% of cases.[1] This cardiac defect is usually associated with the atrial septal defect (ASD), but usually, the only single atrium is present in patients.

The disease is the result of mutations in the EVC1 and EVC2 genes that occur in the 4p16 chromosome.[2] This rare disorder is characterized by; bilateral postaxial polydactyly in hands, disproportionate dwarfism with distal limb shortness, congenital heart disease, and ectodermal dysplasia.[3,4] Approximately 150 cases have been described in the literature. [5,6,7] It is generally reported that the frequency of this syndrome is 1/60000 globally and there is no gender difference. Patient's intelligence is normal.[7,8] The presence of problems such as abnormal frenulum attachments, hypodontia, malformed teeth, malocclusions causes this syndrome to be related to childhood dentistry. Natal and neonatal teeth, delayed onset of permanent teeth, high caries rates are also encountered.[1,9] The most important oral finding in EvC syndrome is the width and multiplicity of labial frenulums in the upper and lower jaws, the absence of gingival sulcus, submucous clefts, dystrophic filtrum, and deep palate.[9,10] Apart from these findings; taurodontism, enamel invaginations, tooth geminations, supernumerary teeth, mesiodens have also been reported. [11,12]

The aim of this article is to evaluate the clinical and radiological findings of a patient with EvC syndrome and to present the patient's oral findings.

Case Report

A seven-year-old girl referred to our clinic with the complaint of abnormal tooth structure. She was the first child of her healthy parents who did not have consanguineous marriage. Firstly, an informed consent form was obtained from the parent of the patient. In her medical history, we learned that she had an atrial septal defect. The patient was short stature (98 cm), prominent ears, low shoulders, and narrowed thorax. The extraoral examination revealed that the head size was larger than the body and the hair was of normal quantity and quality. There was no obvious asymmetry on her face. (Fig. 1) When she applied to our clinic, she had already had polydactyly and it was learned that sixth fingers were recently amputated.

In skeletal radiology of the upper extremity, short metacarpals, ulnar thick fifth metacarpal, ulnar polydactyly were observed. (Fig. 2) The patient's IQ level was normal. Chest radiography showed cardiomegaly. (Fig. 3)

Fig. 1: Patient with Ellis-van Creveld syndrome.

Fig. 2: Radiography of hands showing hexadactyly and small fingers.

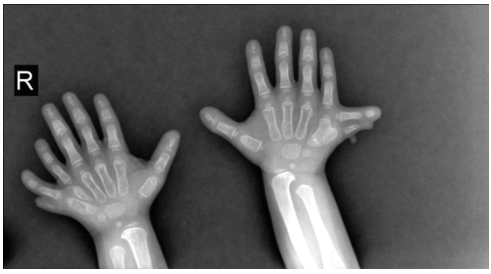
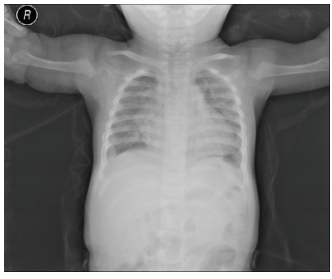


Fig. 3: Chest radiograph showing cardiomegaly.



Orthopantomography showed that mandibular permanent central and lateral incisors, maxillary permanent lateral tooth germs did not occur at all. (Fig. 4) In intraoral examination abnormal frenulum attachments in maxilla and mandible, irregular clefts in the maxillary and mandibular anterior region, and presence of hypodontia were observed. It was seen that

the eruption of the first permanent molar teeth was delayed. There was no evidence of any tooth extraction and spontaneous exfoliation. A conical dental entity_apparently a neonatal tooth_ was observed in the mandibular anterior region. The patient had fissure caries in her deciduous molars. [Fig. 5. (A, B)] The maxillary primary central incisors and all the primary molars were deeply fissured and malformed. Maxilla was found to be slightly hypoplastic. Based on history and clinical examination, Ellis-van Creveld syndrome was diagnosed.

Fig. 4: Orthopantomography showing the absence of incisors and delayed eruption of teeth.

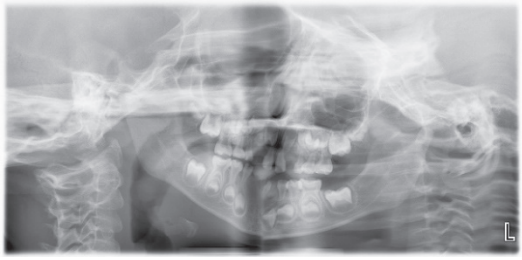
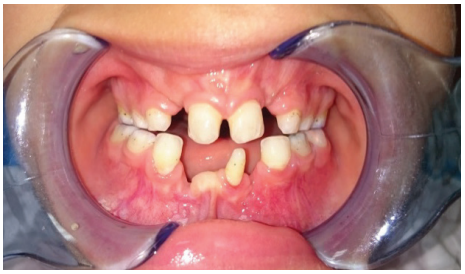
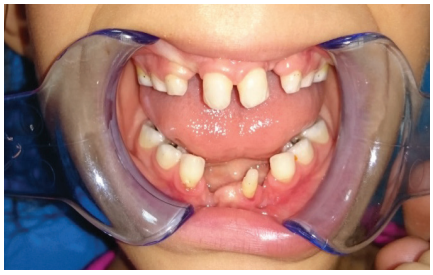


Fig. 5. (A, B) Intraoral photograph showing clefts, hypodontia, neonatal tooth, and decayed teeth.

A

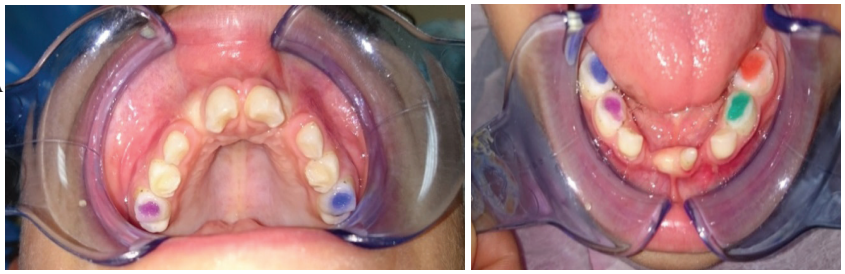


B



Decayed teeth were restored with a compomer filling material. [Fig. 6. (A, B)] It was explained to the parents that neonatal tooth extraction should be performed, along with the implantation of a prosthesis, in order to eliminate tooth deficiencies. Because her parents rejected the tooth extraction, the prosthesis could not be applied.

Fig. 6. (A) Intraoral photograph of malformed teeth. (B) Teeth of the patient restored with colored compomer filling material on her request.

A 

DISCUSSION

EvC is an autosomal recessive skeletal dysplasia. There is no sex predilection, in our case, it is a female. Ellis-van Creveld syndrome can be seen at rates ranging from 1/60,000 to 1/200,000. It is difficult to predict prevalence because the syndrome is very rare in the general population.[13]

This is more common in the Amish population in Lancaster County, Pennsylvania.[12] Characteristic features of the syndrome are bilateral postaxial polydactyly, acromegalic dwarfism, ectodermal dysplasia, malformation of dental issues, and high frequency of congenital heart disease.[14] All of these features were present in our patient.

There is a short-lived disproportionate dwarfism of limbs, shortened in proportion to the trunk in patients with EvC. Unlike hereditary ectodermal dysplasia, the skin is not affected in EvC, sweating is normal. Hair is sparse or normal, the nails are usually small and dystrophic.[7] In our case, disproportionate extremities, short stature, and polydactyly were detected. Meanwhile, the hair length of the patient was normal, while the eyebrows were very thin and sparse. Approximately 30 % of cases have parental consanguinity.[15] Parental consanguinity was not the issue for this patient and there was also no similar sister or family member story.

The newborn tale may include skeletal anomalies at birth as small size, slow growth, and initial symptoms. Natal teeth may be present.[16] In our case, there was a positive newborn story in terms of low birth weight, slow growth, skeletal anomalies, and there was also a natal tooth. Congenital heart defects occur in about 50 % of cases. The most common cardiac defect is the atrial septal defect, while the other is the ventricular septal defect (VSD) and aortic hypoplasia.[7] Presence of atrial septal defect confirmed in our case. Although most patients with EvC have normal intelligence, mental retardation and central nervous system abnormalities have been reported in some cases.[17] Our patient had a normal IQ level.

The absence of gingival sulcus, submucous clefts, dystrophic filtrum,

and deep palate are remarkable findings in patients with EvC. Also, wide labial frenulum defined as partial harelip, multiple small accessory frenula, ankyloglossia, malocclusion, conic-microdontic teeth, hypodontia (usually without permanent mandibular central and lateral incisor teeth) and enamel hypoplasia can be seen.[7,18] Other minor or variable findings include delayed eruption, supernumerary teeth, tooth fusion, dysmorphic roots, taurodontism, wide corrugated abnormal occlusal anatomy, and atypical cusps.[4]

Intraorally, especially in the mandibular anterior segment, the presence of natal and natal teeth and congenital absence of teeth may be seen. Erupted teeth are often malformed and therefore easily affected by caries.[9] In the present case, a natal tooth was present in the mandibular anterior region. Particularly in the jaws where the clefts are observed, permanent tooth germs are congenitally absent. The teeth eruption were delayed, also, size and shape anomalies were observed. All the positive findings of the patient confirmed the EvC diagnosis.

The differential diagnosis of Ellis-van Creveld syndrome includes Weyer's acrodental dysostosis (Curry-Hall syndrome), asphyxiating thoracic dystrophy (Jeune syndrome) and orofacial digital syndrome (OFDS). The features of Ellis-van Creveld syndrome overlap with Weyers acrofacial dysostosis. Similar to Ellis-van Creveld syndrome, Weyers acrofacial dysostosis involves tooth and nail abnormalities, affected people have less pronounced short stature, the delayed fusion of mandibular symphysis and do not have any heart defects. The mutations in the same genes have caused these two conditions.[5] EvC syndrome and Weyer's acrodental dysostosis (Curry-Hall syndrome) are allelic conditions caused by loss of function mutation in EVC and EVC2.[12] Asphyxiating thoracic dystrophy (Jeune syndrome) is a rare, potentially lethal, autosomal recessive disease; characterized by thoracic dystrophy, short limbs which are rhizomelic rather than mesomelic, small stature, polydactyly, and generalized bony dysplasia. Patients with Ellis-van Creveld syndrome and asphyxiating thoracic dystrophy have similar features in hand, pelvis, and long bones. Hence, it is not possible to differentiate between two conditions radiographically. Cardiac anomalies, hypoplasia of nails, the fusion of upper lip to gingiva and neonatal teeth are present in Ellis-van Creveld syndrome. Renal failure with hypertension is present in asphyxiating thoracic dystrophy which will help in distinguishing these two disorders. Presence of multiple gingivolabial frenula is similar to both Ellis-van Creveld syndrome and orofacial digital syndrome. Ankyloglossia, moderate mental retardation, hypoplastic nasal cartilage, and fissured tongue helps to differentiate orofacial digital syndrome from Ellis-van Creveld syndrome.[5,12]

Patients with EvC syndrome with a congenital heart defect are at risk of infectious endocarditis and should be considered to be a triggering fac-

tor for endocarditis if periodontal diseases are not treated, and dental treatment should be performed under antibiotic prophylaxis.[19]

CONCLUSION

The diagnosis of Ellis-van Creveld syndrome depends on clinical and radiological evaluation. Because of both the age of the patient and the absence of deep caries lesions, primary molars were restored with filling material. Oral hygiene information has been given to the family in terms of the importance of the child's oral-dental health. Patients should be routinely called to the controls and the development of teeth and jaws should be monitored. Early diagnosis and treatment of the dentist have an important place when the mouth symptoms are taken into consideration.

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Chapter 14

RETREATMENT OF FAıLED ROOT CANAL THERAPY

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The first stage in the clinical application of retreatmented root canal therapy is the requirement to reach to the apical of the canal. Then, all the principles of endodontic treatment are valid for the completion of renewed root canal treatment. It is necessary to ensure coronal access, remove all previous root canal sealing materials, remove the canal obstruction and remove barriers to reaching full operating size. Only then can irrigation, shaping and sealing processes be carried out as required.

The process stages of renewed root canal treatment are as follows;

• Providing coronal access to the canal,

• Access to the apical of the canal (Removal of the post, removal of the canal sealing material, removal of broken tools, removal of the canal obstruction)

•Shaping the canal,

• Intra-canal irrigation and medication use,

• Sealing the canal,

• Tooth restoration ,

Preparation of The Coronal Access Cavity

The purpose of the access cavity preparation is to provide direct access to the root canal system while preserving as much tooth tissue as possible. For the endodontic access cavity, the first step is to remove the coronal restoration (1, 2). Following the first endodontic treatment, full crown restoration and post-core support are seen in many teeth. The physician and the patient decide together for the removal or protection of the full crown. If the restoration is damaged, it is necessary to remove it, but if the crown is in good condition, the need to remove it should be evaluated. If the restoration is maintained, the cost is lower and the isolation becomes easier, occlusion is preserved and its aesthetic change does not happen much. However, due to the limited visibility with the current crown, the possibility of an iatrogenic error may increase. In addition, it will be more difficult to remove the canal obstructions such as tool fracture, canal obstruction, and post and the physician will be more likely to miss important conditions such as secondary caries, broken instruments or additional canals (3).

It will be useful to use magnification and lighting tools in determining the localization of the canals at the base of the pulp (4). It will also assist in detecting possible dentin colorings and extra canal accesses. However, staining and transilumination methods can be used to determine the localization of calcified canal mouths. During the access cavity process, to avoid damage, it may be useful to use non-sharp burs and ultrasonic devices on the base of the pulp chamber (5).

Providing Access to The Canal Apical

Removal of Post Systems

Root canal treated teeth are restored with post-containing crowns due to excessive crown damage. Today, prefabricated post types are used in prefabricated and cast post types due to their advantages such as time and cost (6). Prefabricated posts are subdivided according to their shape, design and material; It is divided into conical or parallel to shape, active (screwed), passive, perforated, grooved according to the design, stainless steel, gold, titanium, ceramic, zirconium, fiber reinforced composite according to its material.

Since active posts are placed by screwing them into the canal, they are very difficult to remove from the canal. Zirconium and ceramic skins are hard and fragile, and these skins are very difficult to remove.The type of cement that the post is attached to the root canal also affects its removal. The posts, which are cemented with composite resin and dentin bonding agents, are much more difficult to remove than those that are cemented with conventional cements.In addition, the location of the tooth to be removed in the arc affects the difficulty of processing.Post removal is more difficult due to the difficulty of accessing the teeth positioned lower and the distance between the opposite arc (7). One study has shown that heat generation by ultrasonic vibration can help reduce the retention of cements adhered with cement (8). However, concerns about heat-producing periodontal ligament damage can prevent this technique (6).

Removal of Root Canal Sealing Materials

Root canal sealing material consist of canal pastes, cements, gutta-percha, core carrier systems and silver cones.

Root canals can be sealed with different materials and techniques. At this stage, the priority is to determine the canal sealing material with diagnostic tools such as visual and radiography. Thus, suitable techniques and materials can be planned to remove the determined sealing material.

Mechanical and chemical methods such as heat, solvents, ultrasonics, hand files and Ni-Ti rotary instruments, lasers are used to remove root canal sealing (9-12). These methods can be used individually or in combination.

Removal of Gutta-Percha

Gutta-percha is the most used root canal sealing material. The gutta-percha is generally used together with cement or canal paste for canal sealing. The quality of the sealing technique is very important in removing the gutta-percha. For example, in oval ducts filled with single cone tech-

nique or in ducts with poor condensation, gaps occur between the cone and canal walls. In such cases, it is easy to remove the gutta-percha from the root canal (13). If the gutta-percha in ducts that are well condensed is first softened with heat or solvents, a place is prepared for the tools to be used. The gutta-percha density of each manufacturer is different from each other. Production time is also important, the worn gutta-percha becomes more fragile.

Removal of Gutta-Percha with Heat

In order to soften the well-condensed gutta-percha, heat carrier device tips or plugers can be used by direct contact. In addition, heated hand tools can be used for this purpose.The disadvantages of hand tools that they lose heat very quickly are that they need to be heated throughout the process. In addition, electrical devices such as Touch's Heat (SybronEndo, Orange, CA, USA) or System B (SybronEndo, Orange, CA, USA) are also used to soften gutta-percha with heat (13). When working with these tools, it is necessary to avoid periodontal tissue damage due to excessive heat (14-16). For this reason, it is convenient to use the heated tools in the canal for a short time and only on the flat parts of the canal. During the use of rotary tool tips and ultrasound devices without irrigation, gutta-percha can be softened with the heat caused by friction (17-19).

Removal of Gutta-Percha with Solvent

Materials such as chloroform, xylene, eucaliptol, halothane, benzene, turpentine, orange oil, and carbon tetrachloride are used to dissolve gutta-percha (20-22).

The most effective, fastest and most frequently used chemical solvent is chloroform in removing gutta-percha from the root canal (20). Although it is carcinogenic, it continues to be used in dentistry.

In case of contact, it is cytotoxic for periapical tissues. However, with careful clinical use, risks caused by cytotoxic properties can be reduced (21). Solvents are generally cytotoxic to a certain extent and unnecessary use should be avoided. Some solvents such as eucaliptol, xylene, turpentine, ethyl chloroform, halothane, methyl chloride, and orange oil have been studied, which show less toxicity than chloroform. According to the studies on this subject; It is less cytotoxic than xylene chloroform, but the effect of dissolving the gutta-percha is less and slower than chloroform. Eucalyptol is the solvent with the least gutta-percha solvent activity. Turpentine is more cytotoxic than chloroform and its odor is very sharp during use. It has been reported that the cytotoxicity of methyl chloroform is less than chloroform and gives more effective results, but its effect is longer. Although there are studies suggesting orange oil, its effect is slower than

chloroform. Although the effectiveness of halotene gives similar results to chloroform in one study, its effect is slower than chloroform in most studies. In addition, it is not common to use because it is costly and volatile (20-22).

In a study, it was reported that the use of solvent is more effective and faster than the use of heat in softening the gutta-percha (20).

Some studies have shown that using hand files or Ni-Ti rotary tools together with solvent can be used to remove gutta-percha in the root canal (18, 23, 24). In some studies on this subject, they reported that gutta-percha in the root canal cannot be completely cleaned without using solvent (25-27). However, in some studies, they have shown that the walls of the canal are covered with a thin layer of gutta-percha film after the use of solvent in the renewed treatments of the root canals (28, 29). After removing the root canal sealing material, the canal should be filled with solvent and then the paper and the solvent should be removed from the canal.

Thus, it will support the removal of the canal paste and gutta-percha film layer attached to the irregular parts of the canal (30).

Removal of Gutta-Percha with Sonic and Ultrasonic Systems

The gutta-percha in the ducts filled with single cone technique or insufficient condensation can be removed by ultrasonics. In this method, the retention of the gutta-percha is reduced by means of ultrasonic vibration and irrigation, and it is taken out of the canal.

Removal of Gutta-Percha by Lazer

Using Gates-Gliden, K-type file and Nd: YAG laser, gutta-percha removal activities were evaluated and it was reported that gutta-percha was removed in a shorter time compared to other methods used with Nd: YAG laser. In addition, it has been reported that a large number of dentin canals are also blocked (12). Some researchers have reported that caution should be exercised in case the heat generated during the use of lasers can damage peridontal tissues (12, 31, 32).

Removal of Gutta-Percha with Hand Files

Manual hand files are widely used in the removal of gutta-percha from the root canals. For this purpose, the most preferred H-type and K-type hand files.H-type hand file is placed by turning a quarter turn between the cone and the canal wall, After the compression is felt, the gutta-percha is removed by pulling it out. After the use of the file is finished, the process is repeated with a large number of files until the gutta-percha is exhausted in the canal (33). In addition, H-type files can be used to remove the gutta-percha that protrudes from the canal to the periapical region. The file is

turned clockwise to compress the gutta-percha overflowing from the apical to 0.5-1 mm. Then, the file is quickly pulled towards the coronal and the flood is removed. This technique is generally successful, but it is necessary to avoid applying excessive force to the file to reduce the possibility of the subject being pushed deeper or the file is broken (34). Although K-type files and tirnerfs can also be used for this purpose, the tirnerfs are prone to breakage due to their protruding structures and non-durable metal structures. In addition, rigid files such as C File (Dentsply Maillefer, Johonson City, TN) can be used for this process. These files are produced by turning the square-shaped stainless steel wire around itself. Since these files are rigid and their ends are sharp, they can penetrate the gutta-percha area more easily than other manual hand files (35).

Removal of Gutta-Percha with Nickel Titanium (Ni-Ti) Rotary Tool Systems

Ni-Ti rotary file systems were put into use in the field of endodontics in 1990 and have survived to the present day.Ni-Ti alloy elasticity module is very low and it is a material that is very resistant to breakage due to its ability to form easily. Today, the development of Ni-Ti systems that work with high torque and low speed micromotors or endomotors of the same feature increases the success and speed of endodontic treatments (36). Therefore, development studies continue and different companies produce many Ni-Ti file systems with different features. Therefore, the cutting efficiency, flexibility and durability of each system differ. The advantages and disadvantages of Ni-Ti rotary tool systems are (37);

Advantages:

• Since the ends of most tools are rounded, it is unlikely that adverse conditions such as perforation, step formation, transportation will occur.

• Root canal preparation can be done quickly with few tools.

• Possibility of aspirating or swallowing canal device is less.

• It can even be used for shaping extremely inclined root canals.

Disadvantages:

• There is a risk of breaking the root canals when using the tools in the root canals, when the conditions or duration of use are not complied with.

• The use of tools requires careful work.

• Its cost is higher than hand files.

There are numerous endodontic studies in the endodontics literature that investigate the canal forming activities of Ni-Ti file systems. Today,

there are also studies evaluating the properties of Ni-Ti systems to remove canal fillers (18). The reasons why Ni-Ti rotary tools are preferred more are (38, 39, 40);

• Ni-Ti rotary tools are easier to use than hand tools,

• Comparing findings with hand tools in terms of evacuation and shaping of the root canal, obtaining comparable findings,

• More reliable use in curved root canal than stainless steel tools,

• Removing duct sealing material much faster than hand files.

However, in some studies, filler residues were detected on the dentin surfaces of the canals whose root canal sealing was removed with Ni-Ti files andbroken tools were also found in some canals (41-43).

In recent years, many studies have been conducted to evaluate the effectiveness of removing the gutta-percha from the canal with different Ni-Ti rotary instrument systems (44-47). Some of these systems are;

- ProFile (Dentsply Maillefer, Ballaigues, Switzerland),
- Quantec (SybronEndo, Orange, California, USA)
- System GT (Dentsply Maillefer, Ballaigues, Switzerland),
- RaCe (FKG Dentaire, La chaux-de-Fonds, Switzerland),
- Hero Shaper (Micromega, Besançon, France),
- K3 (SybronEndo, Orange, California, USA),
- ProTaper (Dentsply Maillefer, Ballaigues, Switzerland),
- Mtwo (VDW, Münih, Germany),
- Endosequence (Brasseler, Savannah, Georgia, USA),
- Liberator (Miltex, Inc. York, USA),
- Lightspeed (Lightspeed, Texas, USA)

Some studies on the removal of the root canal sealing have revealed that physician and patient fatigue are reduced with a shorter processing time, while some have demonstrated that Ni-Ti rotary instruments are superior to hand files because of the fewer complications from the procedure (18, 48).

As for the removal of gutta-percha from the root canal, some studies have found hand files more efficient (25, 49), while others have reported no significant difference between Ni-Ti rotary tools and hand files (43, 50, 51).

In the recent years, new generation Ni-Ti file systems developed specifically for use in root canal renewal have been introduced. Some of these are those;

- ProTaper Universal-R (Dentsply Maillefer, Ballaigues, Switzerland),
- ProTaper Next-R (Dentsply Maillefer, Ballaigues, Switzerland),
- Mtwo-R (VDW, Münih, Germany),
- R-Endo (Micro-Mega, Besançon, France)
- D-Race (FKG Dentaire, La chaux-de-Fonds, Switzerland)
- Self Adjusting File ((ReDent Nova, Ra'anana,Israel)
- XP-endo Finisher (FKG Dentaire, La chaux-de-Fonds, Switzerland)

Recent studies in mechanical techniques applied to remove root canal sealing material are in the combined use of instruments (52, 53).

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Chapter 15

SPIRITUALITY AND NURSING

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INTRODUCTION

In holistic approach, humans are creatures who spiritual, sociocultural, emotional, intellectual and physical dimensions. These dimensions are interdependent and related to each other (Baldacchino, 2006; Daştan and Buzlu, 2010). Humans possess needs in each dimension. In order to be healthy and have a high quality of life, these needs are required to be satisfied (İnce and Akhan, 2016; McSherry and Jamieson, 2011).

Despite the fact that "spiritual care", which is an important aspect of holistic nursing, is a new concept, nursing theoreticians started to develop an interest in individuals' spirituality towards the end of 1960's. Herein, Travelbeenin (1971) underlined the importance of each level of the caring process, denoting that, "A nurse not only gives care to individuals to diminish their physical pain or in physical sense, but gives holistic care to them." (Daştan and Buzlu, 2010; Kavas and Kavas, 2014).

It has been reported in the literature that spiritual care often starts with "an affectionate approach" and that only such care is able to fully hear and respond to the needs of human spirit. Especially, in situations such as trauma or illness, this unique support can be given by supporting an individual when he/she wishes to pray or engage in any form of religious rite, or simply by being an active listener when required (Herlianita et al., 2018; Tirgari et al., 2013). The number of studies about the importance of spiritual care for patients and healthcare professionals has increased; however, the use and implementation of research findings in practice have not yet reached an adequate level (Balboni et al. 2010; Ross et al. 2018; Selman et al., 2018).

Spirituality

Spirituality, beyond a formal religious commitment, involves an individual's endeavor to understand life and death, embrace his/her place in the universe, and the elements which are his/her stated purpose for living (Arslan and Şener, 2009; Mcsherry, 2000).

The word "Spirituality" originates from the Latin word "spiritus" meaning "to breath, to be alive" (Strang et. al., 2002). While this word means spiritualism in Western languages, TDK (Turkish Language Institute) explains the meaning of it as "sensible, intangible, unfleshly, abstract" ("Türk Dil Kurumu", 2018). "Spirituality", which is the struggle for searching an individual's purpose of life, his/her place in the universe, the relationship with himself/herself and other people, and embracing them, is a result of the knowledge gained throughout life time (Çetinkaya et.al., 2007).

Spirituality is broadly defined as "a part of the ontological base of nursing care and an important humanistic dimension in human health and

prosperity" (Reed, 1992) and it is accepted as an inseparable and fundamental part of nursing.

The Relationship Between Spirituality and Health

Observing the individual with a holistic approach in terms of health revealed the existence of his/her spiritual aspects as well as his/her social, emotional and physical characteristics, thus, a great importance should be given to this field. The studies evaluating the spiritual dimension of the person demonstrated that spirituality has a serious effect on the health of the individual (Kostak, Çelikkalp & Demir, 2010).

One of the factors that positively affect health is the state of spiritual well-being (Eğlence and Şimşek 2014; Sağkal et al., 2017; Yılmaz 2011). Spiritual well-being includes processes such as the effort to understand the purpose of life, realizing the connections that life possesses with higher powers. Individuals in a good spiritual well-being realize themselves, maintain peace of mind and be satisfied with their life (Cooper et al., 2013; Sağkal et al., 2017).

Spirituality and Spiritual Support in Nursing Care

Standard practices for spiritual care targeting nurses has not been developed yet because spirituality is an abstract concept within nursing care and considered as a subjective situation (Kavak et.al., 2014).

In nursing care given to patients by nurses, it is necessary to know spiritual needs of patients and to provide the support compatible with the needs of patients is important (Govier, 2000; Kostak et. al., 2010). In the state of illness, people sometimes resist, sometimes accept the situation and do not fight with it, and sometimes they struggle to defeat the illness. In the state of illness and in stressful times, it is a need to strengthen the beliefs of the patient. In such times, spiritual care provided by a nurse might create a quite healing effect. Therefore, nurses who spend a great amount of time with patients must have competence, ability, and communication about spiritual needs at a professional level (Ergül and Bayık, 2004; Greasley et.al., 2001; Martins et.al., 2015; Pesut 2002).

Nurses should effectively provide spiritual care by combining the importance of spirituality with their caring experiences while considering awareness and flexibility (Swinton and Pattison, 2010). Thus, showing flexibility to a patient and being aware of his/her spiritual needs make it possible to increase the nurses' caring practices and their empathy with the patient. Spirituality, shortly, is a search for hope. In the search for hope, nurses should solve the structure of purpose and demand in their relationship with patients and afterwards, they should technically convey their experiences to their patients in order to provide a better care. Herein, sharing

the nurses' experiences with patients increase spiritual activity for the sake of keeping the patients' hope for life.

The Place of Spirituality in International Council of Nurses (ICN)

In explaining the roles of nurses, the International Council of Nurses Code of Ethics also mentions spirituality in the article "nurses must respect the human rights, values, traditions and spiritual beliefs of the individual, family and society". Nurses should not give random care while caring for individuals, and they should provide care after they determine the current status (psychological, biological, social and spiritual) and needs of each individual. Nurses should take an active role throughout this process (Aştı et al., 2005; Baldacchino 2006; Ergül and Bayık 2004).

The Place of Spirituality in American Nurses Association (ANA)

Spirituality mentioned in "The Standards of Psychiatric-Mental Health Clinical Nursing Practice" published by ANA in 1994. According to these standards, spirituality is a factor that can affect emotional, mental and well-being of society, family or an individual, therefore, the environmental, spiritual, socio-cultural and interpersonal events/conditions must be evaluated in detail.

As stated by ANA standards, people have equal rights in terms of health care regardless of their values and beliefs, and their sexual, developmental, economic, political, cultural, religious, ethnic and national differences (Sülü Uğurlu, 2006).

The Place of Spirituality in North American Nursing Diagnosis Association (NANDA)

In 2003, the list of nursing diagnosis published by the North American Nursing Diagnosis Association-NANDA (North American Nursing Diagnosis Association) included diagnosis such as "potential for strengthening the spiritual dimension", "risk of spiritual distress" and "spiritual distress" (Birol, 2011; Öz, 2004; Sülü Uğurlu, 2006).

The Place of Spirituality in National Nursing Core Training Program (HUÇEP)

The HUÇEP commission started to work for the " National Nursing Core Education Program" in 2002. Through a commission established in 2014, the changes in the primary health problems, the health care needs of the society, the policies and practices, nursing care services and healthcare delivery have been redefined in the scope of "Bologna Process" which consists the criteria set by the EU for nursing education. The reports prepared by HUÇEP have been guided the commission studies that aimed at establishing standards, and psychiatry and mental health nursing undergraduate programs.

The Factors Affecting Spiritual Support in Nursing

Although there are many sources about spirituality and spiritual care in the nursing literature, it is seen that nurses are still not very familiar with these subjects , they cannot provide such care as necessary, they have difficulty and are afraid (Ergül and Bayık, 2004; McSherry and Rose, 2002). Some of the reasons for that nurses do not have sufficient knowledge about spiritual care and spirituality; they are not clear about their own spiritual thoughts, and not being able to spend enough time with the patient (Ergül, 2010). Other important factors affecting spiritual care can be counted as restless working environment, negative communication with other team members, insufficient number of healthcare workers, noise, constant vigil, lack of communication with patients due to hearing loss, dementia, coma, and so on., and prioritizing physical care due to lack of staff and time.

The Nursing Process in Spiritual Care

Definition: Identification of patients' spiritual needs and providing appropriate care to each patient plays an important role in the nursing process. Identification of spiritual needs is more difficult than identification of physical needs because spiritual needs are more abstract and complex than physical ones (Akgün Kostak, 2007).

Individuals should be asked open-ended questions during the identification of their spiritual needs. Answers to those questions gives clues about the individual's behavior, actions, verbal expressions, personal relationships and social environment, spiritual needs.

Such questions might be;

What is the meaning of life for you?

What are the important things in your life?

How do you feel about your illness?

Does your illness cause you to disconnect with your life?

Do you believe in a greater power?

Are you interested in any religion and belief community?

In which situations do you pray?

From whom do you ask for help?

Potential Nursing Diagnosis: Anxiety, fear, hopelessness, weakness, spiritual distress (Küçük, 2012).

Planning: It is very important for nurses to plan their nursing interventions in terms of holistic health care.

The nurse should consider a number of features when planning interferences/practices to meet the patients' spiritual needs. A nurse during planning should;

• help the individual for him/her to satisfy spiritual needs

• support the individual for facilitating his/her inner sources

• respect the personal differences when there is an unpleasant situation for the individual and help him/her on that situation (Abedi, 2011; Leeuwen et.al., 2004).

Practice: As stated in the article by Okyay (2008), Carpetiono summarized nursing interferences as below;

- Tell them you accept different belief and practices.

- Be in an unprejudiced manner

- Be aware of the importance of spiritual needs.

- Show your eagerness in satisfying their spiritual needs.

- Ensure that the individual prays every day and has privacy.

- Cooperate with religious leaders in order to perform religious rituals.

- Encourage the individual to perform spiritual rituals unless they discomfort the health condition of the individual.

- Ensure that the individual prays together with other people.

- Ask him/her questions about his/her previous beliefs and spiritual experienced in order to help him/her to see the illness in a wider frame.

- If you feel good and comfortable about it, pray alone or together with your friends in the team.

- Show that you are eager to listen his/her personal doubts, guilts or other emotions when expressed (Okyay, 2008).

Evaluation: It is checked whether the desired results are achieved after the interferences are implemented in the evaluation section. The evaluation should be clearly stated and new interferences should be planned if the target has not been achieved.

The Role of Nurses in Spiritual Support

The base of a decent nursing care consists of helping an individual to acquire all dimensions of an individual's existence, sustain or protect them.

As a result of the studies conducted by Newman, Parse and Rogers who are nursing theoreticians, the term "Holistic Care" entered to literature in 1980. Throughout history, nurses traditionally have provided care to patients with a holistic approach.

Nowadays, nursing theories evaluated individuals as a bio-psycho-social creature. In the view of Margaret Hutchinson, humans are creatures with three dimensions which are "physical and biological dimension", "psychosocial dimension" and "spiritual/mental dimensions".

- Physical and biological dimensions are related with physical environment and perceived with five senses.

- Psychosocial dimension consists of mental perceptions, moral values and emotions related with the individual and others.

- Spiritual/mental dimension is beyond existence and above psychosocial and physical dimensions.

According to Travelbee (1971), a nurse not only gives care to individuals to diminish their physical pain or in physical sense, but also gives holistic care to them (Bayık and Ergül, 2004; Dover and Bacon, 2001).

In terms of spiritual support, nurses should provide a peaceful environment for their patients and allow them to read and pray their holy books. However, the recent researches demonstrate that nurses are insufficient to satisfy such spiritual needs of their patients and that they need to have trainings about the subject (Baldacchino, 2006; Leeuwen and et.al., 2004).

Spiritual Support in Nursing Education

The spiritual dimension, which is an important component of holistic care, is mostly ignored by nurses and other healthcare workers (Moadel et al., 1999). The reasons for this include factors such as lack of awareness about the importance of spiritual care, using spirituality and religion terms interchangeably, insufficient skills to implement spiritual care, and not accepting spirituality and spiritual care as a scientific approach (McSherry & Watson, 2002).

Although spiritual care is specifically mentioned by international codes and standards as an important part of holistic care, this concept has not been fully integrated into the education programs and nursing practices due to some obstacles. McSherry and Draper (1997) mentioned two of these obstacles. The first of these is the internal factors that include the economic, administrative and political factors in the institution where the nurse works, while the second obstacle is the external factors that include belief and value systems, individual and social principles. Failure to address the mentioned obstacles in detail leads to exclusion of spirituality in nursing education program and practices (Ergül, 2010).

The Studies about Spiritual Support with the Nurses in our country (Turkey)

In the research conducted by Ergül and Bayık (2004) with the faculty members who are nurses, 61.3% of the teaching staff who participated in the study were insufficient to provide the student with knowledge and skills in spiritual care, and 80.0% of them expressed that they heard about the concept of spiritual care.

In the study conducted by Yılmaz and Okyay (2009) with nurses, the percentage of those who stated that they were not informed about spirituality was determined as 65.2%, while the percentage of those who informed about spirituality but found it insufficient was determined as 50%.

In a study conducted with nurses and midwives, the mean score of the midwives and nurses received from "Spirituality and Spiritual Care Rating Scale" was found to be 60.97 ± 7.92.

This percentage shows that the knowledge of midwives and nurses is insufficient in spirituality and spiritual care. In this study conducted by Kostak et al., it is determined that the education level of nurses about spirituality was not effective. Accordingly, 84.9% of midwives and nurses stated that they were not informed about spiritual care, while 62.7% stated that they heard this concept. (Kostak et al., 2010) However, in some studies conducted on a similar subject, it was determined that as the nurses' education level increases, their mean scores of spirituality increase (Özbaşaran et al., 2011; Wong et al., 2008; Yılmaz and Okyay, 2009).

In the study of İnce and Akhan (2016), it was determined that student nurses who are interested in spirituality and spiritual care had a perception on this subject, but they did not have sufficient knowledge. This finding shows that nurses should have the necessary trainings on the subject.

Outcomes of the research conducted by Özbaşaran et al. (2011), determined that the nurses who were single compared to the married ones had high levels of spiritual care and spiritual perception.

The Studies about Spiritual Support with the Nurses in the World

In the study conducted by Baldacchino (2006) with nurses (n = 77) in Malta, the nurses stated that they considered themselves insufficient in providing spiritual care because they did not have sufficient knowledge during the training process.

In the study of Van Leeuwen et al. (2006), lack of education and lack of time were identified as the reasons for nurses (n = 283) not being able to satisfy the spiritual care needs of patients.

Ross (2006) found that the nurses (n = 47) stated that their awareness of the patient's spiritual needs was very limited, they had difficulties in defining spiritual care and their education was insufficient to provide spiritual care. In the same study, Ross determined that nurses tend to focus more on religious needs in spiritual care.

In a study conducted by Lundmark (2006), it was stated that the 98.0 % of the nurses expressed that holistic care is important, 76.0 % of them mentioned that holistic care includes the concept of spirituality, however, 49.0 % of them remarked that they believe spiritual care is given in clinics.

It was aimed to develop a training program regarding spirituality by Lovania and Wallace (2007), and to determine the spiritual care perceptions of nursing students (n=250). The mean score of the student nurses received from "Spirituality and Spiritual Care Rating Scale" was determined to be 64.30±4.88 with the preliminary test result of quasi-experimental study.

In the study conducted by Tiew and Drury (2012) with the nursing undergraduate students, it was stated that spiritual care is a particularly emphasized dimension in nursing education, so that undergraduate students (n = 157) should increase their knowledge and skills in spiritual care until the final year of their education. However, as a result of the research, it was found that the difference between the students' total score on the "Spirituality and Spiritual Care Rating Scale" was not significant.

In a study conducted by Ross et al. (2014) with 530 students, the competencies of midwives and nurses to provide spiritual care and their perceptions of spiritual care were aimed to be determined. Consequently, similar results with previous studies were obtained in the sub-dimensions of "the Spirituality and Spiritual Care Scale".

As a result of the study conducted by Saunders et al. (2016), it was determined that doctors directed 3% of their patients to the religious staff in the hospital while nurses directed 82% of their patients to the religious staff in the hospital.

In the study conducted by Melhem et al. (2016) in Jordan to determine the perceptions of nurses (n = 408) about providing spiritual care, it was found that there was a statistically significant relationship between the total scores of the "spiritual care delivery scale" and the gender factor (Baldacchino, 2006; Baldacchino and Draper, 2001; Govier, 2000; Lundmark 2006; Pesut and Sawatzky, 2005; Ross, 2006; Strang et al., 2002).

As a result; In the review of the literature on the subject, the concept of spirituality is an important concept for nursing, and the holistic process in nursing care should be supported with qualitative and quantitative researches.

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Chapter 16

RECURRENT IMPLANTATION FAILURE

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INTRODUCTION

Live birth rate per in vitro fertilization (IVF) cycle is 26.9% despite the improvements in artificial reproductive technologies (ART) [1]. Two important factors that affect implantation and IVF success are embryo quality and endometrial receptivity [2]. Implantation is a weeks-long process in which the embryo is invaded into the deep layer of endometrium following the attachment to the superficial layer. The ultrasonographic view of the gestational sac confirms a successful implantation process [3]. Implantation failure consists of migration and adhesion defects of embryo in early stages without hCG detection and partial invasion of embryo which is called biochemical pregnancy (BP) with a hCG production without formation of a gestational sac [3, 4]. Recurrent implantation failure (RIF) is a compelling issue for both couples and doctors. There is not a clear data about the incidence or prevalence of recurrent implantation failure due to variations of definitions. Although a common definition has not been agreed [5], the most commonly used one is failure of clinical pregnancy despite the transfer of at least four good quality embryos in at least three fresh or frozen-thaw cycles under 40 years of age [3, 4]. A successful implantation requires a receptive endometrium, embryo in the blastocyst stage, and successful communication between the endometrium and the embryo [6]. This communication is impaired in the presence of intrauterine pathologies, non-receptive endometrium, embryo anomalies, maternal thrombophilia and immune disorders. Maternal and paternal causes, potential treatments and recommendations should be determined in patients with RIF [4, 7].

RISK FACTORS

Maternal Age

Advanced maternal age causes decrease in ovarian reserve and oocyte quality. Mitochondrial and meiotic spindle dysfunctions increase the frequency of aneuploidy as maternal age increases [8]. Implantation and live birth rates decrease in woman age > 35 compared with women age < 35 due to the asynchrony between embryo and endometrium [9].

BMI

Implantation rates decrease proportionally with weight gain. Obese patients (BMI > 30 kg/m^2) have the lowest implantation rates when compared with normal weight patients (BMI 18.5-24.99 kg/m2) [10]. The number of retrieved oocytes are lower in overweight and obese patients despite the use of higher doses of gonadotropins than the normal weight women [11].

Smoking

Smoking has negative impacts on fertility and pregnancy outcomes. Toxic metobolites of cigarette causes lower estradiol levels, number of ret-

rieved oocytes and implantation rates [12]. Miscarriage rates increase due to depletion of oxygen and vasoconstriction [13] and live birth rates decrease [14]. Male smokers had significantly decreased sperm count, motility, normal percentage of morphology [15].

Stress

The patients with RIF have a higher rate of anxiety and depression. The stress hormone cortisol levels are higher in this patient group. It was shown that elevated levels of cortisol cause 2.7 times increased rates of miscarriage. The control of maternal stressors may improve pregnancy outcomes [16].

ETIOLOGIC FACTORS

Embryonic factors

Embryo quality

Oocyte and embryo quality decrease in the presence of advanced maternal age due to the mitochondrial DNA instability and increased risk of aneuploidy [8, 17-19]. In IVF practice, usage of high doses of gonadotropins decrease oocyte quality and fertilization rates also [20, 21].

History of previous surgery, infection, radiotherapy and chemotherapy, DNA repair defects, protamine deficiency and smoking cause sperm DNA damage that impair embryo quality and reduce implantation rates [22-25]. It was shown that the DNA fragmentation rate above 27% is associated with IVF failure [26, 27].

The embryo that contains appropriate number of cells according to the day of development is defined as a good quality embryo. Other criteria are the equal distribution of blastomeres, the cytoplasm without granules and fragmentation less than 10%. In blastocyst stage, embryo inner cell mass is graded according to trophoectoderm expansion [28]. While the implantation probability of a poor quality embryo is 10%, it is 30% for a good quality embryo [29].

Chromosomal abnormalities:

In patients with repeated implantation failure, the incidence of chromosomal abnormalities such as translocation, deletion, mosaicism, inversion is 2.5%, higher than the normal population. Therefore, karyotype analysis is recommended in patients with RIF [30, 31]. The most common chromosomal anomaly in this patient group is translocations. An aneuploid embryo can also develop from a genetically normal oocyte and sperm cell due to the postfertilization molecular mechanisms as meiotic nondisjunction and premature separation of balanced and unbalanced sister chromatids [8]. In cases of RIF, aneuploidy is detected in approximately 50% of embr-

yos [32, 33] and aneuploidic embryos are indistinguishable from normal embryos according to their morphological appearance.

***Zona hardening**:*

The thick acellular matrix surrounding the oocyte is called zona pellucida. It plays a role in sperm binding, formation of an acrosome reaction, sperm and oocyte fusion; prevents the fertilization of the oocyte with more than one sperm. Hatching that means blastocyst expansion and rupture of zona pellucida is essential for implantation. The long-term staying of embryo in culture medium and advanced maternal age causes hardening of zona pellucida, prevents hatching and implantation [30].

Embryo culture medium:

The quality and standardized culture media is very important for IVF success. The culture media should be evaluated with osmolality and pH tests. In some cases, embryo culture mediums may need to be personalized [30].

Embryo transfer technique:

Blood or mucus in the transfer catheter, bacterial contamination, contact of catheter with the uterine fundus, damage to the endometrium, use of a cannula, prolonged or painful procedure reduce pregnancy and implantation rates [30]. Pregnancy rates have been reported to be about 30% less in difficult transfers [66].

Uterine anatomic abnormalities

Uterine pathologies as polyps, myomas, adhesions, septum effect implantation process. These pathologies can be detected by transvaginal ultrasound and hysteroscopy [4]. ***Congenital uterine anomalies*;** occur as a result of defects in the formation or fusion of the mullerian canal [3]. Uterine septum is the most common congenital uterine anomaly with pregnancy complications such as abortion, preterm delivery, intrauterine growth restriction and infertility. Low blood supply of the uterine septum causes a decrease in implantation rate. Hysteroscopic septum resection is recommended because it increases fecundity and reduces pregnancy complications [6]. In the presence of bicornuate uteri and arcuate uteri, surgical treatment is rarely required [3, 6]. ***Submucous myomas*** disturbs the anatomy of the uterine cavity, blood supply to the endometrium and cause an increase in cytokine release, inflammation and uterine contractility. Clinical pregnancy and implantation rates are decreased in infertile patients with submucous myoma compared to infertile controls [34]. When hysteroscopic fibroid resection is performed, the cumulative pregnancy rates doubles [35]. Although the effects of ***intramural myomas*** that are not related to the endometrial cavity is controversial, the meta-analyses showed that implantation rates are lower in patients with intramural fibroids. However, re-

moval of these myomas does not significantly increase clinical pregnancy and live birth rates [3]. ***Endometrial polyp*** is the most common pathology detected in the uterus during hysterescopic examination [6]. Endometrial polyps are thought to negatively affect implantation [3]. Studies show that spontaneous pregnancy rates increase when polyps are removed [36, 37]. It was shown in a randomized prospective study that hysteroscopic polypectomy procedure significantly increased pregnancy rates in intrauterine insemination cycles [38]. Polypectomy is a recommended treatment in infertile patients [39]. ***Intrauterine adhesions;*** can occur after intrauterine surgery, curettage, intrauterine infections and can cause infertility by disturbing sperm migration or the embryo apposition and attachment to the luminal layer of the endometrium [6]. Intrauterine adhesion was detected in 8.5% of patients with RIF [3]. Hysteroscopy is the first choice in the diagnosis and treatment of adhesions [6]. Hysteroscopic adhesiolysis has been shown to affect fertility in a positive manner [40]. The usage of intrauterine device or balloon prevents the risk of repeated adhesions after surgery [41]. ***Adenomyosis;*** affects the junctional region of the uterus and impairs implantation [42]. Since it cannot be diagnosed accurately with ultrasound, its prevalence in patients with RIF is less. MR is a noninvasive test that makes an accurate diagnosis in the presence of adenomyosis [43]. Since there is no real capsule in cases with adenomyosis, its surgery is difficult when compared to myomas. Suppression with gonadotropin releasing hormone agonist is an another treatment option [8]. ***Hydrosalpinges;*** refer to the fluid filling and expansion of the tubes due to distal tubal obstruction [3]. Implantation is impaired as a result of the expulsion of the embryo from uterus due to the mechanical effect of accumulated fluid, direct toxic effects of inflammatory substances on embryo and decreased production of endometrial receptivity markers [3, 6]. In the presence of hydrosalpinx, live birth rates are reduced by 50% in IVF patients [3]. Therefore, salpingectomy is recommended for these patients before IVF [44].

Thin Endometrium:

During the menstrual cycle, the endometrium undergoes biological and morphological changes for a successful implantation. Ultrasonographic evaluation of endometrial thickness and structure is useful in predicting implantation success [7]. If the endometrial thickness is below 7 mm on the day of HCG or embryo transfer, thin endometrium is mentioned. The minimum endometrial thickness sufficient for implantation is specified as 6-8 mm [7]. Implantation, clinical pregnancy and live birth rates have been shown to be higher when endometrial thickness is more than 9 mm. [45, 46]. Thin endometrium despite estrogenic stimulation may be associated with impaired uterine blood flow, congenital or T-shaped uterus, Turner Syndrome, previously received radiotherapy, previous uterine surgery and infections [3, 4].

Endometrial Receptivity:

The implantation process involves a broad signal system with adhesion molecules, cytokines and growth factors. In studies conducted, large differences were detected in implantation related genes expressed between RIF and fertile group [6]. Endometrial receptivity array (ERA) was used to determine receptivity status of endometrium [47]. However, according to the results, it is not the definitive test for RIF cases [4]. Koot et al. demonstrated down regulation of genes that were involved in cell division and cilia formation in RIF patients [48].

In cases of ***endometriosis***, both poor oocyte and embryo quality and decreased endometrial receptivity cause infertility [49, 50]. Implantation rates are lower even in the early stages of endometriosis [51]. The production of implantation markers such as glycodelin A, osteopontin, HOXA10 decreased in patients with endometriosis [52]. Upregulation of estrogen receptors [53] and progesterone resistance due to lack of β subunit of progesterone receptors impair uterine receptivity and cause infertility[54]. Suppression with long-term (3-6 months) GnRH analogue before IVF treatment in women with endometriosis significantly increases ongoing pregnancy rates [55]. It is not recommended to perform laparoscopy for the diagnosis and treatment of a possible endometriosis after repeated failed cycles [3].

During ***ovarian stimulation***, endometrial morphology and receptivity are affected because estrogen and progesterone exceed the normal values [56, 57]. High estrogen values reduce endometrial integrin expression that plays role in implantation [6]. In studies conducted, clinical pregnancy rates were found to be significantly higher in frozen-thaw cycles than in fresh cycles [58] This result shows that ovarian stimulation disrupts endometrial receptivity[4].

Immunological factors

The role of immunological factors in patients with recurrent implantation failure take attention in recent years [5, 8]. ***Natural killer(NK) cells***, a cell of the immune system, have been found to play a role in placental development and maternal tolerance [5]. Peripheral NK cells compose 5-10% of peripheral cells and uterine NK cells compose 70-90% of NK cells. Alterations in the number and activity of natural killer cells can cause implantation failure [59]. It was shown that NK proportions are higher in RIF patients than the control group [60]. Uterine NK cells express ***maternal killer immunoglobulin-like receptors(KIRs***). Interaction between KIRs and fetal HLA-C molecule originated from extravillous trofoblasts play role in the formation of plasenta [61]. ***T-helper cells*** also play a role in implantation process. Tumor necrosis factor-a is secreted by Th-1 and suppresses trophoblastic development. Th-2 cells secrete IL 4, IL 6, IL

10 and inhibit the tissue factor secretion. The increase of Th-1/Th-2 cells proportion is related with multiple implantation failures [62]. ***Leukaemia inhibitory factor(LIF)*** is a cytokine that plays role in endometrial receptivity. It was shown that LIF levels was lower in RIF patients when compared to control group [63]. Immunomodulating treatments are used to increase IVF success rates when any other reason is not found to explain IVF failures [5]. However, there is no consensus on immunoassay testing and immunomodulatory therapy [3, 64].

Thrombophilia

Thrombophilia negatively effects microvascularization and implantation process. The relationship between recurrent pregnancy losses and thrombophilia is clearly determined but there are different opinions regarding the relation with RIF [65, 66]. In RIF cases hereditary and acquired trombophilia were much more detected than the control groups [67]. In a meta-analysis it was reported that the association between antiphospholipid antibodies (APA) and clinical pregnancy and live birth rates is not statistically significant [68]. ASRM committee opinion reported that no association between APA and IVF failure was found in prospective studies despite a minimal relation in retrospective studies and APA assay is not recommended before IVF treatment [69]. The effect of treatment in RIF cases with APA positivity has not been proven yet. [3]. In a comprehensive study it was demonstrated that there was no relationship between hereditary thrombophilia and RIF [70].

Infection

Chronic endometritis (CE) is a silent infection of endometrium with bacterial colonization [71]. It can be diagnosed on histological examination, immunohistochemical CD 138 staining, hysteroscopic visualization and by bacterial culture [72]. In bacterial culture, Group B Streptococcus, Escheria Coli, Enterococcus Faecalis, Myocoplasma and Chlamydia are the potential pathogens [71]. A new faster and reliable diagnostic tool is real-time polymerase chain reaction (RT-PCR) that can identify culturable and unculturable bacterial DNA with a sensitivity of 75% and specificity of 100% [73]. The bacteria colonization disrupts endometrial receptivity by leading a lymphocyte increase in endometrium. It was shown that the prevalence of CE in RIF patients is 14%. [72]. After antibiotic treatment of CE, live birth rate was found 61%, significantly improved when compared the patients that did not use antibiotics [71].

Endocrine disorders

Hipothyroidism, diabetes mellitus (DM) and polycystic ovary syndrome (PCOS) are endocrine disorders related to infertility. It is recommended to infertile patients to sustain TSH levels below 2.5 mIU/L. In subclinical

hypothyroidism that TSH levels are above 2.5 mIU/L, levothyroxine treatment is prescribed to improve embryo quality and pregnancy outcomes. The presence of antithyroid antibodies might have negative effects on fertility [74]. Uncontrolled DM cause implantation disorders and increase the miscarriage, fetal death and malformation rates [8]. Elevated LH levels, insulin resistance and high leptin levels negatively affect oocyte quality, endometrial receptivity and decrease implantation rates in PCOS [75].

THERAPEUTIC INTERVENTIONS FOR RECURRENT IMPLANTATION FAILURE

Embryo factors

Embryo quality is an important determinant in implantation process for all stages of embryo. Blastocyst has a higher implantation rate than cleavage stage embryo (25.4% vs 12.4%) [76]. It was shown that implantation rates were higher in blastocyst transfer when compared to cleavage stage embryo transfer in RIF patients (21.2% vs 6%) [77].

The number of transferred embryo is restricted in many countries by government. If the number of transferred embryos increase, implantation chance increases with an elevated multiple pregnancy risk [3]. Single embryo transfer cycles have lower live birth rates than two embryo transfer cycles. However, recurrent single embryo transfers (two fresh or one fresh and subsequent frozen-thaw cycle) have same cumulative pregnancy rates with two embryo transfers in a single cycle but lower multiple pregnancy risk [29].

In ART practice, frozen-thaw embryo transfer (FET) is becoming a more popular treatment choice despite the debates. It was demonstrated that implantation rate, clinical pregnancy rate, ongoing pregnancy rate were significantly higher in FET cycles when compared to fresh cycles. Impaired endometrial receptivity due to ovarian stimulation return to normalcy in FET cycles and implantation rate improves [78]. However, recently published randomized controlled study showed that there was no significant difference in live birth rates between fresh and frozen embryo transfer of cleavage stage embryos [79].

Sperm DNA fragmentation is a determinant of sperm and embryo quality. Sperm DNA fragmentation rate can be reduced with antioxidant drugs [80], intracytoplasmic morphologically selected sperm injection (IMSI) [81] and testicular sperm use for ICSI [82]. Although there are studies that show higher pregnancy rates with these treatments in RIF, more randomized controlled studies are needed [80, 83].

Preimplantation Genetic Screening:

The aim of preimplantation genetic screening (PGS) is to increase the implantation rates by selecting and transferring genetically normal embry-

os. Biopsy methods are polar body biopsy, one or two blastomer biopsies in the cleavage stage and trophoectoderm biopsy performed in the blastocyst stage [84]. Although cleavage phase biopsy is the most frequently used method, it carries a high incidence of mosaism. Since mosaic embryos can correct themselves, the risk of aneuploidy is lower in blastocyst biopsy compared to cleavage phase biopsy. [85]. In recent randomized controlled trials in IVF patients, it was concluded that PGS did not increase implantation rates in RIF cases and even live birth rates were lower in the PGS group [86]. Damage to the embryo during the biopsy, the presence of mosaic embryos, and the examination of a limited number of chromosomes may be responsible for this results. The American Reproductive Society (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE) concluded that PGS does not increase live birth rates in patients with advanced maternal age, RIF or recurrent pregnancy loss [87].

Alternative approaches to fluorescent in-situ hybridization (FISH) are comparative genomic hybridization (CGH) and single nucleotide polymorphism analysis (SNPs). The CGH microarray provides a broader chromosomal analysis advantage and it is simpler and faster than conventional CGH. SNPs provide information about the genomic structure as well as the number of chromosomal copies [88]. In couples with normal karyotype, cleavage phase biopsy is not recommended in the management of RIF. CGH array of blastocyst may contribute to successful pregnancy formation in RIF group [89]. Next generation sequencing (NGS) is a recently developed technique for aneuploidy screening and simultaneous assessment of aneuploidy, translocations, single-gene disorders, small copy number variations and low level mosaicism. NGS might improve clinical outcomes by detecting mosaicism and triploidy better than aCGH [90, 91].Another advantage of NGS is collecting both nuclear and mitocondrial DNA in the same sequence. While mtDNA is associated with implantation and embryo viability, embryonic mtDNA increases to provide energy as a compensatory mechanism and higher quantities of mtDNA are associated with lower implantation rates [92]. However, mtDNA assessment is controversial due to the conflicting results of studies[90].

In recent studies, it was concluded that PGS for aneuploidy had no positive effect on live birth and miscarriage rates [93]. Due to the conflicting results in the literature, more randomized controlled trials are required in subject of PGS [94]. However, karyotyping is recommended in RIF patients and preimplantation genetic diagnosis (PGD) is recommended in patients with balanced translocations that can lead aneuploidy in gametes [95].

***Assisted Hatching*:**

In order to increase implantation and clinical pregnancy rates, the process of making artificial holes in zona pellucida is called assisted hatching

(AH) [96]. It can be done using piezo micromanipulator or laser and by mechanically, enzymatically or chemically (Tyrode acid). However, this process can damage blastomers, affect embryo viability and cause an increase in monozygotic twin rate. [97]. In a recent metaanalysis, assisted hatching has been shown to increase clinical pregnancy rates in fresh cycles of patients with RIF [98]. While ASRM does not recommend routine use of assisted hatching in all IVF patients, recommends its use in patients with poor embryo quality and poor prognosis with advanced maternal age (≥38) [99].

Embryo culture:

Embryo culture conditions must be appropriate for a healthy development. Various coculture environments have been developed to meet the metabolic needs of the embryo. [30]. In embryo cocultures cumulus-granulosa cells and endometrial cells can be used. The most effective cocultures contain homologous endometrial cells [100].

Although it was detected that embryo quality and clinical pregnancy rates increase by usage of autologous endometrial cells in patients with RIF, most centers did not have sufficient equipment for coculture use [101]. More studies are needed to reveal the advantages of using coculture.

Blastocyst Transfer:

In patients with RIF, blastocyst transfer is recommended to increase implantation and pregnancy rates. Blastocyst transfer is a more physiological procedure that provides the advantage of selecting the embryo with higher implantation potential and performing the embryo transfer when the endometrial receptivity is increased. There are studies that show higher live birth and implantation rates in blastocyst than 2-3 day embryo [102]. As some embryos cannot reach to the blastocyst stage, patient selection should be made carefully to not increase the cancellation rates.

Sequential embryo transfer:

Sequential embryo transfer has been proposed to correct a possible embryo-endometrium asynchronization that may be the basis of implantation failure [103]. Some studies have concluded that a second transfer procedure may impair the implantation process by causing bacterial contamination, endometrial trauma and uterine contractions, while others have concluded that sequential embryo transfer effects this process positively [104]. More randomized controlled trials are needed to prove the effectiveness of this technique.

***Embryo transfer technique*:**

Embryo transfer technique is one of the important step of IVF treatment. Ultrasound guided transfer, aspiration of cervical mucus, performing

trial catheter, transferring with filled bladder, using soft catheters increase transfer success [105, 106]. In women who have a narrow angle between the external cervical os and the cervix, or have a difficult transfer history in their previous trials; it may be helpful to perform cervical dilation before starting controlled ovarian hyperstimulation. In cases where cervical dilatation fails, the use of hygroscopic rods or hysteroscopic dilatation of cervical stenosis may be considered [107].

***Ovarian stimulation protocols*:**

With the aim of improving IVF success rates, several ovarian stimulation protocols are being used by clinicians. GnRH agonist and antagonist protocol are the most popular ones. In a meta-analysis, it was demonstrated that pregnancy rates were lower in antagonist group when compared to agonist protocol [108]. However, in a prospective randomized study, there was no difference in implantation rates between two protocols with a lower OHSS risk and shorter stimulation duration in antagonist group [109]. In cases of suboptimal response to the stimulation protocol, the dose can be increased or the protocol can be changed [3]. Addition of LH may be considered in patients who respond poorly to stimulation [110]. GnRH agonist use before cycle may increase pregnancy rates in cases of endometriosis and adenomyosis [111]. The stimulation protocol decision should be individualized according to etiology of RIF and clinical features [4].

***Embryo assessment methods**:*

Embryo selection according to morphological criteria can be subjective and requires experience. Therefore, new methods are developing to evaluate embryo quality and viability. In time-lapse imaging, embryo development can be monitored continuously without removing it from the incubator [3]. The evaluation of the metabolics used and synthesized by the embryo in the culture medium with the 'Omic' technology can also give information about the viability of the embryo [112]. Vibrational spectroscopy is another emerging method that analyzes culture environments [30].The advantage of using metabolites in patients with RIF has not been proven yet [3].

Anatomic interventions

The diagnostic methods that are used to evaluate the uterus and endometrial cavity are ultrasonography, hysterosalpingography (HSG), sonohisterography and hysteroscopy. Uterine pathologies such as congenital uterine anomalies, fibroids, polyp, hydrosalpinx and thin endometirum can be examined by ultrasonography. HSG has low sensitivity and high false positivity in detecting intrauterine pathologies and is a useful diagnostic method in detecting hydrosalpinx [3]. Sonohisterography is less invasive

than both HSG and hysteroscopy and has diagnostic power similar to hysteroscopy [113]. Hysteroscopy, on the other hand, is the gold standard method in both diagnosis and treatment of intracavitary lesions. The incidence of pathology in the intrauterine cavity in patients with RIF was 25-50% [114] and evaluation of the cavity by hysteroscopy was shown to increase pregnancy rates in these patients [115]. Performing hysterescopy in the proliferative phase provides a better view, while performing a biopsy in the luteal phase before IVF may increase the implantation rates by endometrial stimulating effect [3].

Intracavitary lesions:

Endometrial polyp is the most common pathology detected during office hysteroscopy and it is recommended to be removed in patients with RIF [3]. Recent meta-analyses have shown that submucous myomas also significantly reduce implantation, clinical pregnancy, live birth rates, and increase miscarriage rates . Removal of submucous myomas regardless of diameter increases the clinical pregnancy rates [34]. Several sessions may be required for cases larger than 5 cm and more than 50% embedded in myometrium. Removal of multiple submucous myomas may cause intrauterine adhesion formation [3]. In patients with uterine septum, hysterescopic septum resection is recommended regardless of the size of the septum, as it has a positive effect on fecundity and reduces pregnancy complications such as preterm labor and abortion [116, 117]. Intrauterine adhesions both reduce implantation rates and cause pregnancy complications so hysteroscopic adhesiolysis is recommended [40].

***Myometrial pathologies*:**

The issue of removing intramural myomas that are not related to the cavity is controversial in RIF patients. Patients should be informed that myomas both disrupt the implantation process and that they are associated with various pregnancy complications such as IUGR, ablation, malpresentation, and the decision should be made with patient after explaining the risks such as bleeding and adhesion formation. Uterine artery embolization and ultrasound guided magnetic resonance are alternative treatments to surgical myomectomy [3]. Adenomyosis is not suitable for surgical treatment and ultra-long pituitary downregulation is recommended in these patients [111].

Salpingectomy

Hydrosalpinx should be ruled out in patients with RIF. Hydrosalpinx might not be seen on ultrasonography so HSG should be done to these patients. If a definitive result cannot be reached with HSG, it should be evaluated by laparoscopy [3]. Performing salpingectomy in the presence

of hydrosalpinx increases implantation and pregnancy rates. The benefit of salpingectomy is controversial in cases of hydrosalpinx that cannot be seen ultrasonographically. Ultrasound guided hydrosalpinx drainage is not preferred due to the rapid accumulation of fluid and the increased risk of infection [3]. Proximal tubal occlusion is also an alternative method to salpingectomy, especially in patients with extensive pelvic adhesion [44].

Thin endometrium:

Treatment of thin endometrium that does not respond to estrogenic stimulation is a compelling issue [7]. In the presence of thin endometrium, hysterescopy should be performed with suspicion of intrauterine adhesion and adhesiolysis should be applied if detected [118]. In cases of normal hysteroscopy, treatments such as vaginal or transdermal high dose estrogen, aspirin, vitamine E and sildenafil that increase the endometrial blood flow can be given [119]. ***Granulocyte colony stimulating factor*** (G-CSF) is a treatment option in resistant thin endometrium cases [120]. Gleicher et al. showed favorable effects of G-CSF on endometrial thickness [121]. In a meta-analysis, it was demonstrated that G-CSF use in patients with thin endometrium or RIF, improved implantation and clinical pregnancy rates [122]. ***Endometrial biopsy*** before IVF treatment cycle is thought to increase the implantation rates by initiating the inflammatory response in thin endometrium cases [123]. In a recent randomized controlled trial, higher estrogen and progesterone levels and thicker endometrium were detected in patients receiving luteal support with the GnRH agonist [124]. More studies are needed on the routine use of these treatments. ***Platelet-rich plasma (PRP)*** is an autologous plasma that contains high concentrations of platelets, growth factors and fibronectin that promote tissue healing process. PRP is commonly used in regenerative medicine, orthopedics, aesthetic and reconstructive surgery and dermatology. In recent years, it gained a popularity in reproductive medicine, in the treatment of ovarian failure, implantation failures and thin endometrium. There are some randomized controlled studies about application of PRP in thin endometrium that demonstrate beneficial effects but there is no systematic review that shows efficacy and safety of PRP [125, 126]. Due to the inadequate evidence in literature, PRP should be considered as experimental and should be used in randomized controlled studies [126].

Endometrial injury (biopsy):

Endometrial biopsy that performed in the luteal phase before IVF cycle has been shown to increase implantation, pregnancy and live birth rates in patients with RIF [127-129]. This effect is thought to be the result of increased release of cytokines and growth factors after endometrial local injury. [127]. Endometrial injury can be done using both biopsy catheter or

hysteroscopy. This procedure may reduce the number of IVF attempts needed for a successful implantation. More data is needed about the degree of injury, number of injuries and the timing of injury [130]. It was suggested to perform endometrial injury between day 7 of the previous cycle and day 7 of the embryo transfer cycle to increase clinical pregnancy and live birth rates in a Cochrane review [129].

Endometrial receptivity assay (ERA):

ERA test was developed to determine the receptive status of the endometrium according to the transcriptomic profile. 238 genes were investigated in endometrial biopsy taken LH + 7 in natural cycles or progesterone + 5 in hormone-treated cycles to determine the receptivity status of the endometrium. When the result is non-receptive, a biopsy is performed every month until the receptive period is detected. Successful results can be obtained by timing of embryo transfer with the ERA test, since the available data indicate that there are shifts and transcriptomic modifications in the implantation window in patients with RIF [47, 131]. However, in a recent study, implantation rates were found similar between receptive and non-receptive group (32.8% vs 31.6%). This results indicate that in some RIF patients implantation failure might not be related to a pathological condition and personalized evaluation of implantation window is required for this patient group [132].

Antibiotics for infection

The endometrium has a Lactobacillus dominated flora. It was shown that implantation and live birth rates significantly decrease in patients with non-Lactobacillus dominated endometrium (Gardnerella, Streptococcus and other organisms) [62]. In the presence of chronic endometritis, antibiotic therapy cures the infections and improve the implantation and live birth rates in future IVF cycles. Amoxicillin and Clavulanate twice a day for 8 days for gram positive bacteria, Ciprofloxacin twice a day for 10 days for gram negative bacteria, Josamycin 1 gr twice a day for 12 days with the addition of Minocycline for Mycoplasma and Ureaplasma were used in this study [71]. There are ongoing studies about antibiotics therapy in CE and IVF outcomes [4].

Antithrombotic agents

There is no clear relationship between thrombophilia or autoantibodies and implantation failure, and there is no enough evidence that aspirin and heparin administration will improve the results. [3, 133]. Empirical heparin, aspirin or corticosteroid use is also not recommended in patients with RIF without thrombophilia. ASRM reported that antiphospholipid antibody test and treatment are not indicated in patients undergoing IVF [3].

Immunotherapy

Immunological factors have been emphasized in the etiology of RIF in recent years and immunotherapy is frequently used, although it is not based on evidence [133]. Treatments such as intravenous immunoglobulin (IVIG), 20% intralipid solution, steroid use and allogenic lymphocyte therapy are used to decrease natural killer cell (NK) production and / or activity, to reduce abnormal Th1 / Th2 ratio and NK cytotoxicity. However, there is no clear evidence for the benefit and safety of these treatments in patients with RIF [133-135].

Tacrolimus

Tacrolimus is an approved immunosuppressive drug for immunological allograft transplant rejection. It was demonstrated that implantation rate was 45.7% and live birth rate was 60% in Tacrolimus treated RIF patients with elevated levels of Th1/Th2 ratio whereas there was no live birth in the untreated group. It seems tacrolimus improve the immunological imbalance in some RIF patients [136].

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG) has been used in cases of RIF with an elevated NK cells, Th1/Th2 ratio, an abnormal TNFa/IL10 ratio and auto-antibodies. It was demonstrated in a meta-analysis that implantation rates, clinical and live birth rates were significantly higher in IVIG group when compared to placebo group [134].

Peripheral blood mononuclear cells

B lymphocytes, T lymphocytes and monocytes are called as peripheral blood mononuclear cells (PBMCs) and improve implantation by secreted cytokines. PMNCs are administered in uterine cavity via intrauterine insemination catheter before embryo transfer. Yu et al. reported that in patients treated with PMNCs there was significantly higher implantation rates than control group [137]. In another study, benefits of PBMCs on implantation was shown in patients with four or more previous implantation failure [138].

Lifestyle modifications

Smoking, obesity and stress have negative effects on fertility. Lifestyle modifications as not smoking, having a healthy diet, regular exercise and taking care of mental health may have positive impacts on patients with RIF. These are the first step interventions that should be achieved before expensive and invasive procedures [4].

Conclusions:

RIF is a compelling issue in IVF practice despite the improvements in diagnostic and treatment options. Hysteroscopy, hysterosalpingography, pelvic ultrasonography, parental karyotype are suggested examinations. A personalized approach and recommendation must be preferred according to the characteristics of each patient. The goals of treatments are increasing embryo quality and endometrial receptivity. Treatments should be evidence-based, and undetermined treatments should be used only in scientific studies. In the future, endometrial gene therapy, use of intrauterine adhesion molecules, and development of embryo cultures will create new hopes. Large sample sized well designed randomized controlled studies are needed to introduce safe and efficient diagnostic and treatment modalities in RIF patients.

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Chapter 17

DETERMINATION OF FACTORS AFFECTING DRUG PURCHASE DECISION IN VETERINARY CLINICS: THE CASE OF ELAZIG PROVINCE

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INTRODUCTION

Industrial markets are markets where products and services are taken by non-profit organizations such as businesses, institutions, and hospitals. In other words, it is the market where the products and services marketed are exchanged to enter other production processes, to use them in these processes and to consume or resell them. Since the purchasing volume is high in industrial markets, they take a higher share in the economy compared to consumer markets. One-to-one customer relationships are essential due to a large number of purchases (Arıcı, 2010).

Various features distinguish the industrial market from consumer markets. The industrial product marketer works with a small number of buyers. Few buyers make the bulk of the purchases. Industrial products are purchased by experts. Industrial purchases are direct and personal sales are widely used instead of advertising, as buyers are trying to purchase particularly expensive and technically complicated products directly from their manufacturer (Mucuk, 2004).

Although the veterinary health products market is a component of the industrial market, it is indispensable in terms of the significant productivity increase it creates in animal husbandry and the positive contributions it provides to the lives and lives of pets (DPT, 2007).

The wholesale and distribution of veterinary medicinal products are through veterinary pharmaceutical warehouses licensed (RG 2011), and the retail sale is through licensed veterinary practice, outpatient clinic, and animal hospital (RG 2013).

There are differences between countries in the distribution of veterinary medicinal products by country. Although veterinarians are the only channels in drug delivery in Germany, veterinarians are predominantly involved in distribution in France, the Netherlands, England, Spain, Ireland, Portugal, and Greece. In Italy, Norway, Belgium, and Denmark this authority is in pharmacies. Authorized dealers and cooperatives are competent in France, Ireland, Greece, and the UK (DPT, 2007).

In the veterinary healthcare industry, veterinarians are the mechanism that makes purchases to sell to the final consumer, and also decides which product the consumer will use. This mechanism allows veterinarians the opportunity to buy and sell both medicine and medicine (Arıcı, 2010). One of the main problems of clinician veterinarians is the introduction of commercial concerns in the use of these facilities and the direct sale of medicines and vaccines to pet owners (Yüksel and Özen, 2008).

Today, with the increase in the scale of livestock enterprises, clinician veterinarians act with commercial concerns and increase their income with

the sale of medicines, this situation has caused them to operate as an intermediary between the pharmaceutical company and the warehouse and the producer/patient owner as a commercial enterprise (Şanlı, 1996).

Purpose

The need for clinician veterinarians to know the practical priorities in the drug purchase process. In this study, it was aimed to determine the factors affecting the decision of clinician veterinarians working on farm animals in Elazığ province to purchase drugs from companies and warehouses. At the same time, correlations between income from veterinary medicine sales and factors affecting the decision to purchase drugs were investigated.

MATERIAL and METHODS

The data obtained from the owners of the veterinary clinics operating in connection with the Elazığ Chamber of veterinarians were obtained through the data procurement form. The clinics in which the study will be conducted were determined by a simple random sampling method (Kish, 1965).

The sample of the study consisted of 30 clinics operating in Elazığ and a total of 20 veterinary clinics that responded to the questions entairly and reliably by agreeing to participate in the research and sharing data.

The data supply form consists of two parts. In the first section, information about the gender, age, Faculty of Graduate Studies, year of graduation, the period of working as a clinician, the annual drug purchase turnover of the clinic, the annual turnover of the clinic, the number of warehouses where the clinic purchases drugs were collected.

In the second part, the veterinarians who participated in the study were asked what factors were more active when deciding to purchase drugs. Clinician veterinarians were asked to give a value of 1 to 5 based on the increase in severity to scale the severity of each factor.

These factors were examined under 8 primary headings: warehouse institutional factors, warehouse sales personnel factors, drug-related factors, drug price factors, marketing activities, logistics and after-sales, financial factors and producer/patient owner factors.

The answers to the questions in the data supply form are evaluated in the SPSS 25 Version 25.0 package program (IBM Corp. Released 2017). Statistical analysis techniques, percentage analysis, significance analysis, and correlation analysis were used (Kalaycı, 2016).

RESULTS

The proportion of 21-30-year-olds who participated in the study was 35%, the proportion of 31-40-year-olds was 15%, the proportion of 41-50-year-olds was 35%, while veterinarians 51-60-year-olds were 15% of this group.

When we look at the universities where veterinarians graduated, it was learned that 5% of them graduated from Erciyes University, 90% from Fırat University and 5% from Kafkas University.

When we look at how many years ago the veterinarians graduated, 40% of those who graduated 1-10 years ago, 20% of those who graduated 11-20 years ago, 35% of those who graduated 21-30 years ago, and 5% of those who graduated 31-40 years ago were determined.

When the working time of the veterinarians in the clinic was examined, it was determined that 45% worked between 1-10 years, 35% worked between 11-20 years and 20% worked between 21-30 years.

When we look at how many different warehouses veterinary physicians bought drugs in their clinics, it is determined that 10% bought them from 1, 5% from 2, 25% from 3, 15% from 4, 25% from 5, 5% from 6, 10% from 7 warehouses and 5% from 10 warehouses.

It has been determined that 55% of the annual drug purchase turnovers of clinicians and veterinarians are 0-200.000 TL, 45% over 200.000 TL and average 303.875 ± 71.071 TL / year. They stated that 50% of the annual turnover of veterinary clinics is 0-300.000 TL, 50% of it is over 300.000 TL and the general average is 454.000 ± 87.874 TL / year. In the study, it was also calculated that an average of 66.93% of the annual turnover of veterinary clinics was obtained from drug sales.

Descriptive statistics of the factors affecting the drug purchase decision in the veterinary clinics examined in the study are presented in Table-1.

Table 1. *Descriptive statistics of factors affecting drug purchase profit.*

List of Factors	Min	Max	Mean (0-5)	Standard deviation
Corporate factors				
The image and reputation of the pharmaceutical warehouse in the market.	2	5	3,85	0,875
The financial strength of the warehouse, its business background, and experience.	2	5	3,65	0,988
Social and personal characteristics of the warehouse.	2	5	3,50	0,946

Factors related to the salesperson.				
Personal and social characteristics of salespeople	2	5	3,70	1,129
The level of knowledge and promotion ability of salespeople about the products	2	5	4,05	0,887
The level and quality of social communication with salespeople.	2	5	3,70	0,923
Factors related to the product				
Product diversity and regular /adequate stock keeping	3	5	4,05	0,605
Quality/cost balance of products	2	5	4,40	0,821
Recognition of products by consumers	1	5	3,25	1,251
The level of earnings the products provide	3	5	3,80	0,834
The effectiveness and quality level of the products	3	5	4,40	0,754
Factors Related to Price				
The level of competition of prices by market	3	5	3,75	0,786
Balanced and current price policy	2	5	3,85	0,875
Special discounted price application according to the order size	2	5	3,95	0,999
The activity of marketing				
Advertising, promotion, etc. activities related to the products of the pharmaceutical warehouse	1	4	2,95	0,945
Advertising, promotion etc. activities of the manufacturer/importer company regarding the products	1	4	2,85	0,988
Organizing promotional and training meetings about products	1	5	2,70	1,218
Logistics and after-sales				
Pharmacy warehouses have distribution centers in certain regions	1	5	3,85	1,089
Delivery of the ordered products completely and on time	3	5	4,40	0,754
Providing technical information and support for the products sold after-sales	2	5	3,00	1,056
Financial factors				
General maturity policy of the pharmaceutical warehouse	2	5	3,60	0,883
Special maturity application for the products to be purchased	2	5	3,55	0,887
In general, specific maturity determination for your company	2	5	3,60	0,995
Providing convenience in payments received	1	5	3,25	1,333

Factors related to producer/patient owner				
The purchased drug is known to the owners	1	5	3,05	1,234
Easy application of the purchased drug by the patient owners	2	5	3,65	0,933
Payment power of patient owners in the sale of the purchased drug	3	5	3,60	0,740
Profit margin during the sale of the purchased drug	1	5	3,80	1,105

When Table 1 is analyzed, it is observed that the main factors related to the product, logistics, after-sales, and salesperson are in the top ranks according to the level of importance when making a drug purchase decision.

On the other hand, the quality/cost balance of the products (4.40), the efficiency and quality level of the products (4.40) and the complete and timely delivery of the ordered products (4.40) are the most critcal factors.

The product range and regular/sufficient stock availability (4.05) of the warehouses and the knowledge level and promotional ability of the salespeople (4.05) are essential criteria for the decision to purchase.

Organizing promotional and training meetings on products of veterinary medical pharmaceutical companies and warehouses were found to be the most insignificant factor (2.70) on purchasing decisions. It has been determined that the activities such as advertising, promotion, etc related to the products have a low level (2.85) importance on the purchase decisions.

Correlations between drug revenue and factors affecting drug purchase decisions are presented in Table 2.

Table 2. *Correlations between drug purchase revenue and factors affecting the drug purchase decision*

List of factors	r	p
Corporate factors		
The image and reputation of the pharmaceutical warehouse in the market.	0,041	0,863
The financial strength of the warehouse, its business background, and experience.	-0,089	0,710
Social and personal characteristics of the warehouse.	-0,055	0,819
Factors related to the salesperson.		
Personal and social characteristics of salespeople	-0,210	0,374
The level of knowledge and promotion ability of salespeople about the products	0,180	0,447
The level and quality of social communication with salespeople.	-0,257	0,274

Factors related to the product		
Product diversity and regular /adequate stock keeping	-0,077	0,748
Quality/cost balance of products	-0,327	0,160
Recognition of products by consumers	-0,350	0,130
The level of earnings the products provide	-0,148	0,532
The effectiveness and quality level of the products	-0,082	0,731
Factors Related to Price		
The level of competition of prices by market	0,164	0,490
Balanced and current price policy	0,041	0,863
Special discounted price application according to the order size	0,046	0,846
The activity of marketing		
Advertising, promotion, etc. activities related to the products of the pharmaceutical warehouse	-0,278	0,235
Advertising, promotion etc. activities of the manufacturer/ importer company regarding the products	-0,172	0,468
Organizing promotional and training meetings about products	-0,110	0,644
Logistics and after-sales		
Pharmacy warehouses have distribution centers in certain regions	-0,062	0,797
Delivery of the ordered products completely and on time	0,191	0,419
Providing technical information and support for the products sold after-sales	0,078	0,743
Financial factors		
General maturity policy of the pharmaceutical warehouse	0,070	0,769
Special maturity application for the products to be purchased	0,238	0,312
In general, specific maturity determination for your company	0,166	0,485
Providing convenience in payments received	0,290	0,215
Factors related to producer/patient owner		
The purchased drug is known to the owners	0,455*	0,000
Easy application of the purchased drug by the patient owners	-0,315	0,176
Payment power of patient owners in the sale of the purchased drug	0,082	0,731
Profit margin during the sale of the purchased drug	0,261	0,266

**Correlation is significant at the 0.05 level.*

When Table 2 is examined, a statistically significant statistical relationship was found between the annual drug intake turnover of veterinary clinics and the recognition of the drugs taken from the warehouse by the patient owners (r=0,455, p=0,000).

DISCUSSION

Veterinarians in the veterinary medical products market; are the people who recommend the drug and also sell it. When the distribution channels of veterinary medicinal products are analyzed, 67.9% are sold from the manufacturer/importer pharmaceutical warehouse to the veterinarian, 19.6% are directly sold by company representatives or marketing personnel, and 3.6% are sold as spotlights (Mat et al., 2018).

When the research data are examined, the majority of the veterinarians (85%) participating in the study supply products by working with more than two warehouses. The supply of drugs from more than two points instead of a single supplier reveals that in the purchasing process, medicines are purchased from different warehouses according to the conditions rather than routine purchases in the supply chain.

The income of veterinary clinicians from total drug sales was calculated as 66.93%. In the studies carried out in veterinary clinics, it has been determined that veterinary clinics in Konya, Aydın, İzmir and İstanbul provinces reach up to 24% of drug sales income with auxiliary products (Mat et al., 2018). In a study carried out in Nevşehir province, drug sales revenue was determined to be 27.9% (Erdoğan and Sarıözkan, 2011). In a study conducted only in pet clinics in Ankara province, the ratio of drug sales to total income was reported as 35.93% (Aral et al, 2010). The fact that the income obtained from the sales of drugs within the total income of clinical activities in Elazig province is so high indicates that the medical service lags behind the drug trade.

Since the producer/patient owner in the veterinary pharmaceutical sector does not have enough information about the qualities of the products, the final decision maker on the drugs to be used is veterinarians. Brand awareness refers to the information that the consumer has about the brand at a sufficient level (Çavuşoğlu, 2011). Brand awareness is that the consumer has more information about a brand compared to the brands of other companies. The consumer who knows the brand can distinguish and evaluate the brand from the brands of other companies (Uztuğ, 2002). Also, consumers will prefer a brand they know and trust because they do not want to take risks at the stage of a purchase. It is, therefore, a fact that a known brand is more likely to be purchased compared to an unknown brand (Özyiğit, 2010).

Within the scope of the research, when the correlations between the drug purchase revenue and the factors affecting the drug purchase decision are examined; A positive statistically significant relation was found between the fact that the drug taken from the warehouse is known to the patients ($p=0,000$). This result confirms our hypothesis that veterinarians prefer the

brand they know and trust in order not to take risks during the sale of medicines, veterinarians act as intermediaries acting with commercial concerns when deciding to purchase medicines.

Selling drugs in veterinary clinics intensely brings with it some ethical problems. Studies indicate that the most common ethical violations are the unfair competition in drug sales and that clinicians and veterinarians are moving towards drug sales every day (Kızıltetpe, 2011).

As a result; It has been observed that veterinarians act with commercial concerns when deciding to purchase medicines from warehouses and companies, and tend to reach higher income with drug sales activities rather than medical services since they are both medical and drug dealers in clinics.

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Chapter 18

CLINICAL SIGNIFICANCE OF miRNAS: A VALUABLE DIAGNOSTIC, PROGNOSTIC, THERAPEUTIC TOOL

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Introduction

After the human genome project and the unveiling of the whole genomic map, we discovered that only ~2 % of the DNA includes coding-sequences whereas almost 80 % of it, named as bulk DNA then, is transcribed to produce non-coding RNAs (ncRNAs) (1). We know today that microRNAs (miRNAs), a sub-class of short non-coding RNA (ncRNA) family, are responsible for the regulation of mRNA molecules involved in many normal and pathophysiological events in the cell such as apoptosis, development, cellular differentiation, cell cycle control, signaling, cancer, neurodegenerative disorders, etc. (2-5). Since 1993 when is their first discovery in *C. elegans* nematodes (6), the identified miRNAs have common conserved nucleotide (nt) sequences across species (2, 7). miRNAs are transcribed in many different cell types, including plant and animal cells (2, 3). These oligonucleotides (~19-25 nt-long) provide post-transcriptional regulation of gene activity in the cell by binding to target mRNAs at different complementarity ratio (8). Until today, more than 48,000 miRNAs of which 2693 human-originated, were recorded in miRBase Database, release 22 (9, 10). Emerging evidence has revealed that miRNAs can be used as promising tools for diagnosis, prognosis, and/or therapy of many diseases such as cancer, neurological disorders, and other diseases (7).

miRNA biogenesis

miRNA maturation consists of sequential events and during each single process, specific players are involved (4) as depicted in Figure 1. First of all, miRNA-coding regions in DNA were transcribed by RNA polymerase-II, or -III (5, 7), to synthesize ~500-3000 nt-long primary miRNA (pri-miRNA) as modified with 5'-CAP and 3'-polyA tail in the nucleus (4, 5). Pri-miRNA can have one (mono-cistronic) or multiple (poly-cistronic) hairpin structures (1). Then pri-miRNA is bound and cleaved with double-strand (ds)-specific proteins ribonuclease (RNase) DROSHA enzyme accompanied with DiGeorge syndrome critical region gene 8 (DGCR8), also called PASHA, to synthesize a ~60-70 nt-long precursor miRNA (pre-miRNA) comprising a stem-loop with a single hairpin structure (1, 4). After that, the pre-miRNA is transported through nuclear pores to the cytosol by Exportin-5-RAN-GTP complex and again cleaved from its hairpin loop by another ds-specific RNAase called DICER1 accompanied with TRBP to form a miRNA-miRNA* duplex (4, 11). miRNA duplex is separated from each other by the helicase activity of DICER1, and the mature single-strand miRNA is bound by ARGONAUTE (AGO-2) and GEMIN-3 and -4 to assemble a miRNA-induced silencing complex (miRISC) in the cytosol (4, 11). The opposite or so-called passenger strand miRNA*, the 3'-arm of miRNA (miRNA-3p), is mostly degraded in the cytosol (5,

7). AGO-2 guides the transport of miRNA to complement its seeding sequence (2-8 nt-long) with 3'-UTR of its target mRNA (1, 5). The intracellular levels of miRNA are regulated via multiple mechanisms such as by miRNA itself as a feed-back, other miRNAs, 5'- or 3'-end-modification of the miRNAs, and/or binding of regulatory proteins on immature miRNAs (5, 7). The primary function of a mature miRNA is to knock-down their target genes which can be achieved by degradation, or deadenylation, decapping, and gradual decay of target mRNA, or blocking the translation depending on the complementation rate of miRNA/mRNA pairing (1, 4). Database studies revealed that several miRNAs can bind to a single mRNA molecule or vice versa which makes it complicated to bridge a direct correlation between miRNA and mRNA expressions (5, 7, 10). Furthermore, previous studies have also stated that miRISC complexes can also regulate gene expression via epigenetic control mechanisms at the transcriptional level, e.g. by altering the affinity of transcription factors to the transcription units, or by directly binding to the DNA to change chromatin morphology and its transcriptional availability (12).

miRNAs as potential tools for diseases

The onset of a disease is most of the time initiated by the deregulation of the molecular players and the ensuing imbalance between these regulatory proteins. For instance, the imbalances between oncogenes and tumor-suppressor genes lead to hypertrophy and result in tumorigenesis.

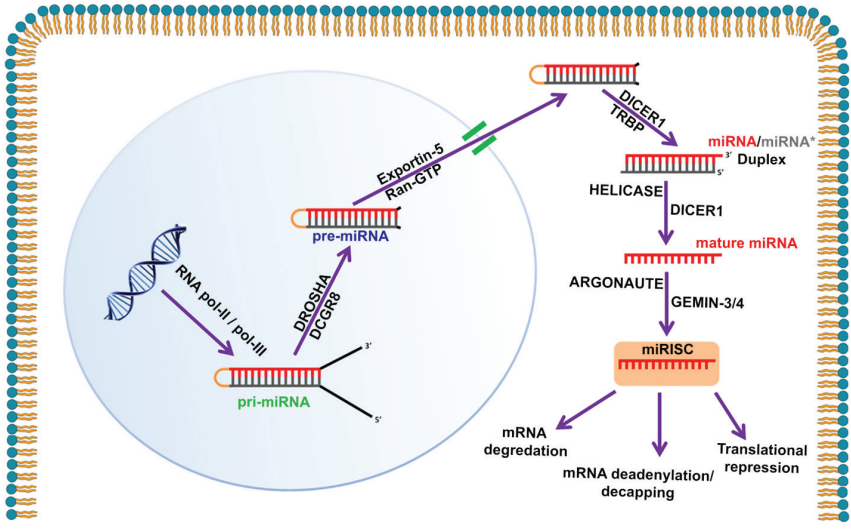

Figure 1. Simplified schematic of microRNA maturation in animal cells. After transcription of pri-miRNA by RNA pol-II/III from DNA in nucleus, microprocessors, DROSHA and DCGR8, cleave pri-miRNA from the 3'-5' free ends till stem-duplex to produce pre-miRNA that is transferred to cytosol by means of

Exportin-5/Ran-GTP carrier complex. DICER/TRBP complex further cleaves pre-miRNA to yield mature miRNA duplex. Mature ss-miRNA (in red) is produced by removing "passenger strand" (in gray) and it is bound with ARGONAUTE/GEMIN complex to assemble active miRISC to silence mRNA through mRNA degradation, deadenylation, or decapping of mRNA, or translational repression. RNA pol-II/III: RNA polymerase-II/III, pri-miRNA: primary microRNA, pre-miRNA: precursor microRNA, ss-miRNA: single-stranded miRNA, miRISC: miRNA-induced silencing complex.

The imbalances do not always arise from the mutations in those particular genes but as we know today, it can stem from the mutations or dysregulation of miRNA-encoded genes that are involved in post-translational control of those aforementioned regulatory proteins (4, 13, 14). Impaired miRNA processing pathways were also linked to tumorigenesis (5). It has been shown in previous studies that the targets of deregulated miRNAs, e.g. miR-21, let-7 family, in several cancer cells were the prominent oncogenes and/or tumor-suppressor genes (4, 14). In a meta-analysis conducted with 23 clinical studies comprising 21 solid tumor samples and 2-serum/plasma, deregulated 42 miRNAs that belong to the miR-379/miR-656 cluster within chromosome 14 were found to be correlated with proliferation, apoptosis, invasion, metastasis, and migration events of several cancer cell types (15).

Most of the miRNAs originally reside intracellularly (10). As to our knowledge, miRNAs can stay stable without degradation long enough for detection in both solid tissues and bodily fluids such as plasma/serum, urine, cerebrospinal fluid, milk, and even tear, which makes them invaluable diagnostic and prognostic biomarkers in pathophysiological conditions (16, 17). Besides, the surveillance of patients is essential during the course of therapy to analyze the efficacy of treatments, e.g. cancer therapy and a likely recurrence. In this context, it is not easy to monitor patients with invasive sampling methods such as biopsy. Thus, the circulating biomarkers, such as miRNAs, gained great importance of diagnosis and following up patients (10, 15). On the other hand, the standardization of miRNA analyses must be achieved by taking great attention to the following parameters; sampling, miRNA isolation, quality control, quantification and data analysis, and interpretation of the data (e.g. biomarker characterization) (10).

miRNA implication in cancer

So far among other diseases, cancer is the most extensively studied disease model regarding the roles of miRNAs as diagnostic, prognostic and therapeutic purposes. In one of the first miRNA-related cancer studies, miR-15 and -16 genes were found to be at the deleted region of the chromosome 13 in the majority of the chronic lymphocytic leukemia patients

and these miRNAs were suspected as the first tumor-suppressor genes (13). But there were also upregulated miRNAs, like miR-155 in lymphoma cases, which then were considered as oncogenes (13). Regarding solid tumors, previous studies declared that serum levels of miR-155, miR-214, and exosomal miR-1246 and miR-21 in breast cancer patients and miR-200 in ovarian cancer patients were upregulated which suggests a differential and early diagnosis of critical cancer cases (1).

During tumorigenesis, the serum levels of several miRNAs are deregulated which can lead to metastasis and relapse of the cancer (7). For instance, while the serum levels of miR-30c-1*, miR-146b-3p, miR-550, miR-566, miR-616*, and miR-939 were upregulated in lung adenocarcinoma patients, mir-486 was found to be diminished in the sera of stage-1 non-small cell lung cancer patients (18, 19). Additionally, the expression pattern of three miRNAs (miR-29a, miR-181a, and miR-652) was utilized for the differential diagnosis of breast cancer patients (20). Dysregulation of miRNAs is also implicated in poor-prognosis of breast cancer patients due to the progression of metastasis, such as over-expression of miR-10b (21).

It can be anticipated that not only deregulated miRNAs but also reduced levels of miRNA microprocessors like DICER1 can be correlated with the poor prognosis in lung cancer since miRNA regulation is directly connected with its biogenesis (5). Similarly, the experimental knock-down of the miRNA microprocessors *DROSHA, DGCR8* or *DICER1* resulted in tumorigenesis in cancer models (22).

The circulating miRNAs can be carried within exosomes (microvesicles, Ø 50-100 nm) together with mRNAs, proteins, lipids, etc. or as bound to lipids or carrier proteins to the target cells (10, 23). It was shown that the number of exosomes carrying miRNAs increases around tumor microenvironment (23). So the tumor cells can modify the neighboring cells for the transformation and metastasis by modulating their fates (23). A study conducted with cancer cell lines demonstrated that cancer exosomes carrying miR-342–3p and miR-1246 could increase migration and invasion of oral tumor cells (24). The primary goal of cancer cells is to gain chemoresistance and mobility for survival by inhibiting apoptosis and inducing angiogenesis. In an in vitro study, the drug resistance of the breast tumor cells was augmented via certain miRNA treatment i.e. miR-222, miR-23a, and miR-24, delivered within exosomes by aiming the expression of tumor-suppressor *p27* and *PTEN* (25). Chemoresistance of tumor cells can also be maintained by cancer stem cell-like cells in the microenvironment via elevated production and secretion of miRNA-containing exosomes to inhibit apoptosis, induce DNA-damage response, and initiate epithelial-mesenchymal transition (23). Another challenge in cancer therapy is the immune resistance of tumor cells. Tumor cells can evade from immune

system by modulating the ingredients of exosomes and alter the immune response and activity of immune cells (23).

miRNAs can also be utilized for surveillance of disease course and treatment response purposes owing to their stable expression and presence in circulation. In clinical studies, the non-reduced miR-210 and mir-221 serum levels exhibited a poor prognosis due to chemoresistance against cancer therapy in breast and ovarian cancer patients (1). In line with these findings, over-expression of miR-222 and miR-29a in breast cancer cell line models were also associated with chemoresistance against different chemotherapeutics, which was achieved by targeting PTEN (1).

miRNA involvement in neurological and psychiatric disorders

miRNAs are implicated in some neurodegenerative and psychiatric diseases too (26, 27). Similar to the development of other organs, brain maturation is also dependent on the spatiotemporal expression of the genes that are regulated by miRNAs (28). In a microarray study, 312 miRNAs were shown to be differentially expressed during different periods of a human lifespan i.e. fetal, post-natal, and adulthood (29). Yet only some of the specific miRNAs have been observed to function in neurogenesis and synaptic activity such as miR-132, miR-134, miR-212, etc. (28). But accumulated evidence has shown that the miRNA levels are deregulated in psychiatric disorders such as post-traumatic stress disorder (PTSD), schizophrenia, bipolar disorder, etc. (30). One of the mental disorders correlated with dysregulation of miRNAs is schizophrenia. Among which miR-137, miR-485-5p, and miR-9-5p in progenitor neurons were reported to be correlated with schizophrenia based on the computational genome-wide analysis (31). A study conducted with 34 postmortem adult schizophrenia cases asserted that miRNA profile in the prefrontal cortex of the schizophrenia patients exhibited a reverted expression pattern such that the miRNA genes that are supposed to be present at infancy of healthy individuals, were found to be upregulated whereas the ones that are normally expressed during puberty of healthy people, were downregulated in schizophrenia patients (28). In the same study, it was further suggested that these deregulated miRNAs were also associated with the known schizophrenia-related genes. In a similar study performed with the postmortem brains of 15 schizophrenia patients, 15 miRNAs out of 16 dysregulated miRNAs were determined to be downregulated, among which miR106b, miR-30b, miR26b, and miR-29b exhibited almost two-fold reduction (32). In the same study, the ratio of pri-miRNA/mature miRNA of these differentially expressed miRNAs was lower compared to healthy controls, thereby indicating a failure of miRNA maturation. miR-132 that is aforementioned

regarding its role in synaptic plasticity and neurogenesis, was found to be diminished in the prefrontal cortex of 100 schizophrenia patients among 854-analyzed miRNAs. The same group also demonstrated *in vivo* that miR-132 levels were regulated by an NMDA-receptor agonist, which takes part in synaptic plasticity and memory, presumably in coordination with miR-132 (33). Besides, in circulation, reduced miR-3130-5p levels in PTSD with depression (34), reduced miR-134 and elevated miR-1908, miR-708, and miR-499 levels in bipolar patients (35, 36), and dysregulated miR-144-5p, miR-34, let-7 family, miR-182, and miR-132 levels in stress and depression patients were found to be significantly correlated with the disorders (30).

Previous studies assessed the dysregulation of miRNAs also in neurological disorders some of which are multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), etc. AD is one of the extensively studied neurodegenerative diseases with respect to diagnosis and therapeutic solutions. But, since there are contradicting outcomes regarding the dysregulation of individual miRNAs, there is yet no consensus about a solid expression pattern during the AD pathophysiology (27). Some of the commonly deregulated miRNAs in AD patients are miR-9, miR-146, miR-181, miR-29, miR-106, miR-34, miR-125b, miR-107, and miR-128 (37). Sohrab et al. stated in 2018 that, 45 miRNAs, analyzed with several samples i.e. whole blood, cerebrospinal fluid (CSF), peripheral blood mononuclear cells (PBMC), serum, and plasma, have been linked to the AD progression (27). Among which, in bodily fluids; miR-27a-3p, miR-29a/b, and miR-181c were found to be reduced whereas miR-146a, miR-155, miR-125b, let-7b, miR-15a, miR-132, and miR-134 families were announced as upregulated in AD patients (27). In the same study, some of the miRNAs, e.g. miR-505, miR-331-5p, miR-626, miR-1826, and, miR-450b-3p, were reported as deregulated in plasma samples of Parkinson's disease (PD) patients. Besides, in another study conducted with PD, miR-29, miR-22*, and miR-1 levels in peripheral blood samples were found to be significantly reduced compared to healthy controls (38). Additionally, the results of an intriguing clinical study suggested that the dopaminergic neurons in the midbrain of PD patients completely lacked the miR-133b expression which is normally required for neurogenesis in healthy individuals (39). Microarray and RNA deep sequences provided valuable data coming from miRNA profiling studies. Among the studies using genome-wide research to find disease-associated genes, various miRNAs (miR-27a-3p, miR-29a/b, miR-146a, miR-155, miR-125b, miR-15a, miR-132, let-7 family, etc.), especially in PBMCs, were identified to be dysregulated during the pathogenesis of systemic lupus erythematosus (SLE) (40). SLE is an auto-immune originated disease with genetic, environmental and epigenetic involvements.

Besides the studies focus on miRNA profiling of SLE patients, a study conducted by Jakymiw et al. ascertained that elevated auto-antibodies in SLE patients were discovered to be also reactive against AGO2 protein which is a central essential microprocessor during miRNA biogenesis and action mechanism. As a result, the disrupted miRNA biogenesis and activity might contribute to the SLE progression with a poor prognosis (41). Similar to SLE disease, there are other immune system-related disorders such as epilepsy and MS. Therefore, any defect in the regulation of both innate and acquired immune systems players due to interference of dysregulated miRNAs can result in complex failures in the immune system and such diseases. An *in silico* study unraveled the connection between various downstream genes of the TGF-β pathway and multitude numbers of miRNAs (42). Besides, it was observed in clinical studies that the targets of the dysregulated miRNAs were mostly the immune-related genes (43). Among which, miR-146a/b, miR-155, and let-7d-5p can be notified as markedly deregulated in epilepsy patients (44). Similar to other autoimmune disorders, the miRNA profile is also abrogated during MS pathogenesis. So far, various sample sources such as blood, CSF, PBMCs, T-cells, plasma/serum were used to assess any correlation between miRNA deregulation and MS (45, 46). In previous studies with MS patients, miR-155 and miR-326, which are involved in Th17 production, were shown to be related to MS pathogenesis (47, 48). In a more comprehensive clinical study performed by Siegel et al. with 4 MS patients and 4 healthy controls, 900 miRNA were analyzed in plasma samples and of which 6 miRNAs, i.e. miR-22, miR-1826, miR-572, miR-422a, miR-648, and miR-614, were detected as upregulated whereas only one, miR-1979, was downregulated (49). Another group also analyzed the expression signature of miRNAs in whole blood and plasma samples of 22 MS patients and suggested that miR-145 exhibited the strongest correlation with the disease with ~3-fold upregulation (50). The alteration of miRNA profile or expression signature also exists in several brain injury-stemmed diseases such as ischemia (51). Besides, it was shown *in vitro* and *in vivo* that post-cerebral ischemia, mesenchymal stem cells transfer exosomal miR-133b to neurons and astrocytes for stimulation of neurogenesis (7, 52).

Role of miRNAs in other diseases

DiGeorge syndrome results from a deletion in chromosome 22 and it was shown that this deleted region includes a gene called *DGCR8* that is responsible for pri-miRNA processing together with DROSHA (4). But the exact association of the deletion with the syndrome is not clear yet. It was shown in a previous study that altered expression of miRNA, due to loss of DICER1 activity and miRNA biogenesis, resulted in deterioration of vision (7). miRNAs are also deregulated in more systemic and metabolic dis-

eases such as heart-, liver-, and muscle-related disorders. Reduced levels of miR-143 and -145 were reported to cause stress-induced cardiovascular disorders (53). Furthermore, as previously reported, the patients with liver fibrosis had increased miR-21 and decreased miR-129-5p levels in liver tissue (54). Besides, miR-214, miR-34a, miR-652, miR-571, and miR-181, were also stated as promising molecules for diagnosis of liver fibrosis (54). The miRNA profiling was conducted with DMD patients and miR-1, miR-31, and miR-133 were upregulated in the serum samples whereas miR-21-5p, miR-23b-3p, and miR-29c-3p levels were detected as down-regulated in urine samples (55). A summary of dysregulated miRNAs in other clinical cases, i.e. skin and metabolic disorders were listed in Table-1.

Utilization of miRNAs as therapeutic targets

The main objective to achieve in miRNA-based therapies is to reverse the dysregulated levels of miRNAs to the normal regular levels by agonistic and/or antagonistic approaches (56). miRNAs can be delivered as therapeutic tools via extracellular vesicles (EVs) to the target cells for modulation of gene expression (7). One of the options to be utilized from miRNAs in clinics is the use of anti-miRNA oligonucleotides (AMOs) which are synthetic oligonucleotides and designed to bind to their target mRNAs for functional blockage (4). By means of AMOs, the specific functions and targets of miRNA can be enlightened and novel therapeutic strategies can be created (4). But there are some obstacles to succeed this aim such as optimization of external miRNAs' lifespans to protect them from early decay and warranting cell-targeted therapy (56). There are many studies reporting that miRNAs can be transferred within viral vectors however, the main obstacles of this method are the early elimination of viruses by innate/adaptive immune system or the high risk of tumorigenesis mediated by viral genes (56). Nevertheless, non-viral vectors such as lipid-based (e.g. liposomes, lipid nanoparticles), polymer-based (e.g. hyaluronic acid, hydrogels), carbon-based carriers, etc. seem to be more promising owing to their easy modification, lack of immunogenicity, more stable features, and less bioactive nature compared to viruses (56). Last but not least, MSCs harbor the pivotal potential for miRNA-mediated therapy. It was shown in a cell culture study that, MSC-mediated secretion of miR-22 in EVs was found to be cardio-protective post-myocardial infarction (57).

Table 1. Some of the dysregulated miRNAs in various clinical disorders.

Dysregulated miRNAs	Clinical sample	Disease	Ref.
CANCER			
miR-15, miR-155, miR-16	Lymphocytes	CLL	(13)
miR-24, miR-155, miR-214, miR-21, miR-1246, miR-29a, miR-181a, miR-652, miR-200, miR-210, mir-221, miR-10b	Serum, tumor tissue	Breast, ovarian cancers	(1),(20),(21)
miR-24, miR-566, miR-616*, miR-550, miR-939, miR-30c-1*, mir-486, miR-146b-3p	Serum	Lung cancer	(18),(19)
miR-221-3p	Tumor tissue, serum	Melanoma	(58)
NEUROLOGICAL & PSYCHIATRIC DISORDERS			
miR-29b, miR-137, miR-485-5p, miR-9-5p, miR26b, miR-30b, miR-132, miR106b	brain tissues, in silico	Schizophrenia	(31),(36)
miR-499, miR-708 and miR-1908 levels in bipolar patients	Plasma, in silico	Bipolar disorder	(36),(35)
miR-3130-5p	Blood	PTSD	(34)
miR-144-5p, miR-34, let-7 family, miR-182, miR-132	Blood, plasma	Stress, depression	(30)
miR-146, miR-9, miR-107, miR-34, miR-125b, miR-27a-3p, miR-29a/b, miR-181c, miR-146a, miR-155, let-7b, miR-15a, miR-132, miR-134 family, miR-106, miR-29, miR-181, miR-128	Brain tissue, CSF, blood, plasma, serum, PBMC	Alzheimer's disease	(37),(27)
miR-29, miR-331-5p, miR-450b-3p, miR-505, miR-22*, miR-133b, miR-1826, miR-626, miR-1	Blood, plasma, brain tissue	Parkinson's disease	(27),(38)
miR-27a-3p, miR-29a/b, miR-146a, miR-155, miR-125b, miR-15a, miR-132, let-7 family	PBMC	SLE	(40)
miR-146a/b, miR-155, let-7d-5p	Brain tissue, serum	Epilepsy	(44)
miR-22, miR-155, miR-326, miR-422a, miR-614, miR-1826, miR-1979, miR-145, miR-572, miR-648	Brain tissue, PBL, blood, plasma	Multiple sclerosis	(47),(48), (49),(50)
OTHER DISEASES			
miR-145, miR-143, miR-24, miR-125b, miR-195, miR-214	Blood, plasma, PBMC	Cardiovascular disorder, heart failure	(53),(59)
miR-21, miR-129-5p, miR-34a, miR-181, miR-214, miR-571, miR-652	Liver tissue, blood, serum	Liver fibrosis	(54)
miR-1, miR-31, miR-133, miR-29c-3p, miR-23b-3p, miR-21-5p	Serum, urine	DMD	(55)

miR-203-3p, miR-146a-5p, miR-221-3p, miR-424-5p, miR-223-3p, miR-29-3p, let-7a-5p	Skin tissue, serum	Psoriasis, Dermatomyositis, Scleroderma	(58)
miR-24, miR-125b, miR-195	Blood	Type-1/2 Diabetes	(59)
miR-125b	Blood	Chronic kidneydisease	(59)

miR/miRNA: Mature microRNA, MS: Multiple sclerosis, CSF: Cerebrospinal fluid, PBMC: Peripheral blood mononuclear cell, PBL: Peripheral blood leukocytes, SLE: Systemic lupus erythematosus, PTSD: Post-traumatic stress disorder, DMD: Duchenne muscular dystrophy, CLL: Chronic lymphocytic leukemia.

Concluding remarks

Overall, since the uncovering of the whole human genome, new insights are being gained regarding the clinical outcome of this breakthrough discovery. As we know today, the diseases are resulting from the imbalances among molecular regulators in biological systems. Until recently, these imbalances have been mostly associated with the mutations in encoding genes of DNA. The encoding genes, however, constitute only ~2% of the whole genome while ~80 % of DNA, formerly designated as "bulk DNA", is used for synthesis of ncRNAs. The evidence is accumulating that ncRNAs have had significant implications in almost every patho/physiological condition such as development, apoptosis, cancer, neurodegenerative disorders, etc. If we try to make projections from today's perspective, it can be barely anticipated the uncovered share and role of ncRNAs, like miRNAs, in biological processes.

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Chapter 19

DENTAL STEM CELLS AND THEIR USES IN DENTISTRY

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INTRODUCTION

Stem cells have an essential role in the repair of all organs and self-renewal capacity of the tissues. Stem cells are able to self-renew and produce different types of cells and may offer the possibility of developing new strategies to regenerate missing tissues and treat diseases (1). In dentistry, a significant increase in stem cell research has been observed in recent years and potential applications of stem cells for the repair and renewal of dental structures have begun to be explored. It is becoming increasingly clear that this approach known as "regenerative dentistry" will be included in the clinical practice of dentistry in the future (2). The oral cavity is a rich and unique source of stem cells and thus, further characterization of these cells is crucial for dental clinicians and researchers in order to develop novel and effective strategies for dental applications (3).

Stem cells are classified as Totipotent, Pluripotent and Multipotent based on their ability to differentiate.

1. Totipotent: These are early embryonic cells which have the potential to differentiate into all cell types.

2. Pluripotent: They can give rise to all cells but cannot develop into an entire organism on their own.

3. Multipotent: They have the capacity to develop into at least two types of specialized cells.

Stem cells can be divided into Embryonic (Fetal) and Adult (Postnatal) according to the sources they are derived (1,4).

Embryonic Stem Cells: Embryonic stem cells were first isolated from mouse embryo (5) and subsequently researchers managed to replicate human embryonic cells in vitro successfully (6). The sperm and ovum unite through fertilization, creating a zygote. The zygote then undergoes cell divisions and doubles its cell number within 36 hours. Embryonic Stem Cells consist of embryos that develop from fertilized eggs (zygote) in vitro and are capable of unlimited proliferation (7). The collection of cells that are formed between days 2 and 11 is called blastocyst. The cells that are derived from the inner cell mass of the blastocyst are called embryonic stem cells. These stem cells originating from the embryo have the potential to form all tissues in the body (8,9).

Adult (Somatic) Stem Cells: These unspecialized cells are found in many tissues and organs and can transform into specific cell types. The sources of adult stem cells (ASCs) are bone marrow, brain, blood, eye, skeletal muscle, lining of the gastrointestinal tract, pancreas, dental pulp, skin and the stem cells found in these tissues are multipotent. ASCs are

located in sites called Niches and these stem cells are believed to reside in specific, three-dimensional anatomic sites (10). Adult stem cells are found in small numbers in each tissue and they need to be grown in the laboratory environment in order to be used for therapeutic purposes (11).

When compared with adult stem cells, embryonic stem cells are pluripotent and capable of unlimited proliferation. Adult stem cells are multipotent unless they are programmed. Embryonic stem cells easily proliferate under in vitro conditions, whereas a limited number of adult stem cells are found in the tissues and harvesting these cells is cumbersome (7,12).

Stem cells obtained from various tissues are used for tissue repair (13-15). The sources of adult stem cells in the oral and maxillofacial areas include mainly Bone marrow (BMSCs), Dental pulpa (DPSCs), Deciduous teeth (SHED), Periodontal ligament (PDLSCs), Dental follicle (DFSCs), Tooth germ (TGPCs), Apical Papilla (SCAP), Oral Epithelium (OESCs), Gingiva (GMSCs), Periosteum (PSCs) and salivary gland (SGSCs) (1). Dental pulp stem cells are widely used in research studies because they can be obtained easily without the need for challenging and complex procedures (12).

The uses of stem cells in the field of dentistry generally include regeneration of damaged coronal dentin and pulp, regeneration of resorbed tooth, cervical or apical dentin and perforations, regeneration of periodontal tissues and regeneration of the entire dental tissue (8).

STEM CELLS FROM DENTAL TISSUES

In the embryonic stage, tooth development (odontogenesis) occurs through differentiation of neural crest cells originating from the ectodermal structure and mesencyhme. Several types of stem cells and progenitor cells are involved in tooth development. These include dental epithelial stem cells, dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from the apical papilla (SCAPs), periodontal ligament stem cells (PDLSCs) and dental follicle progenitor cells (16).

Dental epithelial stem cells

These are undifferentiated cells that are found in the dental epithelial tissue which support continuous regeneration of rodent incisors but disappear after tooth eruption in humans.

Dental Pulp Stem Cells (DPSCs)

These cells which were identified by Gronthos et al. were shown to differentiate into a dentin-pulp complex characterized by mineralized tubular matrices surrounded by odontoblasts and blood vessels (17). Their

ability to differentiate into neural cells and adipocytes in vivo was also demonstrated by the same researchers. Dental pulp stem cells are multipotent clonogenic mesenchymal cells which exhibit a high rate of proliferation and high plasticity (18)

Dental pulp is made up of connective tissue which is found directly beneath the dentin. While dentin surrounds and protects the entire pulp, the pulp both regenerates and nourishes the dentin (19). Studies have shown formation of repair dentin as a result of odontoblastic actions of dental pulp stem cells on the exposed pulp tissue (20). Dental pulp stem cells were demonstrated to transform into both induced pluripotent stem cells and odontoblasts, myocytes, osteoblasts, chondrocytes, adipocytes, neurocytes and corneal epithelial cells (21,22). It was also reported when isolated from the dental pulp under appropriate conditions, these stem cells exhibit immunoregulatory activity and do not induce immune response (23)

Two methods are commonly used for isolation of stem cells from the dental pulp. In the explant method, extracted teeth are mechanically split to remove dental pulp tissue under sterile conditions which is then minced into small fragments, grown in a culture medium and a cell suspension is obtained. In the enzymatic digestion method, the dental pulp is surgically removed under sterile conditions, digested in enzymatic substances and cells are removed from the fragments of the pulp tissue to obtain a cell suspension (24). Primary sources of dental pulp stem cells include extracted third molars, premolars or other teeth extracted for orthodontic purposes and extracted deciduous teeth. Third molars are more commonly used as a source since they have an indication for extraction and their pulpal tissues are rich in stem cells because these are the last teeth to erupt (25,26).

Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs)

Although this type of stem cell has been recently isolated, it led to great excitement.

The stem cells retrieved from the pulp of deciduous teeth were reported to have a higher proliferation rate, with increased cell population and more immature multipotent cells compared to adult dental pulp but unlike adult dental pulp stem cells, they fail to form a complex pulp-dentin structure.

Stem Cells from the Apical Papilla (SCAPs)

They are obtained from apical papilla of impacted teeth or incisors at the very early stages of odontogenesis. These cells have a higher dentin regeneration compared to DPSCs and are difficult to isolate. They have limited indications for extraction. SCAPs were reported to contain more stem cells than adult dental pulp and they can form connective tissue when

used in combination with periodontal ligament stem cells (27,28). However, they were shown to have the ability to differentiate into odontoblast-like tissue and adipocytes and produce dentin-like tissue both in vitro and in vivo (29).

Periodontal Ligament Stem Cells (PDLSCs)

PDLSCs are isolated from root surfaces of extracted teeth and can transform into periodontium-like cells and tissues and cells. They are known to form colonies but have little potential for in vitro osteogenic differentiation. Researchers have reported that this kind of stem cells can induce tissue regeneration and periodontal repair when transplanted into mice (30,31).

In one study, Seo et al. investigated whether human periodontal ligament tissue contained stem cells that could be used for periodontal tissue regeneration. They showed that periodontal ligament tissue extracted from human third molar contains clonogenic and rapidly proliferating cells that can express antigens specific to some mesenchymal stem cells. They reported that when a mixture of these cells with a scaffold matrix, hydroxyapatite/tricalcium phosphate powder, was implanted to immunocompromised mice, a thin layer of cement/periodontal ligament-like structure with dense collagen fibers resembling Sharpey fibers was formed (31).

Dental Follicle Progenitor Cells

The dental follicle contains ectomesenchymal cells originating from neural crest. During the development of the tooth root, cement, periodontal ligament and alveolar bone are formed by dental follicle progenitor cells (18). The dental follicle can easily be isolated after extraction of developing wisdom teeth, making it readily accessible.

Dental follicle stem cells were first isolated by Handa et al. from bovine tooth germs. Handa et al. examined the differentiation capacity of bovine dental follicle cells in immunosuppressed mice in vivo and found that these cells can form a cement-like matrix which was not observed with bovine fibroblasts or alveolar osteoblasts (32) Morsczeck et al. reported that the dental follicle tissue extracted from impacted human third molars contained progenitor cells. These fibroblast-like, plastic adherent and colony forming cells were found to express putative stem cell markers (Notch-1 and Nestin). At 8 weeks after their transplantation into mice using a suitable scaffold, a rigid or fibrous connective tissue was formed with increased osteoblastic antigen expression without any formation of cement or bone (33).

REGENERATIVE TREATMENT WITH DENTAL STEM CELLS

The most important application of stem cells is to derive cells or tissues that could be used for cellular treatment or regenerative therapy. Cellular or regenerative therapy is a form of treatment aiming to restore and maintain the function of damaged organ and tissues using healthy cells (34). Currently, organ transplantation is mostly used for this purpose. However, the low number of donated organs, long waiting time and complications associated with transplantation limit its widespread use. Stem cells which have the ability to differentiate into specific cells may provide a sustainable source of replacement cells and tissues to treat many conditions including Alzheimer's disease, spinal cord injury, stroke, burn, cardiac disease, diabetes, osteoarthritis and rheumatoid arthritis and particularly, for regenerative treatment of tissues lost after tumor resection in oral cavity (35).

The goal of regenerative dentistry is to replace damaged tooth structures to restore normal anatomy and function. Dental stem cells are able to create a microenvironment to promote tissue repair and can stimulate endogenous stem cells or progenitor cells. Appropriate bioactive scaffolds can induce effective dental tissue repair responses through activation of endogenous stem cells and progenitor cells and thereby, obviate the need for exogenous stem cell application (36-38). Such novel strategies would be easier to apply in clinical practice and will possibly raise fewer ethical concerns (37, 39).

The treatment approach involving dental stem cells offers a wide spectrum from specific dental tissues such as pulp or periodontium to formation of the entire tooth.

Regeneration of Pulp-Dentin Complex

Regenerative endodontics is a novel treatment modality that relies on delivery of stem cells into the canal and focuses on restoring dental pulp vitality and promoting continuous root development (40). The first experimental trial using dental pulp stem cells showed that these cells can differentiate into odontoblasts that form a dentin-like structure when transplanted with HA/TCP ceramic powder into immunocompromised mice (17). In recent studies, vascularized tissue was demonstrated after placement of DPSCs and SCAPs into empty tooth root canal (36,41,42). However, this method cannot be included in clinical practice since these studies are carried out in ectopic sites. As a result, investigators have begun searching new ways to use these cells with the aid of bioactive materials and appropriate scaffolds. In 2013, Iohara et al. transplanted DPSCs in combination with granulocyte-stimulating factor (G-CSF) to the root canal of dogs and succeeded in production of new pulp tissue as well as new dentin (43)

Despite all these advances, stem cell-based endodontic treatment is still regarded as an empirical approach (44).

Regeneration of Periodontal Tissue

The stages of healing process should proceed in a programmed and sequential manner in order for regeneration to take place (45). PDLSCs were shown to improve periodontal tissue regeneration when transplanted into immunocompromised mice and have immense potential for future cell-based treatments in dentistry (31). Animal studies have been designed to explore the use of PDLSCs, DPSCs, SHEDs and bone marrow stem cells in the treatment of periodontitis, producing successful results (46,47).

In a 2009 study, d'Aquino et al. grafted a biocomplex of DPSCs with a collagen scaffold for treatment of alveolar bone defect resulting from periodontal disease and showed complete restoration of the alveolar bone (14). However, in a study with long-term follow-up, Giuliani et al. reported that the regenerated bone in the engraftment site was different from the normal alveolar bone (48). A clear understanding of the potential and characteristics of dental pulp stem cells would be of benefit to develop clinical treatment models and can provide a new therapeutic option in dental diseases. In this context, the concept of tissue engineering in dentistry based on a triad of the DPSCs, growth factors and scaffold offers great opportunities for use in regenerative dentistry and merits further studies.

Regeneration of Entire Tooth

Recent research studies have attempted to produce a tooth-like structure using a human being's own tissues. The ultimate goal of such efforts is to obtain a fully functional tooth organ.

In theory, the combination of dental epithelial stem cells and dental mesenchymal stem cells under suitable in vitro conditions would permit the formation of tooth germ to produce a tooth that could develop, erupt and function when seeded in the alveolar bone (49). Different researchers have designed studies with different methodologies. In a study by Yang et al., the tooth buds harvested from miniature pigs were autografted using a platelet-rich fibrin scaffold (50). In another study, dental epithelial and mesenchymal stem cells were implanted with a soluble dental polymer (51). Almost all of the bioengineered teeth have been produced in ectopic sites using human stem cells. However, they lack some essential elements such as correct crown morphology and formation of complete root.

However, recent studies in mice using bioengineering strategies have shown that it is possible to produce functional teeth having roots. The formation of new functional teeth was observed after implantation of the tooth germs that were produced by dental epithelial and mesenchymal cells seeded in collagen to the mandibles of mice (51).

CONCLUSION

In order to achieve clinical success with regenerative treatments, differentiation, transplantation and implantation characteristics of the dental stem cells have to be directed. Thus, the following prerequisites have to be fulfilled for stem cells including sufficient cell proliferation for tissue formation, differentiation into the desired cell type, the ability to survive in the recipient after transplantation and fully function for the duration of the recipient's life.

With advancing technology and knowledge, conventional traditional therapeutic methods can be replaced by alternative strategies through formation of new teeth, bone tissue and periodontal ligament and repair of dental tissues using dental stem cells.

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Chapter 20

Investigation on Atmospheric Allergen Pollens of Thrace (Edirne, Kırklareli, Tekirdağ) in Turkey

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Allergic diseases affect 20 to 30% of our society, and the exposure both restricts the standard of living and causes loss of workforce. Therefore, it creates serious economic effects, whether socially or due to the length of the treatment process. For this reason, allergy is among the major disease groups. Pollen is one of the important factors in the emergence of allergic sensitivity in individuals and clinical manifestations.

Pollen grains are popularly known as flower dust. Their size is usually 5-200 μm in diameter. Pollen are the male reproductive cells of plants and carry a large number of allergic proteins on them. These proteins cause allergic symptoms in sensitive individuals. The main task of the pollen is to reach the female organ of the female flower and perform pollination, that is, fertilization. The most common method for pollination is wind, and this type of plant pollination is called Anemophilous. In addition, pollination can be seen with water (Hydrophilous), insects (Entemophilous), birds (Ornitophilous) or bats (Chiropterophilous) in night flowers. Plants pollinated by insects or different living groups often produce less pollen. However, plants which are pollinated by the wind produce a large number of pollen to guarantee fertilization. For example, a single male cone of the Pine tree (Pinus spp.) can produce 5 million pollen per year, and the tree itself can produce close to 12.5 billion pollen. However, pollen of plants pollinated by insects generally has indented protruding and sticky properties, while pollen of wind pollinated plants are generally dry and flat on the surface. Pollen of the plants pollinated by the wind try to reach the female flower with the help of the air currents formed when they are released into the air by the plants. Pollens that are important in the emergence of allergy tables are those that are 20-60 μm in diameter, which can be carried by wind. Pollen of this size can be transported to very long distances with the help of wind and enter our homes. Therefore, even if these plants are not found in the environment where the individuals with allergic sensitivity live, allergy tables can be encountered. However, the size of the pollen also plays an important role in the emergence of allergic reactions. The large diameter pollen causes mostly upper respiratory tract and conjunctivitis, whereas the small diameter pollen can go down to the lower respiratory tract (alveoli). This causes the appearance of allergic asthma.

Pollen is generally divided into three as tree pollen, meadow pollen and weed pollen. Pollen varies according to the climate and geographical region during the spring and summer, but has a certain distribution. Accordingly, tree pollen in early spring, meadow (grass) pollen in early summer and weed pollen from mid summer to autumn cause allergic complaints.

Our country has different climatic structure and different vegetation in different geographical regions. Therefore, it is expected that the pollen species, density and diversity in the atmosphere will show regional dif-

ferences. Knowing which types of pollen the people will be faced with in what time of year will be the first step in protecting them from pollen. Turkey and the European Union in the west of the country, located in Thrace, Bulgaria and Greece TR21 borders; It consists of Edirne, Kırklareli and Tekirdağ provinces. Thrace continental climate; It reigns in the Thrace section of the Marmara Region. Especially Edirne and Kırklareli are within the influence of the continental climate, which is cold in these winters. Unlike the Terrestrial Climate in Anatolia, it is seen as rainier. Summers are hot and dry, winters are very cold and snowy. Its borders are a little inside the Black Sea coast of Kırklareli province in the east and Büyükçekmece Lake-Rumeli Lighthouse; the southern borders are the Enez-Evreşe-Şarköy line. Today, in many countries, pollen collection tools are placed in provincial and district centers and pollen calendars are created for aeropalinological studies aimed at determining the amount of pollen in the air and which plants they belong to. Pollen collection devices are of two types and in the previous years, gravimetric method was used in which gravity method based Durham device was used. Today, Volumetric method based Lanzoni or Burkard devices, which collects pollen by sucking air, is used.

In the first study in 1966 by Turkey in terms of Özkaragöz aeropalinolojik research is a systematic study describing 55 species and their pollen allergic ridiculous period for the Ankara region[1]. The first research on the pollen calendar in Turkey by the Istanbul University Faculty of Forestry Aytug and colleagues from Istanbul to Belgrade from the forest in 1966, is a 3-year study period was determined by volumetric method of pollen in the air [2]. Later, 2 years in Yurdukoru Samsun province, Gemici and her friends from Ege University, 1 year in İzmir region, İnce and Pehlivan, 2 years in AntalyaSerik district, İnceoğlu and friends from Ankara University made important contributions to the literature for 3 years [3-6]. In the following years, Kırıkkale, Elazığ, Kayseri, Sivas, Aksaray, Ankara Beytepe, İzmir, İzmir-Buca, Erzincan, Adana, Bartın, Zonguldak, Bilecik, Bilecik-Bozüyük, Çanakkale, Denizli, Samsun, Düzce, Karabük, Şanlıurfa, Çanakkale-Bozcaada, Trabzon, Diyarbakır, Kastamonu and Eskişehir-Sivrihisar pollen calendars have been completed [7-32].

Additionally, as Bursa Uludağ University, Isparta, Balıkesir, Burdur, Afyon, Edirne, Kırklareli, Tekirdağ, Uşak, Eskişehir, Kütahya, Rize, Bitlis, Manisa, Sakarya, Aydın-Didim, Muğla-Fethiye, Balıkesir-Savaştepe belonging to different cities of our country. , Pollen studies of Istanbul and Yalova provincial centers have been completed [42-61].

When the findings obtained from the studies are evaluated, the plants with the most pollen in Thrace region, *Pinus* sp. (pine-pine tree), *Quercus* sp. (Oak), Cupressaceae (cypress family, cypress, juniper) and Gramineae (grass-meadow, grass); It was determined that the months with the highest

number of pollen were between March and June.

In some provinces where the pollen calendar is extracted, it is seen that plants with the most dense pollen are as follows: *Pinus* sp., *Olea* sp. (olive-olive tree) and *Platanus* sp. (plane tree-plane tree) generates the top 3 pollen, *Pinus* sp., Cupressaceae, *Olea* sp. The pollen in the top 3 ranks [34-54]. Gramineae, *Populus* sp. (poplar-poplar), Pinaceae in Bartın; Gramineae, Urticaceae (nettle family-nettle, sticky herb), *Juglans* sp. (wallnut-walnut tree) in Bitlis; Pinaceae, *Quercus* sp. (oak), Cupressaceae in Çanakkale, Denizli, Eskişehir and Uşak; *Pinus* sp., Gramineae, *Corylus* sp. (hazel-hazel tree) in Düzce, Gramineae, *Pinus* sp., *Quercus* sp. in İzmir and Sakarya; Cupressaceae, Urticaceae, *Pistacia* sp. in Istanbul; Pinus sp., Fagus sp. (beech), Ostrya sp. (hophornbeam) in Karabük; *Betula* sp., Cupressaceae, Gramineae in Kastamonu; *Pinus* sp., Gramineae, Chenopodiaceae/Amaranthaceae in Kayseri; *Pinus* sp., Cupressaceae, *Platanus* sp. in Kütahya; *Pinus* sp., Cupressaceae, *Morus* sp. (mulberry) in Fethiye,Muğla; *Alnus* sp. (alder), Cupressaceae, *Castanea* sp. (chestnut) in Rize; Pinaceae, Gramineae, *Carpinus* sp. (hornbeam) in Samsun; *Corylus* sp., Gramineae, *Pinus* sp. in Trabzon; *Platanus*, sp. Cupressaceae, Gramineae in Yalova; Pinaceae, *Populus* sp., *Carpinus* sp. in Zonguldak is in the top 3 pollen grains [9,16,18,19,22-26,29,31,46-51,53,55,57,58]. *Pinus* sp., Cupressaceae and Gramineae are taxa with the most pollen in Adana, Afyon, Ankara, Balıkesir, Bilecik, Burdur, Diyarbakır, Elazığ, Isparta, Kırıkkale, Manisa and Şanlıurfa [6-8,17,20,27,30,42-45,52].

If the studies conducted according to Edirne, Kırklareli and Tekirdağ provinces and seasons of the Thrace Region will be evaluated;

Edirne: In the early spring; Cupressaceae, *Fraxinus* sp. (ash-ash tree), *Ulmus* sp. (elm), *Populus* sp., in spring; *Quercus* sp., *Morus* sp., *Salix* sp. (willow-willow tree), *Platanus* sp., *Pinus* sp, *Carpinus* sp., and Gramineae, in the summer period; Gramineae, Chenopodiaceae / Amaranthaceae (chenopod family etc.), Urticaceae and *Helianthus* sp., Compositae (aster family), in the autumn period; Gramineae, Chenopodiaceae / Amaranthaceae, Xanthium sp. (cocklebur), Artemisia sp. (mugwort-wormwood), and Cedrus sp. (cedar-cedar tree).

Kırklareli: In the early spring; Cupressaceae, *Fraxinus* sp. *Alnus* sp., *Ulmus* sp., *Populus* sp., In spring; *Quercus* sp., *Salix* sp., *Platanus* sp., *Pinus* sp, *Ostrya* sp., *Juglans* sp., *Rumex* sp. and Gramineae, in the summer; Gramineae, Chenopodiaceae / Amaranthaceae (chenopod family etc.), Plantago sp. (weed), Urticaceae and *Helianthus* sp. (sunflower), Compositae (aster family), in the autumn period; Gramineae, Chenopodiaceae / Amaranthaceae, *Xanthium* sp. (cocklebur), *Artemisia* sp. (mugwort-wormwood), and *Cedrus* sp. (cedar-cedar tree).

Tekirdağ: In the early spring; Cupressaceae, *Fraxinus* sp. *Betula* sp. (Birch tree), *Corylus* sp., *Alnus* sp., *Ulmus* sp., *Populus* sp., In spring; *Quercus* sp., *Salix* sp., *Platanus* sp., *Pinus* sp, *Morus* sp., *Juglans* sp., *Acer* sp., *Ostrya* sp., *Aesculus* sp., *Fagus* sp., Oleaceae, *Rumex* sp. and Gramineae, in the summer; Gramineae, Chenopodiaceae / Amaranthaceae (chenopod family etc.), Pinus sp., *Plantago* sp. (plantain), Oleaceae, Urticaceae, Umbelliferae, Leguminosae, *Taraxacum* sp. and *Helianthus* sp. (sunflower), Compositae (aster family), in the autumn period; Gramineae, Chenopodiaceae / Amaranthaceae, *Xanthium* sp. (cocklebur-pitrak), *Artemisia* sp. (mugwort-wormwood), Urticaceae and *Cedrus* sp. (cedar-cedar tree)

With the intensification of pollen monitoring studies, the process of detecting atmospheric pollen should be monitored regionally, while the period when the pollen with allergen effect is scattered into the air, the periods when it reaches the highest density and ends, meteorological bulletins should be announced to the public continuously, while the meteorological bulletins are given. It is clear that success will be achieved with the use of regional pollen calendars in the diagnosis and treatment of allergies.

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Chapter 21

GENDER ASSESSMENT STUDIES ON COLUMNA VERTEBRALIS

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1. GENDER ASSESSMENT

Bone remains provide important information about a person's height, gender, and age in identification. One of the most important steps in identification based on the remains of the skeleton is gender determination [1]. Metric measurements and data that can be obtained from bones are important in gender determination [2]. Gender identity is an important starting point as it decreases the number of possible matches at a rate of 50% when a biological profile is developed [3].

When there is a complete skeleton, pelvis and cranium has the most reliable indicators of gender estimation [4]. However, skeletal remains found in archaeological or forensic contexts are frequently damaged or the skeletal integrity might be impaired because of conditions in the grave. For this reason, gender estimation methods are developed from a wide variety of skeletal elements [5]. Estimating the gender of a skeletal remain with non-metric observations is customary among anatomists, anthropologists and forensic experts. Nowadays, gender divergence is based on actual measurements from various bones [6].

Other skeletal parts like cranium and pelvis bones also have a place in gender assessment, and have been examined in many studies. Other studies conducted on many bones in the body like cranium and pelvic bones contribute to gender assessment process. In these studies, appendicular skeleton, clavicula [7], scapula [8], humerus [9], radius, ulna [10] and metacarpal bones [11] and similar upper extremity bones were examined in gender assessment. The bones of the lower extremity femur [12], tibia [13], fibula [14], patella [15] and calcaneus [16] by researchers. In the axial skeleton, on the other hand, studies have been focused on well-known bones [17-19]. Previous researchers chose to use the words "gender estimation", or "gender determination" if the accuracy rates were high in their studies. It is considered that gender assessment methods are useful if they produce at least 80% accuracy [20].

The purpose of the article was to investigate the studies on gender assessment in the columna vertebral bones in the literature, and to investigate the accuracy rates obtained from the parameters used in the assessments and the vertebra.

The bone structures examined in gender assessment were evaluated for morphological and morphometric properties and gender estimation was made [21, 22]. Metric variables are advantageous to morphological methods due to their simplicity and consistency in recording with the standardization of skeletal criteria [23].

2. METHODS USED IN GENDER ASSESSMENT

In gender estimation, statistical examination of metric data in human skeleton has a long history for physical anthropology [24]. The most popular statistical method that may be employed in this metric examination is recommended by many anthropologists "Discriminant Function Analysis" [25]. It was determined that metric measurements may be obtained directly from cadavers, dried bones or indirectly from radiological images [2]. Caliper, radiography, Computed Tomography and Magnetic Resonance Imaging methods were used to obtain metric measurements in skeleton [26-28].

2.1. Caliper

Various measurements can be made by using caliper in many dry bones, which are complete or fragmented in determining the gender in a skeleton. Many researchers obtained data by measuring various parameters on dry bones and used these results to determine gender [13, 29, 30].

2.2. Radiological Imaging (X-Ray)

One of the oldest methods among radiological imaging methods is the X-ray technique. For this reason, many researchers have long used X-ray images to contribute to gender estimation studies [23, 31, 32]. The magnification coefficient is calculated as a solution to the problem as its negative features not giving the values of the measured anatomical structure one-to-one, although the X-ray technique shows the bone structure well. This coefficient is the most important factor that must be considered both in the evaluation of X-ray images and in scientific research on X-rays [33, 34].

2.3. Computerized Tomography

Another method employed in the examination of anatomical structures is the Computed Tomography (CT) Method. In a previous study, in which metric features of the same dry bones were examined by using caliper and CT Method, the data obtained with both methods were compared. According to the results, it was found that there were no statistical differences between the values, and that these data could be easily evaluated [35].

2.4. Magnetic Resonance Imaging

One of the radiological examinations, the Magnetic Resonance Imaging Method was used in studies conducted on gender determination [36, 37]. Gender studies conducted with this method are usually on epiphyses plaques in the bone, and on soft tissues like the brain [38-40].

3. COLUMNA VERTEBRALIS AND GENDER ASSESSMENT STUDIES

When the vertebral column studies were examined, it was found that

studies were conducted on easily recognizable vertebra because of atypical features [41]. Some vertebra were examined alone to determine the gender [42, 43]. In some previous studies, the purpose was to achieve the right gender assessment by examining several vertebra together and increasing the accuracy rate in gender determination [3, 17].

We evaluated the studies which were conducted by evaluating the bones of vertebral column as cervical vertebrae, thoracic vertebrae, lumbar vertebrae, sacrum and coccyx bones.

3.1. Gender Assessment from Cervical Vertebrae

3.1.1. First cervical vertebra

The first cervical vertebra (Atlas) is one of the best-recognized bones in columna vertebralis. Due to the lack of corpus and processus spinosus in this bone, and due to its easy recognition, it is employed in forensics and in anthropological and paleontological studies. The dimensions of the first cervical vertebra show variations in males and females. This difference can also be used in gender discrimination [24].

Marino EA [24] applied the data obtained from Terry Collection by using the 8 parameters (i.e. right upper-lower facet length and width, distance between the upper and lower two facets, anteroposterior diameter, fovea dentis width with the foramen vertebra) to 2 control groups. One of the control groups was 100 first cervical vertebra of Hamann-Todd collection whose gender was known, and the other group was 34 first cervical vertebra found in archaeological excavations with unknown gender and age (23PM5 collection). In his review conducted on three different groups, the anteroposterior diameter for Terry (n=100) and Hamann-Todd (n=100) collections was 2.80 cm (±0.18), 2.68 cm (±0.24), respectively in females; and 3.04 cm (±0.29) and 2.61 cm (±0.50) in males, respectively. He also reported that he measured anteroposterior diameter as 2.37 cm (±0.12) in 23PM5 (n=34) collection without known gender. Gender determination was made with 75-85% accuracy in the Terry collection of 50 adult females and 50 adult males. When he applied the results to 2 control groups, he made gender determination with 60-77% accuracy in Hamann-Todd collection and with 60-70% accuracy in 23PM5 collection. When he examined the 60 first cervical vertebra with known gender from Hamann-Todd collection, he found the accuracy of 8 parameters between the range of 62-80%. Marino reported the number of cases in his study as 20 not as 60, the accuracy rate increased in the gender estimation to 70-90%, and that there was a reverse proportion between accuracy and sample width. He also reported that the area of joint faces and foramen vertebra in Atlas was sexually dimorphic, and was statistically suitable for first cervical vertebra's gender determination.

In their study, Sertel Meyvaci S et al. [18] found that it was possible to make gender determination between 73-80% with the data obtained with CT images with known gender by examining the eight parameters in the first cervical vertebra. They also reported that the distance between the foramen transversarium lateral (FTL) or foramen transversarium medial (FTM) edges in Atlas were sexually dimorphic, and were favorable for first cervical vertebra's gender determination. The cut-off values of the FTM_{AVG} and FTL_{AVG} parameters (4.64 cm, 5.92 cm, respectively), which were obtained by taking the measurements of 6 parameters, were determined with the help of CT. By taking these cut-off values as guide, and when compared with FTM_{AVG}, which was obtained from dry atlases whose gender was unknown, gender determination could be done at an accuracy rate of 76%, and when compared with FTL_{AVG}, the accuracy became 80%. In the light of the findings of 8 parameters obtained from CT images, when the 84 dry Atlases were evaluated, with unknown gender, it was estimated that 49 could be female, 22 could be male, and 11 might be children. The accuracy rate was determined as 78.8%.

3.1.2. Second cervical vertebra

In the study of Wescott DJ [19], a total of 400 second cervical vertebra (Axis) 200 of which were male and 200 female with an age range between 20-79 were investigated by using a 0.1-precision caliper for 8 parameters (i.e. sagittal length, maximum height of the dens, dens sagittal diameter, dens transverse diameter, length of vertebral foramen, maximum breadth across the superior facets, superior facet sagittal diameter, superior facet transverse diameter). He reported that gender determination could be made at an accuracy rate of 80.3-85.6% in axis if the gender was not known.

In their study, Bethard JD and Seet BL [44] tested the accuracy of Wescott's [19] method to determine gender from the second cervical vertebra. The samples were donated by Hamilton County Forensic Scaffolding Center (n=57) and the William M. Bass Skeleton Collection (n=243) consisting of 300 axis of 150 adult female and 150 adult male, and were randomly selected. The ages of these samples ranged from 19 to 101 years in death, and the mean age was 53.1 (±15.2 years). In Wescott's study, 5 parameters were evaluated; maximum sagittal length, superior facet sagittal diameter, superior facet transverse diameter, length of vertebral foramen and maximum height of dens. Wescott's 5 discriminant function analysis developed based on these parameters was then used to predict gender in each case. The accuracy rate for gender classification of the samples varied between 78% (female - function 1) and 90.6% (male - function 5).

In the study of Marlow EJ and Pastor RF [5], the skeletal remains of adult individuals were examined in Spitalfields collection at Spitalfields

Natural History Museum in London. This collection covered 18th and 19th Centuries, and consisted of immigrant European descendants (French Huguenots). Five discriminant function analyses were applied to the cases by using the dimorphic 8 parameters of the second cervical vertebra as recommended by Wescott [19]. It was found that the accuracy rate in gender classification ranged from 70.91% to 78.90% by using Wescott discriminant functions. It was shown that the discriminant functions developed by Wescott had a classification accuracy of 76.99%. When these analyses were applied to Spitalfields data, it provided that gender was properly classified in 83.3% of the individuals with equal accuracy for male and female. As a result of this study, it was shown that the gender determination method from the dimensions of the second cervical vertebra as recommended by Wescott had an important ability to distinguish between the genders. The results of this study also showed that the method could be applied in a correct manner to modern human skeleton remains and in archaeological contexts.

Gamma I et al. [45] used 13 parameters of the second cervical vertebra (i.e. maximum length of the axis, maximum height of the axis, maximum height of the odontoid process, maximum width of the vertebral foramen, maximum width of the axis, maximum distance between the superior facets, maximum length of the superior facet, maximum width of the superior facet, odontoid process transverse diameter, odontoid process sagittal diameter, length of the vertebral foramen, maximum transverse diameter of the body, sagittal maximum body diameter) to measure the gender-based variation and to create a simple predictive model based on logistic regression analysis. A total of 190 samples from the Coimbra Defined Skeleton Collection were used as training examples for Logistics Regression Analysis. The model was also evaluated in an independent test sampling of 47 individuals from Defined Skeleton Collection of the 21st Century (University of Coimbra). The Logistics Regression Model accurately estimated at 86.7% to 89.7% of the cases with known gender. It was shown that the second cervical vertebra was a useful alternative for gender prediction when other skeletal elements were not present or were not suitable for analyses.

3.1.3. Seventh vertebra

In the study of Amores A et al. [17] aiming to examine 7th cervical (vertebra prominens) and 12th thoracic vertebra for gender determination, the study sampling consisted of 121 individuals of known gender, age, and cause of death from San Jose cemetery in Granada (Spain). A total of 8 dimensions (i.e. length and width of superior facets, length and width of inferior facets, length and width of vertebral foramen, length and width of inferior surface of vertebral body) were analyzed, and Discriminant Function

Analysis was carried out for each vertebra to have discriminating functions and examine the correct assignation percentage of these functions. The percentage reliability for gender determination was found to be 65.5% for function 3 in 7th cervical vertebra.

The purpose of the study of Ünlütürk Ö and İşcan MY [1] was to analyze the species variation of the South African white and black population in Pretoria Bone Collection. The samples consisted of 144 full vertebra. A total of 7 measurements were taken from each 7th cervical, first thoracic, 11th thoracic, 12th thoracic and 5th lumbar vertebra bones with a 0.01mm-precision digital caliper. The reason for choosing these bones was that they are the easiest bones to recognize in terms of their position in the vertebral column and anatomical angle. As a result of the analyses, they reported that using 7th cervical vertebra yielded 83.60% accuracy rate in white race and 72.90% in black race in determining the gender.

3.1.4. Cervical vertebrae

Liguoro D et al. [46] conducted a study which included 120 adults, 69 male and 51 female, aged 20-80 years, from French origin, living in the Southwest France. A biometric comparative roentgenological study of the cervical vertebral bodies was conducted according to age and gender with lateral roentgenograms of 120 adult cervical regions. The sexual dimorphism that was already described by many authors was confirmed. As a result, all the means of the measurements significantly differed between male and female.

3.2. Gender Assessment from Thoracic Vertebrae

In McLaughlin SM and Oldae KNM's [47] studies, the 11th-12th thoracic vertebra and 1st lumbar vertebra corpus were examined in a total of 205 skeletons consisting of 97 males and 108 females, ranging in age from 15 to 91. The measured corpus vertebrae for 3 parameters (i.e. posterior transverse diameter, anteroposterior diameter and anterior transverse diameter) using a 0.01mm-precision digital caliper, and reported that gender determination was possible at a rate of 77.6% -83.2% in 11th thoracic vertebra, and at a rate of 70.2%-86.9% in 12th thoracic vertebra.

In the study of Taylor JR and Twomey LT [48], they investigated sexual dimorphism from corpus vertebrae with lateral radiography. They examined corpus vertebrae height and transvers diameter from 6th and 9th thoracic vertebrae by separating age groups in terms of inter-gender difference. They formed 4 age groups between the ages of 2 and 25 and found no significant differences in these parameters between the ages of 2 and 8. They also found that there was a significant difference in groups aged 2–9, 13–16 and 17–25 years old.

In the study of Tsubaki S et al. [49], the purpose was to examine if gender could be determined with geometric features of 10th thoracic vertebra, and 6th and 7th ribs. A total of 600 chest radiographs (300 males and 300 females) were selected to represent patients of 6 age groups (20s, 30s, 40s, 50s, 60s, and 70s) with 100 images (50 males and 50 females). A total of 14 features that included 7 lengths, 5 indices for vertebra, and 2 types of widths for ribs were used and analyzed for gender determination. The dominant features that contributed to gender determination were selected with Stepwise Discriminant Analysis after the variance inflation factors for multicollinearity were checked. The accuracy of gender determination by using a vertebra and rib combination was evaluated by using the selected features with Stepwise Discriminant Analysis. The accuracies of each age group were also investigated in this study. The accuracy of gender determination of 10th thoracic vertebra was found to be 86.5% (519/600). It was concluded that the geometric characteristics obtained with the vertebra and ribs might be useful for gender determination.

In their study, Yu SB et al. [43] examined the 12th thoracic vertebra in 102 Korean cadavers, 52 of whom were male and 50 of whom were female, with an age range of 21-60 and determined their genders. A total of 23 of the 35 measurement parameters of 1-mm thick axial sections obtained by using CT device were found to be sexually dimorphic. A total of 3 of these parameters, i.e. corpus vertebrae's coronal diameter of endplate, ratio of anterior to middle height of the body, the length of left mammillary process and pedicle, were found to be 90% sexual dimorphic by using the equation. However, they reported that gender determination could be made at an accuracy rate that ranged between 62.7% and 85.3% from the 12th thoracic vertebra. These rates were reported to be 66.7%-85.3% for the dimensions of the vertebral body, 66.7%-72.5% for the pedicle, 64.7%-70.6% for the mammillary process, and 62.7%-63.7% for the transverse process.

In Hou WB et al.'s study [50], the purpose was to develop discriminant function equations for gender determination by employing the 12th thoracic vertebra in a modern northeast Chinese sampling. It was also aimed to examine if the differences of the 12th thoracic vertebra between males and females consisted more in shape than in size. A total of 30 linear measurements were obtained from 141 3 dimensional-reconstructed 12th thoracic vertebra models (78 males and 63 females). After this step, 112 ratios were calculated with the abovementioned 30 linear measurements 28 of which were sexually dimorphic. The Univariate Discriminant Function Equations predicted gender with accuracy rates of 56.4% and 90.1%. A total of these 112 ratios, 62 were sexually dimorphic, and their accuracy ranged between 56.7% and 73.8%. A total of 4 variables predicted gender with 94.2% accuracy by using stepwise method of discriminant function

analysis. It was found that the 12th thoracic vertebra of the north-eastern individuals in China was useful for gender determination, and that the size of 12th thoracic vertebra contributed more than the shape in the differences between males and females in 12th thoracic vertebra.

In Amores A et al.'s study [17], which was done to examine 7th cervical and 12th thoracic vertebra for gender determination, the study sampling consisted 121 individuals with known gender, age, and cause of death from San Jose cemetery in Granada (Spain). A total of 8 dimensions (i.e. length and width of superior facets, length and width of inferior facets, length and width of vertebral foramen, length and width of inferior surface of vertebral body) were examined. The Discriminant Function Analysis was made for each vertebra to have discriminating functions and examine the percentage of correct determinations with these functions. In their study, the 12th thoracic vertebra gave an average reliability of 80.2% for gender determination (76.7% for males and 84.2% for females).

The aim of the study of Ünlütürk Ö and İşcan MY [1] was to analyze the sexual variation of South African white and black population in Pretoria Bone Collection. The sampling consisted of the full vertebra of 144 individulas. A total of 7 measurements were taken from each 7th cervical, first thoracic, 11th thoracic, 12th thoracic and 5th lumbar vertebra with a 0.01mm-sensitivity digital caliper. The reason why these bones were selected was that they are the easiest bones to recognize in terms of anatomical angle and position in the vertebral column. As a result of the analysis, they reported that gender could be determined by using first thoracic, 11th thoracic and 12th thoracic vertebra with 88.90%, 81.20%, and 85.30% in white race, and with 84.30%, 76.10%, 81.40% in black race.

3.3. Gender Assessment from Lumbar Vertebra

In their study, Zheng WX et al. [51] aimed to develop a technique for gender assessment from first lumbar vertebra measurements. A total of 29 linear measurements and five ratios were collected from 113 Chinese adult males and 97 Chinese adult females by using digital 3 dimensional anthropometry methods. It was found by using discriminant analysis that 23 linear measurements and two ratios identified sexual dimorphism with predictive accuracies ranging between 57.1% and 86.6%. It was also determined after stepwise method of discriminant function analysis that three dimensions predicted gender with 88.6% accuracy; upper end-plate width, left pedicle height, and middle end-plate depth. The results of this study showed that a single first lumbar vertebra could be employed for this purpose, and that the discriminant equation would help forensic determination of gender in Chinese population.

In the study of McLaughlin SM and Oldae KNM [47], a total of 205

skeletons of 97 males and 108 females whose age ranged between 15 and 91 were used to examine 11th-12th thoracic vertebra and 1st lumbar vertebra corpus. They measured 3 parameters (i.e. posterior transverse diameter, anteroposterior diameter and anterior transverse diameter) of corpus vertebrae by using a 0.01mm-precision digital caliper, and reported that gender prediction ranged between 79.6% and 82.2% in 1st lumbar vertebra.

Oura P et al. [52] conducted another study for cases that had only a skeleton part or individual skeletal elements were available to investigate gender estimation potential of 4th lumbar vertebra in 20-, 30-, and 46-year-old Northern Finns. They used Magnetic Resonance Imaging scans on living subsamples of the Northern Finland Birth Cohort 1966 (scan at 46 years, n = 1363) and the Northern Finland Birth Cohort 1986 (repeated scans at 20 and 30 years, n = 375). After examining the scans for vertebral pathologies, they measured the maximum and minimum widths, depths, and heights of the 4th lumbar vertebra body. The mean vertebral width, depth and height were calculated with vertebral cross-sectional area and volume. Gender estimations were made by using univariate and multivariate logistic regression analysis. As a result of their study, they reported that they detected gender discrepancy in all the examined parameters of 4th lumbar vertebra (80%). They also argued that vertebral width, depth, and height yielded as accurate gender estimates as more complicated vertebral parameters. They also reported high gender estimation potential of 3 easily obtainable dimensions of 4th lumbar vertebra body (i.e. width, depth, and height) in 20-, 30-, and 46-year-old Northern Finns. In addition, they compared 1) other lumbar vertebrae and 2) more complicated measurement parameters by using 4th lumbar vertebra body and its width, depth, and height to justify gender estimation accuracy.

In the study of Ünlütürk Ö and İşcan MY [1], the purpose was to analyze the sexual variation of South African white and black population in Pretoria Bone Collection. The samples consisted of 144 full vertebra. A total of 7 measurements were made in 7th cervical, first thoracic, 11th thoracic, 12th thoracic and 5th lumbar vertebra with a 0.01mm-precison digital caliper. The reason why these bones were preferred was that they are easy to identify in terms of anatomy and position at vertebral column. Their analyses showed that gender estimation was possible with an accuracy rate of 80.00% in white race, and 64.30% in black race by using 5th lumbar vertebra.

In the study that was conducted by Taylor JR and Twomey LT [48] sexual dimorphism from the shape of corpus vertebrae on lateral radiography was investigated. They examined the difference between genders with height of the corpus vertebrae and transvers diameter from 6th and 9th thoracic vertebra by separating into age groups. They also examined the dif-

ference between genders with height of the corpus vertebrae and transvers diameter in the 1st, 3rd and 5th lumbar vertebra by separating these into age groups. They could not find any significant differences in these parameters between the ages of 2 and 8; but found that there was a significant difference in 9-12, 13-19 and 20-35 age groups.

3.4. Gender Assessment from Sacrum and Coccyx

Steyn M and İşcan MY [53] investigated the pelvis of 97 male and 95 female from skeletal collection in Crete Heraklion to perform gender determination. The measurements were made in the pelvis, which had joint integrity, and coxae and sacrum alone. They used Discriminant Function Analysis Method to evaluate sexual dimorphism degree in various parts of the pelvis. A total of 17 parameters were measured by using standard anthropometric techniques. All measurements of the pelvis and the accuracy rates of various combinations in terms of sexual dimorphism were examined with 7 different discriminant function analyses. It was found that the accuracy rate of the parameters measured in sacrum was low (60.9%); and that the accuracy rate was between 79.7% and 95.4% when single coxae was examined. They also reported that since the repeated measurement of incisura ischiadica was difficult, it did not yield reliable outcomes and had a weak accuracy rate. It was also determined that the accuracy rate of the entire pelvis with joint was lower than coxae and sacrum alone. The highest sexual dimorphism rate was in acetabulum diameter with 83.9%.

Mishra SR et al. [6] examined 116 sacra (74 males and 42 females) with known gender in Department of Anatomy, S. N. Medical College, Agra. They examined maximum length of sacrum, maximum breadth of sacrum, curved length of sacrum, anteroposterior diameter of the body of 1st sacral vertebra, transverse diameter of the body of 1st sacral vertebra, length of alae and maximum length of auricular surface parameters in the sacrum. They also examined the sacral index, curvature index, index of body of 1st sacral vertebra, corporobasal index, alar index and auricular index by using these parameters. Their results showed that the length of sacrum and sacral index are important parameters when the gender determination of sacrum is concerned since 71.6% of male bones and 80.1% of female bones could be identified with the demarking points for the above-mentioned parameters. Their study also showed that certain parameters are not significant when the gender determination of sacrum is concerned and some other parameters were much useful. However, not one single parameter could identify 100% of the bones examined. For this reason, it may be concluded that maximum parameter count should be considered to obtain 100% accuracy for gender determination with sacrum.

Flander LB [54] conducted a study and analyzed metric data on 200

sacra with known gender, age and race to determine the usefulness of conventional observations in gender determination with this bone. The sample was divided into 4 age groups to examine whether the effects of lipping biased the measurements (age 20-30, age 31-40, age 41- 50, age 51-60). The fused sacral vertebrae (i.e. segments) count was recorded as code (4 segments = 1, 5 segments = 2, and 6 or more = 3). They examined mid-ventral straight length, anterior straight breadth, at the level of first sacral vertebra, maximum articular surface, maximum depth of curvature, mid-ventral curved length and transverse and anteroposterior diameters of the first sacral vertebra body parameters. The indices were calculated by using these parameters. The univariate analysis results showed that significant gender differences in the sacrum were related with the top part of the bone for white and black populations. However, in the black population, the curvature measurements posed significant gender difference. To classify bones by gender than indices with other measurements, a new index involving first sacral vertebra body to sacral breadth was proposed as a more useful tool in this respect. It was shown in the discriminant analysis that the sample of the white population could be analyzed better with this method compared to an index. Univariate or multivariate method must be chosen based on the condition of bones. This will also be affected by the race from which the sample is obtained to some extent.

Stradalove V [55] conducted a study and examined 15 measurements and 3 indices on a series of 128 sacra of known gender. Their study aimed to find metrical parameters to distinguish the gender on human sacrum most accurately and with greatest probability by using modern multivariate statistical techniques - discriminant analysis and factor analysis that can simultaneously analyze large numbers of variables. The material of their study consisted of 128 sacra with known gender that were 72 males (23-88 years of age) and 56 females (17-84 years of age). Most of the bones were obtained from Department of Anatomy, Charles University, Prague, and the rest were obtained from Department of Anatomy, University J. E. Purkynje in Brno. The bones were from end of the 19th Century and first half of the 20th Century. Some of the parameters they examined in the sacrum were; anterior superior straight breadth, anterior medial straight breadth, anterior inferior straight breadth, mid-ventral straight length, mid-ventral curved length, depth of the base of the sacrum, depth of the mid-ventral curvature, promontorium angle, transverse diameter of the body of the first sacral vertebra, anteroposterior diameter of the body of the first sacral vertebra, maximum length of facies auricularis dextra, maximum breadth of facies auricularis dextra, height of the body of the first sacral vertebra, distance of medial borders of articular surfaces of processus articulares superiores, distance of lateral borders of articular surfaces of processus articulares su-

periores. By using these parameters, they calculated the sacral index, curvature index and corporobasal index. As a result of their study, they chose 11 measurements to form a discriminant function. Similar measurements of sacrum with unknown gender were multiplied by proper coefficients, the products were summed, and the result was compared with male-female dividing point. The accuracy level of gender determination obtained with discriminant analyses was about 85-89% with bones assigned correctly. The following 4 measurements were determined to be adequate for gender determination of the human sacrum; depth of the base of the sacrum, distance of lateral borders of articular surfaces of processus articulares superiores, anterior superior straight breadth and promontorium angle.

In the study of Kimira K [56], the widths of the base and lateral part (i.e. wings) of the sacrum of 103 Japanese (52 males and 51 females) were measured with a sliding caliper at Department of Anatomy, Yokohama City University, School of Medicine in July, 1981; in 100 American Whites (50 males and 50 females); and in 97 American Blacks (49 males and 48 females) of the Terry Collection of the Smithsonian Institution, Washington, in June, 1975. The age of the samples were between 20 and 60 years, and the genders were known. The sacral base transverse diameter, was measured according to Martin and Saller Method (1957). The probability of determining the gender with sacrum was 75.32% in Japanese, 80.88% in American Whites, and 82.70% in American Blacks with the discriminant functions of these dimensions. The overlapping samples between male and female series were 26.2%, 22.0% and 16.5% in each population, respectively in the distribution of the base-wing index.

In the study of Mamatha H et al. [57] conducted on 50 adult normal and fully ossified sacra (25 male and 25 female) obtained from Department of Anatomy, Kasturba Medical College, Manipal, India. By using a digital Vernier caliper, they measured sacrum parameters (i.e. maximum width of the sacrum, maximum height / length of sacrum, width of base / transverse diameter of the body of 1st sacral vertebra, width of base / transverse diameter of the body of 1st sacral vertebra, transverse diameter of the wing, auricular surface length, extensions of auricular surface in relation to the sacral segments and morphology of the sacral hiatus, sacral index and auricular index). As a result, they concluded that all the parameters contributed to gender determination in a positive way, and the most significant was the sacral index and the auricular index, but one parameter might not be useful in determining the gender in sacra. For this reason, it is possible to speculate that maximum parameter count must be included to obtain 100% accuracy for gender determination with sacrum.

Zech WD [58] conducted a study and analyzed the postmortem CT images of individuals with known gender (49 male and 46 female). Post-

mortem CT scan was applied to all individuals at Forensic Institute of Bern University (between 2005 and 2010). The best accuracy in gender determining was 76.8% and 78.9% with two different observers in the Discriminant Function Analysis of the data. It was concluded that measuring the sacrum in postmortem CT to determine gender had moderate accuracy rates, and it should only be used in combination with other reliable methods.

In the study of Sachedeva K et al. [59], the purpose was to examine the reliability of some sacrum parameters for gender determination. A total of 50 adult sacra (40 males and 10 females) that were obtained from Department of Anatomy, Govt. Medical College, Amritsar. The mid-ventral straight length, mid-ventral curved length, ventral straight breadth, transverse diameter of base, transverse diameter of body of first sacral vertebra, anteroposterior diameter of body of first sacral vertebra, breadth of alae were measured in their study. Sacral index, longitudinal curvature index and corporobasal index were calculated and analyzed statistically. Among these, it was determined that the mid-ventral straight length, mid-ventral curved length, transverse diameter of base, anteroposterior diameter of body of first sacral vertebra and breadth of alae were more in males at significant levels; and sacral index was more in females at significant levels. It was found that the corporobasal index was more in females but was not statistically significant. In this context, 5 out of 7 sacrum parameters gave significant differences between genders (i.e. mid-ventral straight length, mid-ventral curved length, transverse diameter of base, anteroposterior diameter of body of first sacral vertebra and breadth of alae), and were more in males compared to females. The sacral index gave significant results, and was more in females. The gender differences were more in line with the robusticity of male skeletons. Although most of the results were in agreement with previous studies, the index of breadth of alae and corporobasal yielded contrasting results. The corporobasal index was more in female individuals, and the alae were wider in males.

In Benazzi S et al.'s study [60] conducted to help improve gender assessment from skeletal remains, diagnostic value of the sacral base was evaluated based on its planar image and related metric data. To do this, 114 adult sacra of known gender and age of two early 20th Century Italian populations were examined, the first from Bologna, northern Italy (n = 76), and the second from Sassari, Sardinia (n = 38). Digital sacral base images were taken with each bone in standardized orientation. Technical drawing software was employed to trace the profile and to measure the dimensions (i.e. maximum transverse diameter, maximum superior breadth, area of the upper face of the body of the first sacral vertebra, and perimeter of the body of the first sacral vertebra). Their measurements were evaluated with Discriminant and Classification Function Analyses. The gender prediction

success was 93.2% for Bolognese sampling, 81.6% for Sassarese sampling, and 88.3% for pooled sampling, which showed that the first sacral vertebra is a good character for gender determination.

Shreekrishna HK et al. [61] examined 150 sacra of known gender in Tamil Nadu in South India for metrical parameters for gender determination. The researchers tried to find the demarking point for each parameter, and compared these with similar studies. As a result of the study, it was reported that sacral index was the most important parameter for gender determination with sacrum because it could identify 56% male and 78% female bones. For this population, sacral index was 99.21 for males and 119.94 for females.

Plochocki JH [62] conducted a study to evaluate the predictive value of anterior sacral curvature for gender estimation from skeletonized remains. The sacra of a sampling of 125 American adults with known age and gender were examined, and 9 measurements describing anterior sacral curvature were used in analyses. Statistical treatment of the data included Univariate Statistics and Discriminant Function Analysis for gender classification. To assess the classification error rates, the Bootstrap Validation Method was used. The sacral curvature was found to be significantly bigger in male than in female at 2^{th}-3^{th} sacral and 3^{th}-4^{th} sacral articulations. Correct classification estimates for the discriminant function ranged between 66-72%. Although they were sexually dimorphic, metric observations of sacral curvature were not as reliable at predicting gender as other skeletal elements. Anterior sacral curvature should only be used for gender estimation when there are no other more reliable indicators.

Kothapalli J et al. [63] conducted a study on certain morphometric parameters of sacrum maximum length and breadth of sacrum, mid-ventral curved length, transverse diameter and anteroposterior diameter of first sacral body, length of ala, maximum length of auricular surface were measured. They also statistically analyzed the sacral index, curvature index, corporobasal index, alar index, index of body of first sacral vertebra, auricular index. As a result of their study, they found that the maximum breadth of sacrum, mid-ventral curved length, anteroposterior diameter of the body of first sacral body, length of ala, sacral index, and alar index were statistically significant. They also found that the width of sacrum and curved length of sacrum were reliable for gender differentiation. They also reported that no single parameter could identify 100% gender of the bones. For this reason, it can be speculated that maximum number of parameters should be taken into consideration for 100% accuracy to determine gender with sacrum.

Gaya-Sancho B et al. [64] conducted a study and included only adults

to measure 170 sacra in modern osteological collection of San José from Granada. Their measurements were based on anatomical regions of the sacra to obtain some regression formulas for gender determination. The results showed that the superior transverse line and right lateral sacral crest were the most dimorphic structures. In univariate analyses, they achieved 74% correct classification of gender. In the analyses, 81.41% correct classification was achieved when the mentioned variables were combined. As a result of their study, it was shown that this method could be applied with other methods simultaneously to determine the gender of individuals in forensic and archaeological contexts.

Bhandarkar U et al. [65] conducted a study on sexual dimorphism and regional differences of varied features of sacrum in West Bengal population, and compared the important anthropometric indices with similar observations in India. The study consisted of 50 adult sacra (35 male and 15 female). The study was done at Anatomy and Forensic Medicine Department of ICARE Institute of Medical Sciences and Research and Haldia Institute of Dental Sciences and Research, Haldia, West Bengal. Different parameters were measured (i.e. mid-ventral straight length, mid-ventral curved length, ventral straight breadth, transverse diameter of base, transverse diameter of body of first sacral vertebra, anteroposterior diameter of body of first sacral vertebra, breadth of alae); and indices (i.e. sacral index, longitudinal curvature index, and corporobasal index) were calculated and analyzed in statistical terms. As a result, they reported that sacral index was the most useful criterion to identify gender after breadth of alae, corporobasal index and the ventral straight breadth. They also reported that 7 out of eleven sacrum parameters yielded statistical significance between two genders. For this reason, it can be speculated that gender determination based on sacrum with 100% accuracy might be possible only when maximum number of parameters were considered.

Torimitsu S et al. [66] conducted a study examine the skeletal sexual dimorphism of the sacrum and coccyx with CT images in a modern Japanese population to obtain discriminant function formulae for gender determination. The data were collected from 230 cadavers (115 males and 115 females) in postmortem CT and subsequent forensic autopsy. In CT images of each subject, a total of 7 measurements were made (anterior and posterior sacral length, anterior and posterior sacrococcygeal length, maximum anteroposterior diameter, maximum transverse diameter, maximum breadth of sacral alae) in the sacrum and coccyx. The results were evaluated with Descriptive Statistics and Discriminant Function Analyses. There were statistically significant sexual dimorphism in all measurement except one. According to discriminant function analyses, the maximum gender determination rate was 83.5%. Based on the results of this study, it

may be argued that Discriminant Analysis of the sacral and coccygeal traits might be beneficial in gender determination in skeletal remains in Japanese population when other methods like morphological trait evaluation of other bones are used.

Zhan M et al. [67] conducted a study to estimate stature and gender based on sacrum and coccyx measurements with Multidetector CT in a Chinese population today. A total of 9 measurements were made for every sacrum and coccyx in CT images of 350 Chinese individuals. The sampling consisted of 190 males and 160 females and the ages of the participants varied between 55 males and 50 females. In estimating gender, the Discriminant Function was employed, and Regression Analysis was employed to estimate the stature from these two bones. The gender classification accuracy rate was 84.9% in stepwise analysis of all measurements. The accuracy of the classification of univariate discriminant function analyses were 58.3%-76.9%. The study provided indications that the sacrum and coccyx were important bones in estimating gender, and could be employed effectively as an alternative in forensics if the skull and pelvis are not present.

3.5. Studies in which cervical, thoracic and lumbar vertebrae were examined together

Amores A et al. [17], which was done to examine 7th cervical and 12th thoracic vertebra for gender determination, conducted a study with 121 individuals whose genders, ages, and causes of death were known in San Jose cemetery in Granada (Spain). Analyses were made on a total of eight dimensions (i.e. length and width of superior facets, length and width of inferior facets, length and width of vertebral foramen, length and width of inferior surface of vertebral body); and discriminant function analysis was also made for each vertebra for discriminating functions and for examining the percentage of correct assignation of these functions. The accuracy was around 80% for both vertebra; however, this rate varied according to gender, and was higher for 7th cervical in male individuals, and for 12th thoracic in female individuals. As it was reported in some other populations, it was found that the biggest dimorphism values were in the length of inferior surface of vertebral body, in the width and length of vertebral foramen of 7th cervical vertebra, and in the length of the inferior surface of the vertebral body of 12th thoracic vertebra.

Ünlütürk Ö and İşcan [1] conducted a study and aimed to analyze the gender variation in South African white and black population in Pretoria Bone Collection. They examined full vertebral column of a total of 144 individulas, which included 37 white male, 36 white female, and 35 black male and 36 black female. They made seven measurements from each of

the 7th cervical, first thoracic, 11th thoracic, 12th thoracic and 5th lumbar vertebra bones with digital calipers. These were chosen because of being the easiest vertebra to recognize with anatomical structures and positions. The gender differences were evaluated with Discriminant Function Analysis. The measurements made on bones consisted of anterior height, posterior height, superior transverse breadth, inferior transverse breadth, middle transverse breadth, inferior anteroposterior diameter, superior anteroposterior diameter. As of the vertebral body structures, the middle transverse width measurement was not taken from the vertebra of the cervical colon. They reported that first thoracic vertebra had the highest rate of gender determination, and 5th lumbar vertebra had the lowest gender determination rate in both races. In the analyses, it was found that 88.90% correct determinations were made when the superior anteroposterior diameter and posterior height parameters, which were selected for first thoracic vertebra, were used. Again, in analyses on first thoracic vertebra, it was found that gender determination was observed in female at a rate of 86.11%; and 91.67% in male. The lowest sexual dimorphism was observed in 5th lumbar vertebra with an accuracy rate of 89% in white individuals, and 84% in black individuals. When considered in general terms, it was found that gender determination could be made over the vertebra with an accuracy rate of 89% in white individuals, and 85% in black individuals. These vertebral bones have advantages over the others, because there are many of these at crime scenes. The difficulty in studies conducted on the vertebral colon is to sort and identify each bone when many are missing.

In the study of McLaughlin S and Oldae KNM [47] conducted on a total of 205 skeletons (97 males and 108 female) between the ages of 15 and 91, they examined the 11th-12th thoracic vertebra and 1st lumbar vertebra corpus. They used a 0.01-mm precision digital caliper and measured the corpus vertebrae in terms of 3 parameters; posterior transverse diameter, anteroposterior diameter, and anterior transverse diameter. They reported that gender estimation can be made in 11th thoracic vertebra between 77.6%-83.2%, in 12th thoracic vertebra between 70.2%-86.9%, and in 1st lumbar vertebra between 79.6%-82.2%. In this respect, they reported that 11th thoracic vertebra and anteroposterior diameter constitute the most successful combination in gender estimation, and when evaluated in the same person, gender determination can be made with an 89.2% accuracy rate.

4. CONCLUSIONS

It might become extremely difficult to determine gender from the skeletal remains especially when the bones are severely fragmented. For this reason, it is important to be able to define gender even from one single bone [2].

We examined the studies conducted on gender assessment in the vertebral column bones. It was found that there were many studies conducted in the literature regarding sacrum bone, and also that these studies were conducted with different populations. We also found that studies on other vertebra and especially on recognizable vertebra were insufficient. Although the reproducible and reliable method that is used widely by researchers is the Discriminant Function Equations, its practical use requires experience. For this reason, practical applications that will contribute to gender assessment are needed. As a result, since each recognizable vertebra is 1 in number in human skeleton, these bones have direct contribution to gender assessment in humans. In addition to conducting studies on different populations by using these unique bones, i.e. vertebral column, practical and applicable methods must be selected to make important contributions to the identification process.

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Chapter 22

OBESITY, ADIPOCYTOKINES and CANCER

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INTRODUCTION

Adipose tissue is a tissue formed when cells are covered with 95% fat droplets, and called adipocytes. Adipose tissue is the largest energy store of the body[1,2]. As body weight increases, adipose tissue expands. Firstly, triglycerides accumulate within the cell and thus an increase is seen in the size of adipocytes. In the next stages of adipose tissue, evident differentiations start to be seen as stromal vascular division of pre-adipocytes like the formation of adipose tissue[3].

Obesity is characterised by intense accumulation and storage of fat in the body[4]. Various epidemiological studies have shown that obesity, which increases rates of morbidity and mortality, causes an increase in cancers of the breast, endothelium, oesophagus, heart, colonorectum, gall bladder, pancreas, kidneys and liver[3].

It has been shown that for every increase of 5kg/m2 in BMI, there is a 30% increase in mortality and a 10% increase in cancer-related death[5].

Adipocytokines

Adipose tissue is not only responsible for fat storage but also it is an organized endocrine tissue originating from cells which have the ability of secreting polypeptide cytokines and hormone-like molecules[3].

Adipocytokines were found as a group of hormones derived from adipose tissue, when the first member of the family, leptin, has been defined at the beginning of 90s[6,7]

Adipocytokines are classified in three groups:

1. Hormones that are simultaneously secretedin other tissues or organs substantially by adipose tissue production (for example TNF- α),

2. Hormones that are substantiallysecreted by white tissue. However, adipocytes are not the only source for production. Hormones that are secreted by other cells of adipose tissue, for example the onessecretedby the cells having the ability of immune response (for example, resistin),

3. Hormones that are secreted mainly or only by adipocytes of white tissue (for example, leptin and adiponectin).

One group of adipocytokines is synthesized by adipocytes, but the restis synthesized by stromal-vascular components of adipose tissue including pre-adipocytes, lymphocytes, macrophages, endothelial cells and fibroblasts[8]. It has been known that adipocytokines have paracrine and endocrine effects[9,10].

Adiponectin

Adiponectin is the C1q-related and collagen-like plasma protein which is synthesized by adipose tissue and has a molecular weight of 30kDa (GBP28, adipoQ or ACRP30). They are the most abundant adipocytokine. Adiponectin gene is localized to chromosome 3q27 and it coded from gene transcript-1 (OpM1) region. It is 15.8 kb in length and has 82 SNPs[11-13]. It is mainly secreted by visceral adipose tissue. There is an inverse relationship between the level of adiponectin and BMI, and it is an insulin-sensitive hormone that has contributionto the regulation of anti-angiogenic, anti-inflammatory, apoptosis and inflammation[14-16].

Adiponectin has an anti-carcinogen activity which significantly reduces the plasma concentration level of adiponectin in obese individuals[17].

Leptin

Leptin is a glycoprotein that is secreted by adipocytes (>95%) and the molecular weight of leptin is 16 kDa. Leptin is a pro-angiogenic hormone which is specific to adipose-tissue and regulates appetite and weight gain. It acts as an indicator of sufficient energy intake[18]. Leptin, which acts as an energy detector under normal conditions, regulates energy consumption and gain weight and causes the sensation of satiety[19].

Visfatin

The visfatin gene is located on the long arm of chromosome 7, encoding a polypeptide of 491 amino acids with a molecular weight of 52 kDa [20]. Fukuhara et al. (2005) has been showed that visfatin is secreted by adipose tissue. Both the synthesis of visfatin from adipose tissue and the plasma level of visfatin increase in parallel with obesity. It has been shown that visfatin decreases glucose plasma level by acting like insulin[7].

Resistin

It has been called resistin because of its resistance against insulin[21]. In humans, resistin is mostly found as a variant with several connections and it is polypeptide of 114 amino acids. When resistin is secreted in adipose tissue, it causes insulin-resistance on adipocytes.

Apelin

Apelin is a short peptide released from adipocytes upon stimulation. According to this, the plasma levels of apelin increase in associated with insulin-resistance and hyperinsulinemia in obesity. It was firstly isolated from bovine stomach tissue[22,23] and it was derived from a pre-proapelin precursor with 77 amino acids. It has been found that apelin exists in multiple molecular forms in various tissues such as apelin-10, 13, 16 and 36[24].

TNF-α

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine which has a significant role in the pathogenesis of various inflammatory diseases. TNF-α has a critical role in host defense against bacterial and especially mycobacterial infections. TNF-α is a pro-inflammatory cytokine which stimulates acute phase reaction. It induces cell death and inflammation but it inhibits tumor development and viral replication. It is synthesized by active macrophages and T-cells as a transmembrane precursor protein. TNF-α stimulates the secretion of inflammatory cytokines (IL-1 beta, IL-6, IL-8) and thus causes an increase in the level of some critical chemokines (MCP-1, MIP-2, RANTES, MIP-1 α). Moreover, it is a strong activator of endothelial adhesion molecules (ICAM-1, VCAM-1, E selectin)[25,26].

IL- 1

IL-1 was firstly defined as a leukocytic pyrogen in 1940. It has a molecular weight of about 10,000 Daltons. It causes fever, increase in the secretion of colony-stimulating factors (CSF), neutrophilia, loss of appetite, sleep and synthesize of acute phase proteins. At higher doses, it induces hypotension, leukopenia and an increase in cardiac flow [27]. TNF has strong in vitro effects on immune system like IL-1which includes T-cell proliferation, increase in the expression of MHC class 1 and 2, and the stimulation of other cytokines (IL-1, IL-2, IL-6 and IL-8)[28].

IL-6,8,10

IL-6 is a multi-poietic protein that induces the growth and differentiation of tumor cells[29-32] and the expression of other cytokines[31]. IL-6 acts by its receptor called Janus kinase JAK [33,34]. IL-8 induces endothelial cell proliferation in vitro and chemotaxis[35]. IL-8 is the most potent chemotactic for neutrophils. IL-10 is an anti-inflammatory and anti-angiogenic cytokine. It has been found that the level of IL-10 increases by obesity[36].

Relation between Adipocytokines and Cancer

Adipocytes may induce angiogenesis and tumor development in cancer cells[2]. These incidents are thought to be associated with cytokines including adiponectin, leptin, TNF-α, receptor agonists (IL-6, IL-8, IL-10 and IL- 1)[2,37].

Adiponectin

It has been found that adiponectin has two molecular forms with low and high molecular weight, and adiponectin with high molecular weight is more closely associated to cancer risk [14,15,38].

In a prospective study performed by Wei et al, it has been found that there is an inverse relationship between the plasma level of adiponectin

(ADP) and the risk of colorectal carcinoma (CRC) in men. Also in the same study, a relationship has been found between the low ADP plasma level and development of colorectal adenoma. It has been known that increased BMI and reduced levels of ADP increase the cancer risk more than six times[16]. Brackenhielm et al. was reported that adiponectin had an anti-angiogenic characteristic and thus reduced anti-angiogenic activity and increased angiogenesis might result with tumor development[39]. A correlation has been found between the reduced levels of adiponectin and progressive disease and high-grade prostate cancer[40].

In another study, an inverse relationship has been observed between the level of adiponectin and development of endometrial cancer[3].

It is thought that adiponectin stimulates tumor development in epithelial tissue cells by various signal pathways including the activation of leptin, NFkB and JNK, and causes cancer in colon tissue[17, 41, 42].

Leptin

It has been found that the circulatory level of leptin is associated with lipoidosis and colon cancer. In vitro studies confirmed the increase in cell proliferation, angiogenesis and metalloproteinase in esophagus and colon cancer cells[43]. The pro-carcinogenic effects of leptin do not only arise through the stimulation of signal pathways affecting cell division but also the possible reduction of apoptotic response is also responsible for this response in breast cancer. In breast cancer cells that were incubated with leptin a long-time; a significant reduction was observed in the expression of p21 and p53, and *bax* protein[44].In obese breast cancerpatients, the elevated levels of leptin causes high risk in the metastasis of lymph node and the size of tumor is bigger in these patients when they are compared with non-obese patients[45]. Leptin is effective in the progression of prostate cancer cells[46]. Ribeiro et al.has been found a relationship between the risk of prostate cancer and progressive disease (partially), and the polymorphism of leptin gene which is related to the synthesis and secretion of leptin[47].

Visfatin

The expression of visfatin has been increased in different cancer types[48]. The elevated levels of visfatin expression have been showed firstly in colorectal cancer[49,50]. Hypoxic regions within the central region of solid tumors trigger angiogenesis which has an important role in cancer progression[48]. The expression of visfatin increases in the presence of tumor which makes a contribution to the progression of the cancer[51].

Apelin

There are a few numbers of studies showing the relationship between apelin hormone and cancer. Berta et al. searched about in vitro and in vivo

effects of apelin in human non-small cell lung cancer (NSCLC). They found that in vitro administration of apelin did not affect cell proliferation, but in vivo study; it was found that apelin increased the development of tumor cells by inducing angiogenesis[52]. In a study performed on human tongue cancer cells, Heo et al. reported that apelin increased cell proliferation[53]. Moreover, the mRNA expression of apelin has been shown in malign ductal and lobular tumor cells[54]. So it can be concluded that apelin may have a significant role in the development of breast cancer.

Resistin

The relationship between resistin and inflammation, and the stimulating effect on nuclear transcription factor of resistin show that this level of adipocytokine may be effective in the development and/or partially progression of cancer stage. The relationship between certain cancer types (breast cancer, lung cancer, colorectal cancer) and resistin has been evaluated in studies performed so far[55,56]. Elevated serum resistin levels were found in patients with colorectal cancer in comparison with control group[57]. In a study performed by Kang et al., it was found that serum resistin level was higher in breast cancer patients than the control group [58].

TNF-α and Interleukins

It has been suggested that the TNF causes DNA damage and inhibits DNA repair mechanisms by increasing the synthesis of NO and free oxygen radicals in macrophages that phagocytosis cancer cells or tumors. Besides, it is thought that TNF makes a contribution to tumor development by enabling the survival of cells when NFκB activation and apoptosis are blocked in the cells[59].

The studies showed that the genetic variations in the inflammation-related genes such as Interleukin (IL)-6, IL-8 and IL-10, have been associated with colorectal adenoma and colorectal carcinoma risk. It is observed that IL-6 enhances tumor development by stimulating cell proliferation and inhibiting apoptosis with a paracrine and autocrine mechanism. Moreover, it was indicated that the level of IL-6 reflected the progression of the disease and related to common metastatic disease[60].

A correlation was found between the elevated levels of LI-6, TNF-α and CRP and the tumor size[61]. In various studies performed recently, the high levels of IL-8 in prostate cancer have been provided direct proofs regarding the regulatory role of IL-8 in cell proliferation and metastasis[35, 62, 63].

It is supposed thatIL-10 has a pro-tumorigenic potential which enables their escape from immune system by means of their anti-inflammatory properties. IL-10 also increases tumor development and angiogenesis both

in animal and in vitro models[64]. While the high levels of IL-10 synthesis are associated with genotype, the lower levels are associated with increased sensitivity and advanced cancer stages (skin, malignant melanoma and renal cell carcinoma) [65].

IL-10 is substantial cytokine acting like a double-edged sword, however, the underlying mechanism is not known. IL-10 suppresses tumor immunity and supports cancer cell tolerance[66, 67].

It has been shown recently that adipocytes are not only energy-storing cells but they are also responsible for the synthesis of hormones, cytokines, growth factors and other bioactive substances. In conclusion, adipocytokines are significantly involved in cancer process, and the most of the positive data, not all, has been shown on cancer cell lines in vitro studies. The effect of obesity in risk enhancement has been well documented in certain types of cancer such as colon, breast and prostate cancer[68].

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Chapter 23

RECENT STUDIES ON ANALYSIS OF TRANS FATTY ACIDS IN TURKISH FOOD PRODUCTS

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INTRODUCTION

Carbohydrates, proteins and fats are the most important building blocks and energy sources for sustaining of the organisms. Fats, which per unit gives the highest energy in weight, it is an essential ingredient in human and animal diets since they are very suitable for energy storage. In general, compounds with different structures, which are insoluble in water while soluble in organic solvents such as ether, benzene, chloroform are collected under the name of oil (Ozdemir & Denkbas, 2003). It is a group of compounds dominated by triglycerides, consisting of fats, oils, glycerol and fatty acids (FA). The physical and chemical properties of the fats determine the composition of the FAs' contain. According to these features, it provides the use of edible fats, soap, perfumery and other industries.

Glycerol, which constitutes the oil, is the same in all fat plants, whereas FAs, which constitute the other fats, are in a different composition in each fats plant (Baydar, 2000). The composition of the FAs they contain determines the usage areas of the fat. The FA is a straight hydrocarbon chain that carries a carboxyl group (-COOH) in its structure and is the most important element of the fat (Figure 1).

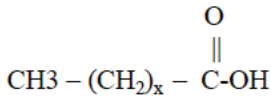

Figure 1: *The general formula of a fatty acid*

The dominant FAs in fats contain a double carbon atoms and a carboxyl group. (Nas et al., 2001; Kayahan, 2003). FAs that in the hydrocarbon chain, separated from each other in terms of carbon number, whether there are double bonds between carbon atoms, location and number of double bonds. (Baydar, 2000). As the properties of the fats change according to the types of FAs' contain obtained, it is necessary to produce for consumption purposes.

Classification of Fatty Acids

FAs are generally straight chain derivatives and classified into 2 types as saturated and monounsaturated fatty acids (MUFAs). FAs, which consist of a single covalent bond (-C-C-) between carbon-carbon atoms and are generally solid at room temperature, are called saturated FAs (Kumeli, 2006).

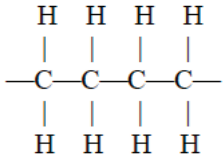

Figure 2: *The general formula of a saturated fatty acid*

Lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20: 0) and behenic acid (C22:0) are the most important saturated fatty acid (SFAs). Especially C16:0 and C18:0 are the most common SFAs found in the plant fats.

FAs containing one or more covalent double bonds at various locations on the carbon chain between carbon-carbon atoms are called unsaturated fatty acid (UFAs).

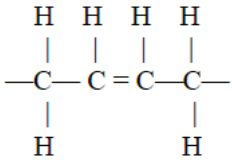

Figure 3: *The general formula of an unsaturated fatty acid*

Due to the double bonds in their structure, UFAs are more reactive than SFAs. This reactivity increases with the number of double bonds in the FA chain (Nas et al., 2001).

In UFAs, it is divided into two as cis and trans according to the configuration of hydrogen atoms bound to carbon atoms at the double bond. When the hydrogen atoms standing on two carbon atoms next to each other lie on the same side of the bond, the second looks like a putative bond at this point with two hydrogen atoms extending out of the bond. This condition or isomer is known as a cis double bond (Başoğlu 2002, Webb ve O'Neill 2008).

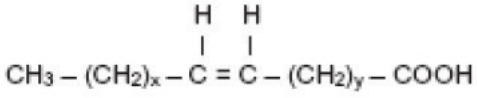

Figure 4: *The general formula of a cis isomer*

When two hydrogen atom bonds lie on opposite sides, a weak node is formed and this is defined as a trans isomer.

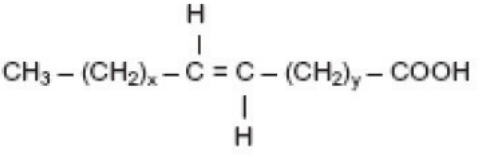

Figure 5: *The general formula of a trans isomer*

Different sequences of cis and trans isomers cause FAs to have different characteristics such as different melting point and thermodynamic stability, different crystallization degrees and ability to participate in chemical reactions (Kayahan 1998, Kara 1999).

Trans Fatty Acids in Nature

The vast majority of FAs in nature are in cis form. It was thought that trans fatty acid (TFA)s were not found in foods naturally in the past years and were caused by hydrogenation of unsaturated fats. However, researches carried out as a result of developing sensitive analysis methods have shown that TFAs are found naturally in most foods, although production and storage conditions affect the amount of discovery (Kıralan et al. 2005). TFAs occur in three forms that biochemical hydrogenation, partial hydrogenation and high temperature applications (Tekin 2007). TFAs occur by direct activity of microorganisms present in the rumen of mammals and biohydrogenation of polyunsaturated fatty acids (PUFAs) in dietary triacylglycrols (Kıralan et al. 2005).

Firstly, TFAs and conjugated linoleic acid (C18:2) increase in their stomach by hydrogenation of UFAs in the diets of mammals throughout the bacterial fermentation. The first step of this biohydrogenase is the isomeration of C18:2 by mainly anaerobic bacteria Butyrivibrio fibrisolvens (Fritsche and Steinhart 1998, Bessa et al. 2000, Palmquist 2001).

In an oxygen-free environment, bacteria use the double bonds of FAs as acceptors for hydrogen produced during metabolism. This process leads to the saturation of UFAs and the formation of TFAs. The amounts and types of trans isomers found in animal fats do not differ as much as industrially partially hydrogenated fats (Bessa et al. 2000, Palmquist 2001).

The intestinal systems of single-stomach animals and humans cannot do this. Therefore, TFA isomers are not synthesized in the human body. FAs exist in cis form in the human body and mammals. However, due to the nutrients taken in the fats of mammals, trans and conjugated FAs can be found even in a small amount. These FAs are also thought to cause hard plaques to form in the vessels (Çiftçioğlu 1997, Stachowska et al. 2004).

TFAs are found in partially hydrogenated oils as well as normal oils. Different factors such as the type of oil, processing and method conditions depend on the level of TFA forms during partial hydrogenation. Theoretically, the trans-cis balance form is around 75% of the total double bonds (Gürcan 2000).

During hydrogenation, the number of cis double bonds naturally found on the carbon chain decreases. It is the first stage conjugate conversion in the hydrogenation process. Then, the cis trans transformation and double

bond slipping intertwined and walk together. During the process, 60% of the total FAs of the product turn into cis and trans isomers. According to the researchers, during the hydrogenation reactions, 2/3 of the UFAs in the environment form a balance that turns into a trans and 1/3 to the cis form. In addition to the hydrogenation process, various isomers can be formed by bleaching and oxidative reactions applied in the refining stages of crude oil (Kayahan and Tekin 1994, Kayahan 2002).

Trans isomers can also be found as a short-lived intermediate in the biosynthesis of SFAs and as a durable final product. In addition, some plants contain little amounts of FAs in trans form. In a study, related to TFA which consists of 70% of punicic acid (9c, 11t, 13c C18: 3), was found in 6 types of plant, including pomegranate (Feldman et al. 1996).

A small amount of TFAs can be found at low levels depending on the food eaten in poultry and lard (Semma 2002). It is found that TFAs in the food, they received from the environment during the feeding of the chicken passed to the eggs significantly and also appeared in the embryo tissue. It is also found in hydrogenated vegetable and marine animal fats and in low amounts in land animal fats and meat and dairy products (Manteca and Noble 1993).

Effects of Trans Fatty Acids on Human Health

The physiological effects of dietary fats on plasma lipids have been investigated since 1856. In the 1970s, results began to be obtained that TFAs act like SFAs, cause cardiovascular diseases due to nutrition and increase triglyceride and levels in plasma (Mansour and Sinclair 1993, Erkkila et al. 2008).

TFAs are constantly found in people's diets, as they are contained in ruminant meat and milk in low amounts. TFA distribution in plasma lipids is known to increase the risk of coronary heart disease, since TFAs melt at a higher temperature, such as cis forms, at a higher temperature of 25 ± 5^0C (Kayahan2003), as they have a negative effect on nutrition physiology and cardiovascular diseases. Recently, there has been an intense scientific interest on this subject and it has been stated by scientists that caution should be exercised in the consumption of foods containing high levels of TFAs (Hayakawa et al. 2000, Marangoni et al. 2008). It is reported that it is associated with sudden heart attack deaths and diabetes consumption of TFAs (Counil et al. 2008).

TFAs such as elaidic acid increase the level of LDL cholesterol, since increase serum cholesterol level while decrease the amount of HDL cholesterol, increase the risk of coronary heart disease. Total diet / HDL cholesterol and LDL / HDL cholesterol increase as a result of diet with TFAs. These rates strengthen the possibility of more coronary heart disease than total

cholesterol or LDL cholesterol alone. In studies, TFAs also increase plasma triglycerides and also increase the amount of VLDL. Since these diseases increase the risks, high amount of TFA consumption is an important issue to be emphasized (Judd et al. 1994, Sundram et al. 2003, Erkkila et al. 2008).

The increase in the total serum cholesterol level for each 1% energy from the daily carbohydrate foods replaced by TFA is approximately 0.028 mmol / L (1.1 mg / dL). Most recent studies have shown that trans oleic acid (*t*-C18:1), which causes low density lipoprotein cholesterol (LDL), which causes vascular stiffness, is found in high concentration hydrogenated vegetable oils. Trans C18:1, which replaces 1% of energy from carbohydrates, increased the LDL cholesterol level by approximately 0.034 mmol / L (1.3 mg / dL). Trans C18: 1 has no positive effect on high-density lipoprotein (HDL) as compared to carbohydrates, on the contrary, HDL decreases compared to C18:1 (Gürcan 2000, Kabagambe et al. 2008).

The European Union FAIR project evaluated the effect of TFAs on factors related to coronary heart disease and as a result, TFAs were found to have more negative effects compared to cis form (Kıralan et al. 2005).

In recent years, the effects of TFAs on cancer formation have been investigated and it has been determined that it promotes tumor formation as a result of animal experiments (Ip and Marshall 1996, Bakker et al. 1997, Innis et al. 1999). In addition, It is reported that there is a linear relationship between TFA intake and breast cancer. In another two studies, it was determined that it caused 63-75% of colon cancer. According to the data obtained as a result of the studies conducted so far, it has been reported that TFA intake does not cause prostate cancer. The conclusion reached in one of the recent studies is that there is a direct proportional relationship between TFA intake and brain cancer risk in post-menopausal women (Ip and Marshall 1996, Bakker et al. 1997, Katan et. al. 1998).

It is known that PUFAs, especially n–3 and n–6 FAs, are caused by allergy, eczema and asthma in children. However, there is insufficient information about the effect of the configuration of these FAs (cis, trans) on these ailments. A group of researchers found a high correlation between the intake of TFAs and conditions such as asthma and allergy as a result of their studies between children aged 13 and 14 (Yildirim, 2002).

Consumption of Trans Fatty Acids

TFAs have been in the diet of people since ancient times because of the low amounts in ruminants and fats of ruminant animals such as cows and sheep. Nevertheless, large-scale commercial production of oils with high TFA content and consequently a significant amount of TFA consumption started with the developing margarine industry (Ovesen et al. 1996).

The main foods that cause TFA intake are 37% commercially-produced oil-fried buns, 36% oil-fried products such as chicken or potatoes, 11-49% margarines and chocolate oils (Feldman et al. 1996).

The nutritional statistics of many countries include the TFA content of foodstuffs. In many publications in which TFAs are investigated in various foodstuffs, the daily TFA intake amounts are determined based on various studies (Stender et al. 2008).

The increase in the use of vegetable oil for frying and cooking processes after the 1970s led to a decrease in animal oil consumption. In addition, the presence of margarines with low trans fat content in the markets caused reductions in TFA intake (Holley and Phillips 1995). In the examinations carried out in the 1980s, it is stated that the average amount of TFAs taken corresponds to 2.1% of the total energy and this ratio varies according to the nutritional habits (Willett and Asherio 1994).

The use of partial hydrogenated oils in the food industry is decreasing to reduce the intake of TFAs. Also, with the introduction of new methods developed in margarine and shortsening productions, decreases in TFA contents are determined in these products. Frying oils are not subjected to long-term hydrogenation after 1985. On top of that, products with zero or very low trans content started to be produced. However, in many parts of the world, hydrogenated oils obtained by partial hydrogenation method remain important in margarine and shortsening formulations. Various fast food restaurant chains also use frying oils containing 25-35% TFAs for frying. Snack and fast food products, chocolate and other similar products, especially containing the fats obtained by using partial hydrogenation technique, are consumed at high rates by children and young people (Willett et al. 1992, Anonymous 1996, Marangoni et al. 2008).

Although there is no limit value for the consumption of TFAs, the conditions mentioned for their negative effects on cardiovascular diseases are seen only in high consumption. It is stated that the negative effect can be seen in consumption at normal levels is related with other risk factors. In a study, consumption of 4% TFA did not cause any adverse effects in LDL and HDL cholesterol concentrations in hypercholesterol patients. 4 g per day. taking TFAs is not at a risk to health; however, it is stated that intake of TFA should be reduced in patients with fat metabolism disorders, pregnant women, lactating women and infants. FAO and WHO recommend the consumption of oils containing less than 4% TFAs in 1994 and encourages the food industry to reduce the amount of TFAs in their products (Yildirim 2002, Diraman and Hisil 2004). As a matter of fact, it is stated that the level of TFA taken with dairy products in the daily diet constitutes 6–25% of the total trans FA daily (Diraman 2004).

Consumers who are familiar with the subject are skeptical of TFAs. Thus, they pay more attention to the FA composition and the content of saturated fat and TFA in the products they buy and they want this information to be included on the food packaging (Endres 1994).

As the requirement of the saturated acid content to be included in the food packaging by the FDA since May 1994, the researchers working with TFA have increased their demands for the trans isomer contents to be on the label day by day. Because food producers can go to increase the amount of TFA in order to decrease the amount of saturated fat and to obtain the product with the same feature. Researchers working with trans FA expressing their opinions to the FDA about the need to have information about trans isomers on the food label. However, considering the fact that the subject is new, its arguments are inadequate, and the public is not informed about this new subject, the application has not been accepted (Taş 1998, List 2004).

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FDA has announced the criteria for keeping information about TFA content in foodstuff labels, and since January 1, 2006, companies have been obliged to legally indicate the amount of trans fats on their labels. The FDA reports that the TFA, which can be found in products up to 0.5 g, and in Canada up to 0.2 g, is negligible. (Rozema et al. 2008). This legal obligation has directed large food companies to reduce the amount of TFAs in their products. For example, since December 2005, the amount of TFA has to be written on the label in Canada, since TFA intake has decreased (Hawkes 2008).

REVIEW OF ANALYTICAL METHODS

Daglioglu et al. have studied the FA composition and total TFAs in cereal-based Turkish foods with using GC-FID. The total fat contents of the samples, which are 13 foods, which contains grain, manufactured by Turkish companies, varied between 1.8 and 37.9%. While the largest values of

fat content belongs to wafer (37.9%), the smallest fat content belongs to Classical Turkish white bread and bulgur (1.8% and 2.3% respectively). The primary FAs were C16:0, C18:0, trans C18:1, C18:1 and C18:2 in the all samples. The whole UFA composition changed from 49.0 to 80.3% of total FAs, and the higher FAs proportion was found at the bulgur. Apart from bulgur, compound of TFA's at the the samples involved (weight percentage of methyl esters) changing between 0.1 and 31.0% of the total. The TFAs level of bulgur could not detect whereas white bread and corn chips included little: 0.1% and 0.7% respectively. (Daglioglu et al., 2001).

Daglioglu et al. have investigated of FA content of classical fermented and unfermented of Turkish corn breads by GC-FID. Non-fermented samples were prepared from corn flour, and fermented samples were prepared from corn flour-wheat flour mixture (1/1). As a consequently, non-fermented and fermented samples have the similar saturated and unsaturated acid formation as the flour used in the recipe. Saturated and unsaturated acid content of corn breads were not influenced with Dough fermentation. Still, Saturated and unsaturated acid content of the baked bread modified gently. The amount of C18:2, Linolenic acid (C18:3), C20:0 and eicosenoic acids declined while C16:0, C18:0 and C18:1 raised. Trans C18:3 was determinated in both unfermented and fermented corn bread at a degree of 0.1% as well. Gentle changes in the FA content and structure of trans C18:3 were as a consequence of baking temperature (Daglioglu et al. 2003).

Karabulut et al. have researched that FA contents of consumed foods in Turkey were investigated by GC-FID. 134 samples were practised that were classed as meat products, chocolates, bakery products and others. Apart from chicken-based foods, the meat products contains 1.45 g/100 g FAs. The conjugated C18:2 composition of meat products were detected less than meat and chicken doner. Chocolate samples involved TFAs less than 0.17 g/100 g FAs, with the remarkable national product of chocolate bars and hazelnut cocoa cream (2.03 and 3.68 g/100 g FAs, respectively). The maximum TFA compositions were determinated in bakery products and varied from 0.99 to 17.77 g/100 g FAs. The average TFA contents of milk-based products of ice-cream and infant formula were determinated 1.50 and 0.79 g/100 g FAs, respectively. Within the investigated samples, it was obtained that coffee whitener and powdered whipped topping had the maximum composition of SFA, with an average content of 98.71 g/100 g FA (Karabulut 2003).

Basol et al. have investigated of the FA and TFA content of Turkish shortenings by GC-FID. The different varieties of SFA, MUFA and PUFA compositions were detected 27.4–48.8, 33.8–56.7 and 8.4–35.3% of total FA, respectively. The whole TFA varied between 2.7 and 23.9% of total FA. Among the all studied products, the total trans C18:1 acid was the

major of total TFA with 82.3 94.5%. The total trans C18:2 ranked between 0.2 and 2.9% of total FA, whereas the total trans C18:3 was only detected at very low degrees (0.1% of total FA) in the products of two companies. To sum up, partly hydrogenated vegetable oils with high compositions of TFA are still the primary raw materials used in the production of Turkish shortenings. (Basol et al. 2007).

Cakmak et al. have determinated of the FA components and TFA composition of chocolate and chocolate wafers in Turkey by gas chromatography equipped with a flame-ionization detector. Total 62 chocolate samples were investigated which of them 35 samples of chocolate and 27 samples of chocolate wafer were categorized Broadly, the major compounds of FAs were C16:0, C18:0, and C18:1 in the whole samples. Showed a variation in chocolate wafers samples from 0.00 to 7.92%, TFAs were detected as 0.00–6.23% in the chocolate samples. In brief, it was demonstrated that TFAs composition in chocolates were nearly lower than those of other countries (Cakmak et al. 2010).

Cakmak et al. have investigated of the FA and TFA components in crisps and cakes in Turkey's markets by GC-FID. In this research, 57 crips and 50 cakes were studied in order to detect proportion of SFAs, MUFAs, and PUFAs of crips and cakes. C 18:1 was the primary FA in all crisps and cake samples. The percentages of SFAs, MUFAs, and PUFAs varied from 27.98–46.57, 35.73–47.57, to 9.86–35.90 g/100 g FAs in crisps and 35.41–54.03, 25.89–44.87, and 10.52–26.97 g/100 g FAs in cakes, respectively. Total TFAs varied from 0.02 to 1.35 g/100 g FAs in crisps and 0.00 to 5.05 g/100 g FAs in cakes, respectively (Cakmak et al. 2012).

Cakmak et al. have determinated of the TFA's and conjugated C18:2 composition of Turkish ice creams" by GC-FID. In this research, 27 ice creams belonging to 4 different brands were studied for their FA composition, with relevance on their TFA and conjugated C18:2 compounds. Apart from cacao and chocolate ice creams, C16:0 was major FA in whole samples. Other predominant FAs were C12:0, C18:0 and 18:1 ω9 C18:1. SFAs were in the highest percentage in the whole samples followed by MUFAs and PUFAs. The total percentages of SFAs followed by MUFAs and PUFAs varied from 61.56-80.99, 13.30-210.86 to 3.68-8.02 %, respectively. TFAs and conjugated C18:2 compositions in ice cream samples were from 0.71-2.66 to 0.17 0.91 %, respectively. As a consequence of this research have shown that TFA and conjugated C18:2 content of ice creams determinated in Turkey are positive levels when compared to many countries (Cakmak et al. 2011).

Ergonul et al. have investigated of the FA contents, TFAs and cholesterol compounds of cheese-flavored crackersby by GC-FID. So as to

obtain FA compositions, TFAs and cholesterol contents of cheese-flavored crackers sold in Turkish markets were studied. As a result of this study, C18:1cis had the largest (40.2%) amount of UFAs followed by C18:2 cis (14.6%), while the amounts of TFAs in C18:1tr and C18:2tr were 0.2% and 0.4% of total FAs, respectively. The average cholesterol composition of the samples were 0.59 mg/100 g. In this study shows that cheese-flavored crackers, which are sold in Turkish markets, did not consist of remarkable amounts of TFAs. The PUFA/SFA proportions of the samples were under the limit of 0.45% and closely half of the fat fraction of the crackers was consisted of SFAs (44.0%) (Ergonul et al. 2012).

Yilmaz et al. have studied of the determination of FA content and total TFAs in some meat products by GC-FID. Total fat compositions of the 22 meat products viewed between 11.60 and 42.50%. While sucuk (soudjuk) the highest 42.50%, Salami had the lowest fat content 11.60%. C16:0, C18:0, trans C18:1, cis C18:1, and C18:2 were the primary FAs in the samples. Total UFA compositions have varied between 38.73 and 70.71% of total FAs, and sausage had the highest proportion at the whole samples. The majority of samples contain TFAs and the level viewed between 2.28 and 7.95% of the total FAs. The maximum amount of total TFAs was obtained in kavurma (Cavurmas) (7.95%), and total TFAs of meat products like pastrami included more than 5% of the total FAs (Yilmaz et al. 2009).

Yilmam et al. have studied of the TFA compounds of crude soybean oils industrially determinated by solvent extraction with hexane and were studied by GC-FID. In this study, the compositions of total TFA were within the ranges of 0.05 +/- 0.02-0.17 +/- 0.05% of total FAs. In addition, total Linolelaidic acid (C18:2 trans and total C18:3 trans acids of the studied samples were obtained 0.01 +/- 0.00-0.06 +/- 0.02 and 0.02 +/- 0.01-0.09 +/- 0.04%, respectively. Regarding the total C-18:1 trans acid, its levels were shown less than 0.02 +/- 0.02% of total FAs. Moreover, C18:1 trans acid was not detected in any samples. As a result of this study, the TFAs were probably formed by heat treatment of soybeans before or during the solvent extraction processes. Even though very low values of the TFAs were obtained in the crude soybean oils, the existence of these FAs can be caused difficulties to produce refined soybean oils with or without low level of TFA (Yilmam et al. 2008).

Demirbas et al. have determined of the TFA content of edible margarines in Turkey by capillary gas-liquid chromatography. 8 different oil samples normally used in margarine manufacture were used in order to obtain FA content. TFAs contents were detected in 6 soft-type and 6 hard-type Turkish margarines. TFA content varied between 5.6 and 6.7 (average 6.2%), whereas in hard-type consist of TFA concentration in fatty spreads ranged between 16.0 and 18.3% (average 17.2%) (Demirbas et al. 2000).

Yildirim et al. have investigated of the FA content and TFA form in extracted oils from French-fried potatoes and classification of samples using chemometric technics by GC-FID. In this study, FA content and TFA form are studied in oils extracted from French-fried potatoes, which were produced in the laboratory and collected from different restaurants. Potatoes were fried at 180 °C at particular frying times (1, 7, 13, 19, 25, and 31 min) and the FA content of the extracted oils was obtained. C16:0, C18:0, C18:1, and C18:2 were detected at the samples. C18:2 trans was obtained in the oil samples extracted from potatoes fried in margarine. 3 principal compounds (PCs) and 4 main clusters were found from the chemometric analysis, which characterized the samples. 3 PCs were obtained to be explanatory of more than 84.96% of the total variability in the data set (Yildirim et al. 2015).

Sahin et al. have evaluated of the isolated impact of TFAs on short dough product by gas chromatography. In this study, the effects of TFAs on rheological properties of dough (elastic moduli (G'), loss moduli (G''), complex modulus (G*)) and textural properties of dough and cookie were investigated. 2 different groups of fat samples having different TFA content but similar solid fat content (SFC) were prepared. The first group (group 1) had TFA amoung 0.0 to 56.23 %, whereas the second group (group 2) consisted of trans isomers varying between 0.0 and 44.40 %. Texture measurements, which were performed for texture measurements of different doughs and cookies prepared with different fat samples indicated, which hardness values of doughs raised between 3950 ± 420 and 5498 ± 506 g in group 1 and 4700 ± 501 to 6787 ± 369 g in group 2 with raised amounts of TFAs. A spesifically high, about three-fold raise in complex modulus values was wieved in the dough samples consisting of the highest TFA levels compared with samples consisting of 0.0 % TFA. Even though not important difference, mean hardness and relative sound intensity values of cookies showed an initial decreasing trend and then both parameters had maximum values while the TFA compound was highest in both groups. (Sahin et al. 2016).

CONCLUSION

TFA intake, which functions of physiologically important for health, should be limited because of excessive intake or negative effects of some FAs on metabolism. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) make recommendations on the application of food processing to reduce TFA production. These new criterias legally introduced the requirement that TFA content should be indicated in the labeling substance from 1 January 2006. Accordingly, if the TFA content in the product portion exceeds 0.5 g, it must be stated on the prod-

uct label. If it is below 0.5 grams, "TFA does not contain acid" can be used. The consumers are advised that the amount of TFA should be as low as possible. For this reason, many food industry organizations are also working on alternative alternatives to produce reduced amounts of TFAs. In addition, The Turkish Food Codex General Labeling and Nutritional Labeling of Foodstuffs Regulations Amending the Communiqué on the situation in less than 1g in 100 g. "does not contain TFAs." It is stated that it can be written on 23 August 2007.

Trans fats are frequently used in the production of ready-made food products thanks to their alternatives such as high physical stability, low cost and longer shelf life of the product. Since the fast-food products are widely consumed by humans, such foods can contribute significantly to daily TFA intake. Nowadays, TFAs are used in the production of cakes, biscuits, cookies, mayonnaise, chips, puff pastry, pizza, wafers and similar products and in the preparation of deep-fried fast food foods. There are also differences in the FA and TFA content of these products.

It is known fact that trans isomers have negative effects on human health. For this reason, the amount of trans fats in foods have to kept under control and it is important that they have to be below the specified limits. Many food products are now on the Turkish markets including TFAwith high concentration. The analytical methods used are insufficient, although there are studies on the subject in the literature for Turkey. Turkey attach great importance to studies on trans fats found in foods, the mentioned subjects show that it is an area open to research and study, but its importance is increasing.

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Chapter 24

Metabolic Acid-Base Disorders and Fluid Therapy in Cats and Dogs

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INTRODUCTION

The body fluids are arranged in dynamic but orderly functional compartments. Maintenance of these compartments in terms of volume and composition is essential for maintenance of normal physiologic and biochemical events. Maintenance of these compartments in terms of volume and composition is vital for maintenance of normal physiologic and biochemical events. The electrolytes dissolved in the body fluids fulfill vital roles in virtually all of life's processes (Carlson, 1997). Fluid therapy in states of hypovolemia is to correct cardiac output to improve microcirculatory perfusion and tissue oxygenation (Alastair, 1988; Yuruk et al., 2007). The total body water (TBW) of most domestic animals is approximately 60 % of body weight (0.60 liter/kg) (Davis, 1982; Sensior, 1983). Water is freely diffusable in the body and can readily move from the intracellular space (ICS) to the interstitial space (ISS) to the plasma space (PS) or vice versa (Figure 1) (Garvey, 1989). The distribution of body fluids is summarized Figure 1 and Table 1 (Davis, 1982; Saatçi, 1982; Sensior, 1983; Hilî, 1984; Chandler et al., 1999).

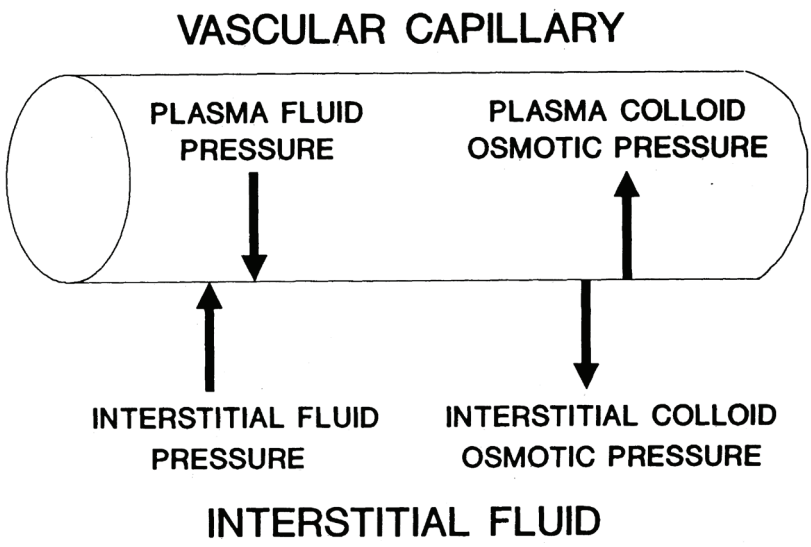

Figure 1: *Four major fluid pressures that affect the movement of body water between the vascular space and the interstitial space. Colloid osmotic pressure draws water in; fluid pressure pushes water out. The sum of the four forces determines the net movement of the water (Garvey, 1989).*

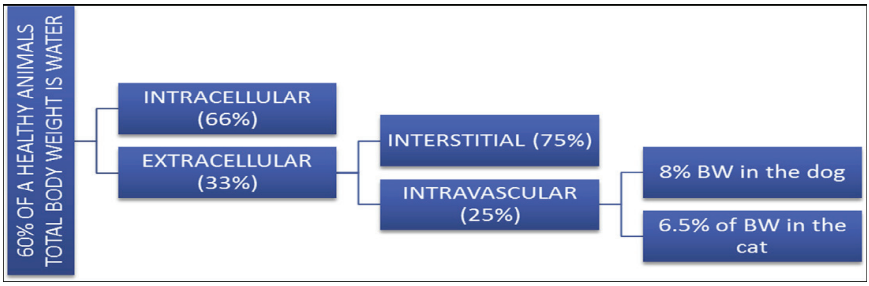

Figure 2. *Total body of water and fluid compartments within the body characterized by percentage of body weight (Tello and Perez-Freytes, 2017).*

Table 1.: Body fluid compartments

Compartment	Fluid to Body Weight ratio	Cations	Anions
Total fluid	% 60		
Intracellular fluid	% 40	K^+ , Mg^{++}	HPO_4, Protein
Interstitial fluid	% 15	Na^{++}	Cl, HCO_3
Intravascular fluid	% 5	Na^{++}	CI, HCO_3, Protein

The regulation of body fluid balance and electrolytes in compartments maintains optimal cell function. Fluid loss extends to from the intravascular compartment to interstitial compartment and the intracellular compartment's fluid reservoir and causes a particular deviations in the concentrations of the main electrolyte in compartments (Gross, 1989). Physiological differences in the amount of body fluid are associated with the animal's age, fattening status and sex. In dogs and cats, 55-60% of the body weight generally constitutes body fluids (Michell, 1974; Davis et al., 2013). This is one of the reasons why dehydration develops faster and has more serious consequences in offspring (Cullen, 1991). As seen in Table 2, the animal's nutritional status has a significant effect on the total amount of body fluid (Michell et al.,1989; Davis, 1982).

Table 2 The Effect of fattening condition on the body's fluid composition

	Water (%)	Fat (%)
Normal Weight	**60**	**18**
Cachectic	**66**	**12**
Adipose	**47**	**35**

As a result of this distribution, adipose patients are more affected by fluid loss than normal body-weighted or cachectic patients. Apart from age and fattening condition, another factor affecting the fluid rate in the body is gender. Male animals with normal body weight have more fluid and less fat than females (Michell et al.,1989; Dibartola, 2000). Body fluid contains

ions and molecules that are partially free to move from one fluid compartment to another as well as the particles dissolved in the water molecule, but partially returning to their effect under the use of energy in a certain compartment, which are frequently bound or in concentration change. The continuity of body water and electrolyte concentrations is ensured by the balance between uptake and excretion (Cullen, 1991, Verbalis, 2003).

Water loss through normal feces, expiration air and sweating is described as "insensible losses" because there are uncontrollable (continuity in disease conditions including health and dehydration) (Davis, 1982; Sensior 1983.). Insensible losses are due to extarnal factors such as temperature and humidity (skin and respiratory water loss); Total loss is 20 ml/ kg / day (including 5 ml feces, 15ml respiration + cutan). This amount is equal to 1/2 of daily water intake (Humm et al., 2008). Depending on its role in the regulation of heat in dogs, a significant amount of water loss through breathing develops, while skin losses are minimal due to the incomplete development of the sweat glands. Urine fluid losses (sensible losses) are controlled by the kidneys that regulate extracellular fluid volume and electrolyte concentration. The fluid lost by urination is approximately 20-40 ml/kg / day in healthy cats and dogs and is covered by the other half of daily fluid intake (Pond,1982; Sensior,1983; Muir,1990). In order to preserve body fluids and electrolytes at normal levels, the amount of fluid (ml) and electrolyte (mEq or mg) to be taken should be equal to the amount of losses. Daily total requirement is approximately 40-60 ml/kg (average 50 ml/kg) in adult dogs, depending on the ambient temperature, humidity and the animal's renal concentration ability. This amount is higher in small breed dogs, adolescents, lactating or pyrexic animals and at high ambient temperature. The need to add 1 mmol /kg /day Na, 2 mmol /kg/day K to the daily water requirement has been reported (Schaer, 1989; Muir, 1992).

The daily water requirement of cats and dogs can be formulated as follows.

Insensible Losses (Gastrointestinal + Skin + Respiratory) = 20ml/kg/day

Sensible Losses = 20-40 ml / kg / day

Daily requirement = 40-60 ml / kg / day. It is reported that the amount of daily fluid 60 ml large and 40 ml small for dogs that must be taken to ensure the maintenance of body fluids and electrolytes (Schaer, 1989; Muir , 1990).

Electrolyte disorders

Electrolyte disorders are common in patients with vomiting, diarrhea, or both. A large volume of fluid containing electrolytes (sodium and chloride) is secreted and reabsorbed by the normal GI tract (Brown and Otto

2008). Vomiting or malabsorption and diarrhea cause loss of these electrolytes and can thus lead to severe electrolyte abnormalities. Hyponatremia and hypochloremia commonly result from loss of these fluids. The typical findings of hyponatremia and hypochloremia are most common when only gastric contents are lost (Nelson et al., 1983).

Dehydration

Dehydration literally refers to the loss of pure water. However, since its clinical use is often accompanied by dehydration and some electrolyte losses, fluid electrolyte losses are understood to decrease extracellular fluid volume (Gross,1988; Muir, 1992). It is a dehydration circuit that progresses in the deficiency and shows clear clinical symptoms.

The dehydration, which is the indicator of the negative fluid balance in the organism are seen in the course or result of many diseases (mix water and electrolyte deficiencies) (Cullen, 1991, Michell, 1985). The osmolality of a fluid is determined by the electron binding capacity of different dissolved parts and the amount of dissolved parts and is expressed in mEq/kg or mmol/kg. Depending on the osmolality of the lost fluid, dehydration is divided into three. Water loss or hypotonic fluid loss results in hypertonic dehydration, isotonic or hypertonic fluid with water also results in isotonic dehydration when the osmolality of the lost fluid is equal to that of the body fluid. Extracellular volume decreases in hypertonic dehydration that develops as a result of hypotonic fluid or predominantly water loss (eg febris or high ambient temperature). The concentration of Na increases. When the intracellular fluid passes into the extracellular region, the disorder is compounded (Chandler et al., 1999). As a result, a decrease in intracellular fluid volume and cell shrinkage develops (Sensior 1983). The most important reason is insufficient water intake and thirst. Apart from this, it is observed in this type of dehydration due to severe diarrhea due to severe diarrhea and severe water loss over the skin surface and lungs without severe sweating, with high fever hyperventilation (Beienger et al., 1990). Hypertonia in the extracellular region increases the reabsorption of water from the tubules by stimulating vasopressin secretion (Verbalis, 2003). Hypotonic dehydration develops as a result of hypertonic (predominantly Na^{++} loss with water loss in diarrhea) and isotonic fluid loss after completion of water deficiency.The amount of fluid lost is removed by increasing the water intake, but the electrolyte deficiency cannot be compensated. This situation results in hypotonia in the extracellular region and consequently an increase in the volume of intracellular fluid, the flow of water to the cells and edema in the cells. Hypotonic dehydration is the most common form of dehydration and generally develops following isotonic dehydration. In isotonic dehydration resulting from the loss of water and electrolyte at the same rate, the osmolality of the blood does not change.

The osmolality of the lost fluid is the same as that of the body fluids, only a reduction in the total amount of fluid. Mostly at the beginning of diarrhea; this type of dehydration is observed in the loss of gastric fluid (osmolality equals that in the blood.). Clinically, thirst, stagnation, tachycardia and decreasing vena stagnation are observed. This classification is used to correct dehydrations (theoretically, hypertonic dehydration is corrected using isotonic solutions of hypotonic dehydration, hypertonic and isotonic dehydration) (Muir, 1990).

Weight Points of Clinical Examination in Patients with Dehydrated

The diagnosis of fluid balance abnormalities needs the informed and reasoned interpretation of clinical and laboratory information (Roumelioti et al., 2018). Before the infusion treatment, it is important to determine the type, amount, speed and way of the fluid to be given for the clinical examination of the patient to determine the type and degree of the disorder in fluid balance. Examination includes anamnesis, physical examination and laboratory tests. In addition, dogs and cat should be evaluated and fluid treatment should be applied according to the following criteria (Pond, 1982).

a. *Type of fluid loss (diarrhea, vomiting, diuresis. Ascites, polyuria, anorexia, lack of water, excessive salivation).*

b. *Duration of disease causing fluid loss.*

c. *The magnitude of fluid loss.*

d. *Gastro intestinal losses (Vomiting and diarrhoe)*

e. *The amount of urine (Polyurie, oligurie).*

f. *• Exposure to heat, trauma or hemorrhage.*

g. *Febris, Polypnoe.*

h. *• Questions about food and water consumption are posed.*

Learning the way of fluid loss from the anamnesis enables determining the main disorders (losses or accumulations) and the type of the disorder.

In the context of the anamnesis, the first information about the severity of dehydration and the character of the disorders in the fluid-electrolyte balance is learned from the answers to the above questions. If an infusion therapy has been previously applied to the patient, the composition, amount, administration route and frequency of adverse effects are possible in terms of possible adverse effects, periodic examination of the efficacy and prevention of excessive infusion in this way (Schaer, 1989; Verbalis, 2003).

Signalement: Factors affecting the fluid rate in the body, such as age and gender, are important not only for the recognition of the patient, but

also for the evaluation of dehydration and infusion (Schaer, 1989; Verbalis, 2003; Gross, 1988; Saatçi 1982; Hilî, 1984).

General condition: The general condition in the patient with dehydration may be intact or slightly moderate or severely impaired. Body temperature: In hypovolemic and traumatic dehydrated animals, body temperature has dropped from normal body temperature to 3-4 °C. In shock situations, a decrease of 10 °C is observed than normal body temperature (Michell, 1985; Chandler et al., 1999).

Circulatory system: The frequency of the heart and pulse, the quality of the pulse, the fullness and tension of the vena, the control of the skin temperature, and the color of the conjunctiva and mucous membranes are evaluated. Depending on the degree of fluid loss in dehydrated patients; tachycardia, rapid and weak pulse, decreased blood pressure, cooling in the extremities and pale and cyanotic color in the conjunctiva and mucosa are the most important symptoms specific to the circulatory system (Clark, 1988). Abnormal K^+ concentration and disturbances in acid-base balance often cause cardial arrhythmias. Irregularities in the heart rhythm can be noticed by auscultation, and its quality can be fully demonstrated by the electrocardiogram findings (ECG) (Cullen, 1991; Gross,1988; Verbalis, 2003).

Respiratory system: In the examination of the respiratory system in a patient with dehydration, especially respiratory number, type and depth, and lung auscultation findings are evaluated. In patients with severe metabolic acidosis, as in hypovolemic animals, hyperventilation and associated increase in the number and depth of respiration are determined. Diabetic ketoacidosis in cats and dogs are the cases where these changes are seen (Cullen, 1991; Thomas et al., 2008).

Capillary filling time: Capillary refill time (CRT) is an indication of the adequacy of tissue perfusion. CRT gives information about the state of peripheral circulation (Schaer, 1989). In healthy cats and dogs, the normal light red color of the gum resumes within 1.5-5 seconds after pressure is applied. CRT being more than 2 seconds is moderate (7-10% loss) and in CRT over 3 second accompanied by tachycardia and cooling in the extremities. It is an indicator of hypovolemia due to fluid loss of 12-15% (Chandler et al., 1999).

Evaluation of mucous membrane color: Color and humidity are checked. It may be an indicator of pallor or cyanotic color, decreased humidity and dehydration to the mucosa. Drying in the oral mucosa may be an indicator of fluid loss from the body, as well as nasal congestion or runny nose, although hydration is normal in case of breathing through the mouth (Cornelius, 1980).

Skin elasticity: It is known that the degree of dehydration is determined by controlling the skin elasticity from the lumbar region. In evaluation, skin elasticity decreases as a result of loss of protein and fat in cachectic animals, and elasticity is the norm in fat animals despite dehydration! It should be kept in mind that the elasticity does not change (YVaterman, 1984), and 5% less body weight losses (Cornelius, 1980; Saatçi, 1982).

Abdminal palpation: Abnormalities such as fluid accumulation in the body cavities (ascites) and fluid accumulation in the intestines can be detected. It is known that the the urinary bladder is reduced by abdominal palpation in dehydrated animals (Cullen, 1991).

Body weight: 1 kg loss in body weight is equivalent to 1 liter of fluid loss. If the normal (healthy) body weight is known, the amount of dehydration fluid loss (normal body weight-actual body weight) can be determined by determining the actual body weight. On the other hand, losses in body weight can be detected by evaluating clinical symptoms (Table 3) (Saatçi, 1982; Cullen, 1991). The type and severity of clinical symptoms depend on the type and path of fluid loss (Cornelius, 1980; Schaer, 1989).

Table 3 .: Clinical symptoms of dehydration

Body weight fluid loss compared (%)	**Clinical findings**
< 5	No clinical symptoms are observed
5	Oral mucous membranes dried out
6-8	It is called moderate dehydration. Since the oral mucosa has dried, its elasticity has decreased, the amount of urine has decreased and its concentration has been intensified a little.
10-12	It is called severe dehydration. In addition to the above findings, there are shock findings that develop. There is tachycardia, cooling of the extremities, fast and weak pulse.
12-15	**Death exists. Blood pressure and sentral venous pressure decrease.**

Laboratory findings

Laboratory examinations are important in determining the type and degree of the disorder in fluid balance and determining the effectiveness of the treatment (Michell, 1985). Laboratory examinations are of great importance in the presence, degree and type of dehydration, diagnosis of electrolyte and acid-base balance disorders, determining the prognosis of the patient and determining the path to be followed in treatment. The packed

cell volume (PCV), Hb, TP, urea, creatinine, electrolyte and blood gases analyzes, total protein, urine specific gravity, glucose and the electrocardiogram (ECG) findings must be regarded as being of importance in the management of disturbances of fluid (Gross,1988; Michell et al. 1989).

Table 4.: Values of some Parameters in cats and dogs (Michell, 1985, Clark, 1988; Jones et al.,1989).

Parameter	Dog	Cat
PCV (%)	**37.0-50.0**	**30.6-46**
Hb (g/dl)	**9.0-16.0**	**8.1-13.5**
TP(g/dl)	**5.5-7.5**	**5.5-7.5**
Creatinine (mg/dl)	1.**0-1.7**	**0.9-1.7**
Urea (mgr/dl)	**1-10**	**5.40**
Na+ (mEq/L)	**137-150**	**135-150**
K- (mEq/L)	**3.3-4.8**	**3.4-4.5**
HC03 (mEq/L)	17-**24**	**16.5-21**
PCO2 (mmHg)	**29-42**	**26-40**

Hematocrit value (Hct) is the most important parameter in determining the degree of dehydration. Hemoglobin (Hb) and plasma total protein (TP) such as Hct mostly increase in patients with dehydration (Schaer, 1989). In cases where there is no increase, a pathological change should be considered before fluid loss occurs. Before fluid loss, anemia or hypoproteinemia may result in dehydration-related increase in specified parameters, resulting in normal limits (Gross,1988). Therefore, it is safer to evaluate both parameters together. If the mean erythrocyte amount does not decrease due to hemorrhage or hemolysis, the percent increase in PCV is equivalent to the decrease in extracellular fluid volume. For example, 20 % reduction in extracelluler fluid (ECF) causes a 20 % increase in PCV and TP. The importance of this calculation limits the loss of erythrocytes and protein, which are accompanied by fluid loss. Plasma urea concentration also increases with nitrogen. However, it should be remembered that the increase may also be of renal and postrenal origin. Urine analysis in dehydrated animals; It includes the presence of glucose, protein and blood, specific gravity and pH. The urine specific gravity in fluid losses increases up to 1060 in dogs, up to 1080 in cats, more prominent in cats. When the kidneys are the main fluid and electrolyte regulatory organ, irreversible impairment will develop in the kidneys unless this condition is corrected in a short time. Although the pH of urine reflects plasma acid-base status, in the presence of metabolic aicalosis developing in patients vomiting, urine may be acidic, as expected (Michell et al.,1989). Determination of plasma electrolyte Na^{++}, Cl^{-} and K^{+} concentrations may not always reflect the amount of these ions in the body. While low plasma Na, Cl^{-} and K^{+} concentrations always reflect significant losses, the presence of these electrolytes

within normal limits does not mean there is no loss. Plasma Na values give information about the relative deficiency of water and electrolytes in extracellular fluid and intracellular fluid status. Increased plasma Na concentration is indicative of severe net water loss. Since most of the K^+ in the body is located in the intracellular region, the plasma value of this electrolyte gives little insight into the body's deficiency. Despite the severe deficiency, the plasma value of K^+ can remain normal. On the other hand, hyperkalemia may develop with inhibition of normal renal excretion due to renal failure and obstruction, although intracellular potassium is normal or low (Gross,1988; Schaer, 1989; Chandler et al., 1999).

Acid - Base Balance

The organism has a certain pH imperative in metabolic events. The pH of the blood, which constitutes the most important buffer system of the organism, varies between 7.35-7.45. The organism must maintain this pH. The proportion of bicarbonate (HCO_3) in the blood to carbonic acid (H_2CO_3) is determined. In routine applications, the Carbonic acid - Bicarbonate buffer system is preferred in the laboratory diagnosis because it is the most important buffer system and the changes in this buffer system can be easily determined with dehydration, deviations often occur in the buffer systems of the organism (Michell, 1985).

Metabolic Acidosis

Metabolic acidosis is described by a primary increase in plasma H^+, decreased HCO_3^- concentration H^+, decreased pH, and a secondary, or adaptive, decrease in PCO_2. Metabolic acidosis was the most common acid-base disturbance in dogs and cats (Dibartola, 2000). The diagnosis and treatment of metabolic acidosis is most naturally possible by evaluating blood gas analysis findings (Sensior, 1983). The condition needs blood pH 7.1 that is taken into account in calculating the missing buffer ion amount in body. Sodium bicarbonate, lactate and Trispuffer are the best buffering agents in the correction of metabolic acidosis (Chew et al., 1991).

Treatment of metabolic asidosis

The buffer to be used can be calculated in two ways: In cases of metabolic acidosis requiring treatment, HCO_3-deficit and $NaHCO_3$ amounts to be used are calculated from the following formulas.

HCO_3-Gap (mmol) = 0.5 (0.3) × Body Weight (VA) × Base Gap (BE)

$NaHCO_3$ (mg) = 0.5 (0.3) × VA × BE x 84

*1. Correction of metabolic acidosis according to blood gas analysis results***:**

a) Sodium bicarbonate ($NaHCO_3$):

Formula: HCO_3 / Patient (mEq) = -Base Deficience (BD) x 0.3 x Body Weight (kg)

* 1 ml of 8.4 % $NaHCO_3$ solution 1 mmEq HCO_3 4.2% of $NaHCO_3$ solution of 4.2% 1 mEq HCO_3-*** 6 ml of 1.4% NaHCO3 solution contains 1 mEq HCO3.

If it is desired to calculate the amount of $NaHCO_3$ to be given, the following formulas are used. $NaHCO_3$ (mg) = -BD x 0.3 x Body weight (kg) x 84 $NaHCO_3$ (mg) = -BD x 0.3 x Body weight (kg) / 12

* 1gr NaHCO3 = 12 (11.9) mEqNa + 12 (11.9) mEq contains HCO_3.

Example 1: When blood gas analysis was done in a diarrhea dog weighing 30 kg, BD = -10. How many ml and how much $NaHCO_3$ or HCO_3, 8.4, 4.2 and 1.4% $NaHCO_3$ solutions are required to correct metabolic acidosis in this patient

1) How much HCO3 is required?

mEq HCO3 / Patient = 10 x0.3 x 30 = 90 mEq is required.

How many ml of $NaHCO_3$ Solutions of 8.4, 4.2 and 1.4% should be given?

To this patient

8.4% $NaHCO_3$ 90 ml of solution

4.2% $NaHCO_3$, 180 ml of solution

1.4% NaHCO, 540 ml of solution should be given.

3) How much grams of $NaHCO_3$ are required?

$NaHCO_3$ (mg) / Patient = 10 x 0.3 x 30 x 84 = 7560 mg = 7.56 g or NaHCO3 (gr) / Patient = 10 x 0.3 x 30/12 = 7.5 g are required.

If the plasma HCO_3 value is unknown (when blood gas analysis is not performed), the amount of $NaHCO_3$ to be given is calculated by evaluating the anamnesis data and clinical and other laboratory findings. In this way, the findings must be interpreted completely and accurately in the elimination of metabolic acidosis. Otherwise, excessive HCO_3 administration causes a metabolic alkalosis and correction of its consequences may be more harmful than the desired metabolic acidosis (Adams and Polzin,1989; Adams and Polzin,1990).

Metabolic Alkalosis

Metabolic alkalosis is characterized by an increase in serum bicarbonate (HCO_3) concentration or an increase in baseexcess (Ha et al., 2013), de-

creased H^+, increased pH, and a secondary or adaptive increase in PCO_2. Metabolic alkalosis was the third most common acid-base disturbance in dogs and cats. Metabolic alkalosis can be caused by loss of chloride rich fluid from the body via either the gastrointestinal tract or kidneys or by chronic administration of alkali (Muir, 1982). In the normal animal, renal excretion of exogenously administered alkali is very efficient, and it is difficult to create metabolic alkalosis by administration of alkali unless there is some factor preventing renal HCO_3 excretion (Ha et al., 2013). Most cases of metabolic alkalosis in small animal practice are caused either by vomiting of stomach contents or by administration of diuretics (Dibartola, 2000).

Table 5.: Causes of metabolic alkalosis (Muir, 1982; Dibartola, 2000; Ha et al., 2013; Tello and Perez-Freytes, 2017).

Chloride Responsive	**Chloride Resistant**	**Alkali Administration**	**Miscellaneous**
Vomiting of stomach contents Diuretic therapy Posthypercapnia	Primary hyperaldosteronism Hyperadrenocorticism	Oral administration of sodium bicarbonate or other organic anions (e.g., lactate, citrate, gluconate, acetate) Oral administration of cation exchange resin with nonabsorbable alkali (e.g., phosphorus binder	Refeeding after fasting High-dose penicillin Severe potassium or magnesium deficiency

Treatment of Metabolic Alkalosis

Metabolic alkalosis has been stated to be the most common acid–base disorder in several studies of hospitalized human patient (Ha et al., 2013). Diagnosis and definitive treatment of the responsible disease process are vital to the successful resolution of acid-base disorders. However, it must be remembered that alkalosis persists until chloride is replaced if vomiting of stomach contents or diuretic administration is responsible for the metabolic alkalosis. The goal of treatment in chloride responsive metabolic alkalosis is to replace the chloride deficit while providing sufficient potassium and sodium to replace existing deficits. Definitive treatment of the underlying disease process (e.g., removal of a gastric foreign body) prevents recurrence of the metabolic alkalosis (Dibartola, 2000). Patients with chronic pulmonary disease that have hypoxemia and hypercapnia are

at greater risk from metabolic alkalosis than others because super imposition of metabolic alkalosis can further reduce ventilation and lead to worsening of hypoxemia. Thus, metabolic alkalosis should be treated appropriately if present and avoided if not present. Giving oxygen to patients with metabolic alkalosis should also be avoided if possible because this may impair ventilation and further aggravate hypercapnia. Potassium without chloride (e.g., potassium phosphate) corrects neither the alkalosis nor the potassium deficit because administered potassium is excreted in the urine (Muir, 1982). A chloride salt must be given for alkalosis to be resolved and potassium retention to occur. Provision of chloride as either the sodium or potassium salt corrects chloride-responsive metabolic alkalosis. This therapy permits the kidneys to reabsorb the sodium the body needs with chloride to maintain electroneutrality. Thus, a NaCl solution (0.45% or 0.9%) with added KCl is the fluid of choice for dogs and cats with chloride (Dibartola, 2000)

Infusion Therapy

Fluid therapy plays an important role in veterinary medicine in the treatment of dehydration, and in providing volume support during anesthesia and hypovolemic states. Both crystalloids and synthetic colloids are widely used and there are a number of guidelines for fluid resuscitation (Adamik et al., 2015). Treatment of dehydration, which develops during or as a result of many diseases, is carried out with three steps:

I-Correcting the existing deficiency

a) Correction of the blood volume in the circulation,

b) Remedy of the remaining deficiency.

c) Regulation of existing fluid losses, electrolyte and acid-base balance disorders.

d) Meeting daily needs.

e) Covering fluid and electrolyte losses

Fluid Requirement (ml) = Deficiency = Body weight x% dehydration degree x 1000 + insensible losses (40-60 ml / kg / day) + extraordinary losses.

For these stages to be carried out effectively and rationally, the answers to the following questions must be known (Sensior 1983; Gross,1988; Schaer, 1989).

1-Which Solution (s) should be given

Crystalloids, colloids, blood products, hemoglobin-based oxygen carrier and total parenteral feeding solutions are used for fluid therapy in cats and dogs (Öcal and Ünsüren, 2009). Which solution or solutions to be

given in a patient requiring infusion treatment depends on the degree of volume deficiency and the quality of fluid loss. For example, the correction of intravascular volume as soon as possible in a patient in shock is the way to be followed in treatment. In the lack of volume due to blood loss, the best is blood transfusion (Clark, 1988). A colloidal solution should be used initially if there is no blood or in the absence of volume that does not result from blood loss. Isotonic electrolyte solutions can be used to increase plasma volume, but Na^{++} is distributed to the extracellular region and only 20-25% of the volume infused remains in the intravascular part (Sensior 1983; Schaer, 1989). For this reason, the isotonic electrolyte solution is given at the start of shock, until plasma or other colloidal solution is found. Depending on the cause and severity of the fluid loss, it is necessary to regulate the defect in electrolyte and acid-base balance other than eliminating the fluid deficiency (Sensior 1983; Cullen, 1991; Kozat, 2018).

Colloidal solution: Plasma expanders are used to reinstate the circulating volume of a hypovolaemic patient. Typically, colloids are used to expand the plasma volume, although combinations of hypertonic crystalloid and colloid have recently been used (McCahon and Hardman, 2007). These solutions contain macromolecules with a molecular weight greater than 25000 daltons (Yuruk et al., 2007). Due to their high molecular size, these solutions never leave the intravascular area or leave only after they are destroyed. Dextran 40 or Dextran 60, among the colloidal solutions described as plasma expanders are the most recommended (Clark, 1988). These solutions, by increasing oncotic pressure, absorb fluid from the intracellular and interstitial region to the intravascular region, and it is possible to increase the blood volume rapidly (Kozat and Voyvoda 2006; Öcal and Ünsüren, 2009). Due to their colloidosmotic properties, they bind water and hold it in the intravascular area when the oncotic pressure they create exceeds arterial hydrostatic pressure (Michell, 1985). The hyperoncotic solution (6% Dextran-60 solution) draws from the extracellular space to the intravascular space with the balance of oncotic pressure and water binding capacity, thereby increasing the plasma volume (Kozat, 2000; McCahon and Hardman, 2007, kozat, 2018). Release of inflammatory mediators secondary to hypovolemia is another important factor causative to microcirculatory dysfunction. The volume replacement strategy can moderate inflammatory activation, generation of reactive oxygen, and leukocyte adhesion to the microcirculatory endothelium (Boldt, 2006). Saline solution look like to be the most pro-inflammatory fluid, whereas certain colloids (especially when dissolved in a balanced solution) may be more beneficial in controlling the inflammatory process (Kellum et al., 2006; Boldt et al., 2009; Matharu et al., 2008).

Crystalloid solutions: A crystalloid fluid comprises of water and var-

ious forms of electrolytes (including salt) or sugar crystals (Table 6). Some crystalloid fluids also contain buffers (eg, acetate, gluconate, and lactate) that are metabolized to bicarbonate to increase serum pH (Mazzaferro and Powell, 2013). These solutions can be collected in two gaps, extracellular fluid regulators and maintenance solutions. Solutions used as extracellular fluid regulators are solutions that regulate the volume of extracellular fluid without changing its composition. To achieve this goal, Na^{++}, Cl^{-} and HCO_3 or precursors in solution must have plasma levels. Since the lack of volume is mostly caused by hyperkalaemia. The K^{+} concentration in the solution should not be higher than in plasma (Clark, 1988). In particular, solutions containing $HC0_3$ provide plasma pH regulation of the kidneys in the treatment of acidosis as well as by increasing volume-decreasing renal perfusion (Cullen, 1991). The saline (0.9 % NaCl), which is the ECF acidifier, improves acidosis by increasing the ECF voium and ensuring the renal regulation of plasma pH. Isotonic solutions have osmolality equivalent to that in serum (280-310 mosmol / L). Of these solutions, 0.9% NaCl, Ringer and Ringer lactate solutions can be counted. Isoionic solutions (Ringer's solutions) contain cation combinations similar to those in serum (Schaer, 1989).

Crystalloid-free solutions: For the addition of free water, a solution containing 5% isotonic glucose is mostly used. In this solution, after the glucose is metabolized, only water remains that do not have an osmotic effect in the fluid compartment. In vomiting, diarrhea and burns, a fluid suitable for the change in ECF composition is selected. It has been reported that 5% dextrose solution is not sufficient to eliminate the deficiency in electrolyte losses (Clark, 1988). The patient's daily energy requirement = (30 x Body weight (kg) +70) x disease factor (1.8) = kcal / 24 hours) glucose to the patient by choosing the solution that is sometimes used in the treatment of fluid and closing the fluid gap but needing total parenteral nutrition. Hypertonic total parenteral solutions consisting of amino acids and lipids should be applied (Öcal and Ünsüren, 2009).

Tablo 6: Composition of solutions used parenterally

Solüsyon	Electrolyte concentration							
Colloidal Solutions	**Na^{+}**	**K^{+}**	**Ca^{+}**	**Mg^{+}**	**Cl^{+}**	**Buffer (Emq/L)**	**pH**	**Osmolalite**
6% Dextran in 0.9% salt	154	-	-	-	154	-	4.5-7	300-303
Plasma	145	4.2	5	2.5	108	20	7.4	290
Crystalloid solutions								
Lactated Ringer's	130	4	3	-	109	Lactated 25	6.5	273
Ringer Solution	147	4	5	-	159	-	5.8	310

NaCl	154	-	-	-	154	-	5.4	308
Maintenance Solutions								
Dextrose %2.5 veya NaCl %0.4	77	-	-	-	77	-	4.8	280
Dextrose %2.5 / ½ Str. Lactated Ringer's	65	2	1	-	54	Lactated 14	5.0	263
Electrolyte free solutions								
Dextrose %5	-	-	-	-	-	-	5.0	252

2-At what speed should be given

The rate of development of fluid loss and the degree of clinical symptoms are important in determining the rate of delivery of the selected solution (Sensior,1983). Due to the limited uptake capacity of the therapeutically accessible intravascular region, the maximal infusion rate is limited by circulating capacity and distribution kinetics in the interstitial region. As the fluid or volume deficiency increases, the infusion rate of the fluid to be increased increases (Chandler et al., 1999). While large infusion is practically not possible in large animals, the risk of excessive infusion increases in small animals, especially in young people and in the presence of cardiovascular diseases. Hypertonic solutions should be given slower in isotonic solutions. In the event of sudden and severe blood loss and hypovolemic shock, correction of intravascular tissue perfusion is rapid initial intravenous application is required (Sensior,1983). In veterinary practice, 7.2-7.5 % hypertonic salt solutions for resuscitation are administered to dogs in 4-7 ml/kg and cats 2-7 ml/kg in 5-10 minutes. Taking into account the risks of bradycardia and hypotension of cats and dogs, it is reported that hypertonic salt solutions should not be given faster than 1 ml/kg per minute (Öcal and Ünsüren, 2009). If fluid application is performed slowly in the specified situations, insufficiency of tissue perfusion for a long period of time, exacerbation of hypovolemic shock and makes the patient susceptible to acute renal failure. It is reported that they are resistant to rapid fluid administration in dogs without cardiovascular disease, and they tolerate the administration of electrolyte solution at a rate of 90 ml / kg hour. However, cardiovascular and renal functions should be checked in rapid infusion. Symptoms of excessive infusion are distress, tachypnea, vomiting, cough, dyspnea, lung edema, serous runny nose, ascites, polyuria, diarrhea and aexophtalmus (Cornelius, 1980; Gross,1988; Chandler et al., 1999).

The first signs of excessive infusion that started were restlessness and tachypnoe in the patient with apathic status due to dehydration. It should be remembered that infusion should be stopped when vomiting develops,

cough due to pulmonary edema and dyspnoe will worsen the patient's condition (Michell et al., 1989; Cullen, 1991). Exophtalmus and ascites; It is reported that it is rarely seen as a sign of excess infusion, and cats are more susceptible to excessive infusion than dogs. Rapid infusion also stimulates diuresis. The maximal infusion rate recommended in cats is 40 - 50 ml/kg/hour. The cause of excessive infusion is the distribution of different compartments of the body. The infusion treatment first regulates the deficiency in the intravascular region. This is about 60 minutes it takes place inside. In the intravascular region, approximately 8 % of the body weight is contained with erythrocytes. While intravascularly applied electrolytes pass rapidly in the interstitial compartment, it takes time to correct the intracellular fluid loss. To maintain fluid balance in dehydrated cats and dogs, half the amount of solution to be applied should be given within six hours. Fluid balance should be maintained in a 24-hour period. It can be given to the dog at a rate of 90 ml/kg/hour., intravenouse fluid application should be done more slowly in patients with mild dehydrated. Fluid deficiency can be corrected over a period of 4-6 hours. Drip infusion 30-60 drops / min, depending on the size and body weight of the patient, 80 drops / min for very large dogs can be given up. In severely dehydrated patients, solutions should be taken at a higher drop rate at the start of infusion therapy (Veech, 1986; Muir, 1992).

3-The amount of fluid to be given

The amount of fluid to be give determining by evaluating the anamnesis data, physical examination and laboratory test results. The degree of dehydration from skin elasticity, in other words, fluid loss from the body can be detected high in cachectic, older, low in adipose and young animals. if patient's body weight, PCV, and TP values are known the before dehydration develop, volume deficiency can be calculated more objectively. 1 kg decrease in body weight is equivalent to 1 liter of fluid loss from the body (Sensior,1983; Schaer, 1989). Let's explain the volume of fluid required for fluid loss with an example.

Example 1: 6 month-old male entire Labrador retriever, body weight 20 kg, present with a history of 3 days of vomiting and diarrhoea that has been getting progressively worse. He is unvaccinated and a diagnosis of parvovirus is confirmed.

Physical examination

- Depressed
- Heart rate:170, cardiac auscultation is unremarkable
- Pulse quality weak/moderate

- Mucous membranes pale with a CRT of 2.5 seconds.
- Respiratory rate and effort are wthin normal limits as isauscultation of the lungs
- When raised, the skin over the back of the neck falls back more slowly than normal.
- Moderate (8%) dehydration

Initial plan

The patience requires fluids. The hyopperfusion should be addressed first as it is potentially life-threatening.plan to administer a fluid bolus of 50ml/kg isotonic replacement and crystalloid over 1 hour, The aim of normalizing of perfusion 50x20=1000ml/h for 1 hour.

Assessment 1 hour later

Need to calculate and sum for 24 hours.

1. Replacement of hydration fluid deficite: Dehydration (%)xBody weightx10:8x20x10:1600ml
2. Maintence fluid requirement: 50mlxkgxday: 50mlx20=1000ml/day
3. Ongoing losses: Diarhoe+vomiting

Estimated Diarhoe volume/episode: 100mlx 5 episode/day: 500 ml/day

Estimated Vomiting volume /episode:50mlx5/ day: 250ml/day

Total ongoing losses: 250+500= 750 ml/day

Daily fluid requirements:Replcament+maintence+losses ongoing: 1600+1000+750ml/day

3350 ml/day/ 24 hour:140ml/h

A. Correction of hypovolemia: In all fluid losses, hypovolemia should be corrected first. The lack of volume that develops without changing the ECF composition is the most common condition. The amount of fluid required to correct the blood volume in the circulation can be calculated from plasma loss. Plasma loss is 1/8 - 1/12 of total loss. It is ideal to deliver plasma or plasma equivalents to correct the circulation volume in shock (Veech, 1986; Schaer, 1989). This amount should be given as quickly as possible. One liter of infused electrolyte solution remains in only about 200 ml of intravascular region. Therefore, approximately 4 times the calculated plasma loss of Ringer's lactate or 0.9% NaCl solution should be given to increase blood volume. In this case, PCV drops below the critical value (PCV <30%). Pulmonary edema may develop with decreased mito-

chondrial diffusion and low oncotic pressure (Clark, 1988). It is reported that it is effective to give hypertonic NaCl solution 4 ml / kg in dogs with severe hemorrhagic shock. Hypertonic NaCl solution increases the Na^{++} concentration in the extracellular region, allowing water to pass from the intracellular region to the extracellular region. In this way, it is possible to increase the blood volume rapidly, but there is an improvement against the intracellular region. The disadvantage of this application is short. Serum Na is contraindicated in severe dehydration with increased concentration of Na and in cases where serum osmolality increases (diabetic ketoacidosis). The required amount of infusion, the amount required to meet (eliminate) the existing deficiency in the patient and meet the daily requirement can be determined from the degree of dehydration. Up to 80 - 120 ml / kg of fluid can be given in mild isotonic dehydration (> 5 %). The amount required to meet the daily need consists of normal fluid losses (respiration, urine, feces, sweat) and is directly proportional to metabolic activity, not body weight. Therefore, the larger the animal, the lesser the amount of fluid required. Mild to moderate electrolyte and acid-base disorders can be corrected by normal body compenzatoric mechanisms after correction of fluid volume (Schaer, 1989).

B. Compensating the current loss: Following the elimination of hypovolemia, the loss of the remaining fluid is provided by giving a slower rate. It is ideal to cover the total loss over 24 hours. The rest of the required amount is 1/2 in the first 6- 8 hours and the rest in 24 hours. Crucial acid-base balance disorders should be corrected early in the treatment. Metabolic acidosis is likely if the patient is known to be hypercaemic (plasma K^+ concentration > 6 mmol / L) or if the urinary bladder is ruptured from the anamnesis or detected from complete urinary obstruction. Dehydration and correction of metabolic acidosis K^+ free fluid and $NaHCO_3$ (1-2 mmol / kg HCO_3 is unknown) and regulatory functions must be regulated. More specific treatment is required if hyperkalaemia is severe (plasma concentration > 8 mmol L) and cardial arrhythmia. The 10 % calcium gluconate dose of 0.5 - 1.0 ml /kg and given intravenously intravenous and corrects the effect of potassium on the heart will be removed (Veech, 1986; Chandler et al., 1999).

Example 2: It was stated in the anamnesis that a dog weighing 30 kg at the age of 9 has no appetite for 4 days and vomiting 6 times a day since the last 2 days and there was no urination. In the clinical examination, it was determined that vomit contained bile, radiography found foreign body in the intestine, and Htc was 62 %. How much fluid should be given to the patient in the light of these data?

Calculating the amount of fluid to be delivered from the anamnesis

a) 4 days water loss = 20 ml / Body weight (Kg) / day formula = 20 x 30 x4 = 2400 ml

b) 2 days of urine loss (before Oligurie develops) = 20 ml / Body weight (Kg) / day = 20 x 30 x2 = 1200 ml

c) Loss from vomiting = 4ml / Body weight (Kg) / vomiting / day = 4 x 30 x 6x2 = 1440 ml

Total Fluid Loss = 2400 + 1200 + 1440 = 5040 ml

Extracellular fluid loss (1/3) = 1680 ml

Plasma loss (1/4 of extracellular fluid loss or 1/12 of total loss) = 420

Calculation of Fluid Loss from Hct:

Normal (desired) average Htc = 45%

Patient's Htc value = 62%

Increase (difference) 62-45 = 17%

Taking into consideration that 10 ml / kg fluid is required for each unit increase above 45% of Htc:

Total Fluid Requirement = 17 x 10 x 30 = 5100 ml.

The required amount of infusion can be found from the deficiency and meeting the daily need. The amount of fluid lost can be calculated in the degree of dehydration. Mild (up to 6% decrease in body weight; 60 ml in isotonic dehydration, Medium up to 8% decrease in body weight; 80 ml / kg in degree dehydration and Severe 8% from in body weight; 80-120 ml / kg in severe dehydration (Cullen, 1991, Gross,1988).

4-Route of administration: The way in which fluid electrolytes are administered is determined by considering some factors. In cases where rapid volume increase is required intravenouse application is recommended. It can also be used intraperitoneally or subcutaneously (Kozat and Voyvoda 2006; **Öcal** and **Ünsüren**, 2009), but absorption from the subcutaneous tissue may not be sufficient or severe in severe dehydration. For oral fluid administration, the gastro intestinal system must be functional (Schaer, 1989). Meeting daily needs, daily normal fluid need is between 40-60 ml / kg (**Öcal** and **Ünsüren**, 2009), and it can increase up to 2 times the specified amount due to urinary excretion in disease states. This amount of fluid should be given daily until the patient can freely drink. For this purpose, hypotonic solution is used. In the course or result of many diseases in cats and dogs, the extracellular fluid volume electrolyte composition and the solutions to be applied in the disorders of the acid-base state are known as summarized as follows (Cornelius, 1980). The amount

of subsequent abnormal losses can be estimated from clinical findings and added to the daily need. While pleural, peritoneal and effusion losses can be measured exactly, vomiting is 1-4 ml/vomiting. In diarrhea, fluid loss up to 200 ml/kg one day and the amount of abnormal losses following can be estimated from clinical findings and given by adding to daily need. The pleural, peritoneal and effusion-induced losses can be measured precisely, vomiting up to 1-4 ml/kg/vomiting and diarrhea up to 200 ml / kg / day can be estimated (Schaer, 1989, Alastair, 1988; Veech, 1986).

Table 7.: Solutions applied in different disorders

Disorder	Solution(s) to be given when fluid is required
Severe vomiting	Ringer's solution or 0.9% NaCl+KCI solution
Diarrhea	Ringer Lactate solution, KCl solution
Severe and prolonged bowel obstruction	Colloidal Solution Ringer's lactate + HCO3
Urethra obstruction or urinary bladder rupture	Ringer Solusyon veya 0.9 NaCl+%5 Dekstroz
In severe bleeding	Colloidal solution or Ringer's lactate
Peritonıtis, Pancreatitis	Colloidal Sol. -Ringer laktat HCO_3 2-3 mmol/kg;
In severe liver diseases	Dextrose solution, KCl solution
Anorexia	3 days 10 days 5% Dexstrose + amino acid mixture follow-up to meet abnormal losses

Monitoring fluid therapy

A fluid therapy plan includes the type, quantity, and rate of fluid to be administered (Öcal and Ünsüren, 2009). The primary goal is to give the least amount of fluids possible to reach the desired end-points of resuscitation. Fluid resuscitation and maintenance helps restore perfusion and hydration while preventing volume overload. Diligent patient monitoring is required in reaching and maintaining these endpoints (Lichtenberger, 2004; Öcal and Ünsüren, 2009).

Body weight

Acute changes in body weight largely do not represent changes in the body's fluid content. Fluid therapy improves the patient's body weight and restores dehydration (Kozat, 2000).

Central venous pressure

Central venous pressure (CVP) has stood a main tool in assisting clini-

cians with fluid therapy during resuscitation efforts for decades (Hutchinson and Shaw, 2016). CVP measurements should theoretically be reflective of a patient's intravascular volume status (Boag and Hughes, 2005) and CVP could be thought of as a reliable guide to volume therapy (Dellinger et al., 2013). Central venous pressure (CVP) is a measure of the hydrostatic pressure in the central venous compartment and therefore provides the most accurate assessment of vascular filling (Hutchinson and Shaw, 2016). Typically, the tip is measured with a catheter placed percutaneously into the jugular vein in the cranial vena cava. Catheters inserted into the caudal vena cava through saphenous or femoral vessels can also be used, but tend to be less predictable and less accurate readings (Gookin and Atkins, 1999). Normal CVP is 5-10 cmH_2O and A low CVP means insufficient, a high CVP refers to intravascular volume overload, right-sided cardiac dysfunction, or increased intrathoracic pressure (eg pleural effusion) (Byers, 2017). The change in CVP following a fluid bolus can be a useful guide for the need for increased fluid loading, especially in patients with volume overload, for example in patients with possible anuric / oligural renal failure. If CVP is low, it increases after a fluid bolus, but then quickly returns to pre-bolus levels, more fluid therapy is required. On the contrary, if it rises and stays high, it means that the vascular volume is sufficient and hypovolemia is not the cause of poor urine output (Hutchinson and Shaw, 2016).

Urine output

It represents the filtration rate (GFR) representing the glomerular balance and the balance to the tubular fluid reabsorption. The urine output of the dehydrated animal should be increased to the range of 0.5-2.0ml / kg / h. This amount is obtained by providing the patient with adequate fluid therapy (Öcal and Ünsüren, 2009).

Plasma lactate level

The value of blood lactate measurement as a prognostic indicator for survival has been studied in several animal species, including dogs. The clinical value of blood lactate measurement in predicting prognosis and outcome, determining tissue perfusion and evaluating treatment response in human intensive care units has been well accepted (Stevenson et al., 2007). Both the initial blood lactate concentrations and the duration of hyperlactatemia can be used to predict survival or death in human trauma patients (Manikis et al., 1995). In septic human patients, serum lactate levels have been closely related to severity of illness and metabolic acidosis (Hagman et al., 2009). In cat and dog medicine practice, determination of blood lactate lev-

el is used in evaluation of hypovolemia and prognosis and diagnosis in some disease groups (Başer et al., 2016). While the average lactate value in dogs varies between 0.3-2.5 mmol / L (Hughes 2000), it increases up to 5.33 mmol / L in cats (Tynan et al., 2015).

Termination of fluid treatment

As a result of fluid treatment applied to patients with dehydration; general condition is good, blood pressure is normal (Systolic> 80-90 mm Hg), serum lactate concentration is normal (<2.5 mmol / L), central venous oxygen saturation is higher than 70 %, HCT is higher than 25%, urine excretion is 1 ml Intravenous fluid in patients who have started taking orally all the fluid requirement and 2/3 of their daily energy need, more than/kg/h, pulsoximeter value above 93%, central venous pressure (CVP) at 5-10 cm H_2O level (Byers, 2017) application is terminated (Öcal and Ünsüren, 2009).

In conclusion

Approximately 60-70% of the body weight of cats and dogs constitute body fluids. Many diseases in cats and dogs cause disorders in body fluid balance. All metabolic events in the body occur within narrow pH limits. When there are deviations from these limits, significant changes occur in enzyme activities, electrolyte balance, pharmacology of organ systems, primarily respiratory, cardiac and central nervous systems, and drugs. Decreasing fluid volume and type of dehydration should be determined to ensure effective resuscitation in fluid therapy. In this context, the composition of the solutions used in fluid treatment is important according to the metabolic disorders that occur. In addition, the importance of the applied solution in resuscitation and monitoring of fluid therapy form the basis of effective fluid therapy.

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Chapter 25

PLACENTAL ADIPOCYTOKINES AND THEIR ROLES IN PREGNANCY

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INTRODUCTION

The placenta is a non-embryonic tissue where all types of substance exchange take place between the mother and the fetus. The placenta is formed by combination of fetal and maternal parts. The part belonging to the mother is known as placenta maternalis, while the part of the fetus is placenta fetalis (Hassa & Asti, 1997). The placenta has several life-related functions. In the early pregnancy period, the nutrition and energy required for the embryo and fetus are obtained from the glycogen, cholesterol and fatty acids synthesized by the placenta. Transfer of the oxygen, carbon dioxide and carbon monoxide gasses is also performed via the placenta. Other than these, various nutritional substances, electrolytes, drugs and drug metabolites, various infection factors, some waste products and hormones also pass through the placenta. The placenta also has the function of hormone secretion (Moore, Persaud & Torchıa, 2016).

Adipose tissue serves as storage for triglycerides and a source of free fatty acids, but it is also considered as a significant part of the energy metabolism with the several enzymes, cytokines, growth factors and hormones it secretes (Lau et al., 2005). It was reported that adipose tissue is an endocrine organ due to the secretions of mature adipocytes in its structure and plays a role in several metabolic reactions by synthesizing some mediators (Ferroni, Basili & Falco, 2004). Adipocytokines are hormones synthesized by adipose tissue. Adipocytokines were discovered in the nineties by identification of the first member of the family, 'leptin'. As adipose tissue is considered as the energy storage of the body or a mechanical protector, studies have been mostly on brown adipose tissue (Rıcquıer, 2005). On the other hand, after the discovery of leptin towards the end of 1994 by Friedman et al. (Zhang, Proenca & Friedman, 1994), studies on white adipose tissue gained significance, and until now 20 known adipocytokines have been identified. Adipocytokines are categorized under 3 different groups:

1. Hormones secreted in other tissues in addition to adipose tissue (like TNF-α).

2. Hormones mainly produced by adipocytes in white adipose tissue but can also be produced in other cells in adipose tissue (hormones like resistin that are synthesized from cells with immune capacity).

3. Hormones that are only produced by adipocytes in white adipose tissue (like leptin and adiponectin).

Besides these, there is also another physiological classification related to adipocytokines. According to this classification, adipocytokines may be divided into two groups: the first one is "insulin resistance-inducing factors" like resistin, TNF-α and interleukin 6, while the second one is "insu-

lin-sensitive factors" like leptin, adiponectin and visfatin (Fukuhara et al., 2005). Adipocytokines show their main effects on the immune system, adipocyte metabolism and vascular functions. For this reason, adipocytokines have significant roles in many diseases such as obesity, insulin resistance, metabolic syndrome, hypertension and dyslipidemia (Weiss et al., 2004). This review presents information on the effect mechanisms of adipocytokines produced in the placenta in pregnancy and their relationship to pregnancy complications.

SOME ADIPOCYTOKINES SYNTHESIZED FROM THE PLACENTA AND THEIR FUNCTIONS

1. LEPTIN

Leptin is a hormone that was discovered in 1994 during studies on the ob gene (Kaminski et al., 2006). Leptin is produced more by white adipocytes and has a molecular weight of 16 kDa. The transferring leptin concentration is usually proportional to the total adipose tissue mass, that is, while it increases in obese individuals, it decreases in underweight individuals (Rohner & Jeanrenaud, 1996). Leptin is a protein with 167 amino acids from the cytokine family, and it is coded by the ob gene found at the position of 7q31.3 (Geffroy et al., 1995). Leptin achieves appetite control in the region known as the "gut-brain axis" and releases a signal of fullness by affecting the CNS receptors in the hypothalamus (Konturek et al., 2004). It was reported that there was no fullness signal in the gut-brain axes of mice with mutated ob genes (ob/ob mice), and for this reason, they showed severe obesity symptoms (Pelleymounter et al., 1995). It is known that, in addition to adipose tissue, leptin is also synthesized in humans in the placenta, fetus, brain, heart, lungs, stomach, pancreas, spleen, small intestines, colon, skeletal muscles, kidneys and testes (Alemzahed & Lifshitz, 2003). Long- and short-form leptin receptors were also shown in the placenta. It is thought that leptin may have effects on placental functions through the autocrine and paracrine routes (Lea et al., 2000; Bodner et al., 1999). Studies have stated that leptin receptor expression is different in normal and diabetic pregnant women. In the placenta of women with Gestational Diabetes Mellitus (GDM), it was determined that, as opposed to transmembrane receptors, the expression of the soluble receptor isoform increased (Challier et al., 2003). It was reported that the plasma leptin levels in pregnant women were higher than those at the same age but not pregnant (Kautzky-Willer et al., 2001; Lewandowski et al., 1999). It was stated that this increase in leptin levels had a correlation with changes in leptin-binding proteins (Lewandowski et al., 1999). It is known that the maternal leptin levels increase 2-3 times in pregnancy, and they reach their peak at the 28th week of pregnancy (Schubring et al., 1998). Several stud-

ies reported that serum leptin concentrations increase during pregnancy (Henson & Castracane, 2000; Sagawa et al., 2002; Bajoria et al., 2002). It was stated that leptin secreted from adipose tissue in pregnant women showed a strong correlation with the mother's body weight and BMI (Body Mass Index) (Cortelazzi et al., 2007). Increased leptin levels in pregnant women were associated with changes in maternal fat storage and the glucose metabolism (Schubring et al., 1998).

In a study where the sex-specific effects of leptin application on the placenta and the metabolic phenotypes of offspring, male and female offspring born out of mothers treated with leptin firstly showed growth retardation, and after they left nursing, they showed a wave of growth. The body weights of male offspring fed with a standard diet were observed to increase. It was stated that leptin application prevented development of hyperglycemia in obese offspring of both sexes, and the placentas of male and female fetuses were different in terms of size and insulin-like growth factor expression. It was reported that leptin injection reduced both sexes' fetal weight, male fetuses' placental weight and the placental gene expression of the GLUT1 glucose transporter in female fetuses (Denisova et al., 2020). When the relationship between age of pregnancy and leptin levels was investigated, placental, cord blood and maternal serum leptin levels were checked, and it was reported that maternal leptin concentration was positively correlated with maternal weight before and after pregnancy in all groups. The cord leptin levels were lower in the group with younger ages of pregnancy and higher in the group with older ages. The placental leptin levels were higher in the group with younger ages of pregnancy in comparison to other groups. It was stated that placental leptin levels showed an inverse correlation with placental weight independently of maternal weight and age of pregnancy. It was reported that placental leptin expression may affect placental growth, and therefore, birth weight (Lazo-de-la-Vega-Monroy et al., 2017). Among Gestational DM and healthy pregnancies, it was reported that leptin and insulin receptors were activated in the placentas of pregnant women with GDM. It was stated that stimulation of trophoblasts by low-dose leptin and insulin in the placentas of GDM pregnant women strengthened signal activation, but stimulation of high-dose leptin and insulin affected signal activation negatively (Pérez-Pérez et al., 2016). Similarly, it was reported that leptin expression significantly increased in placental endothelial cells in women with preeclampsia (Park et al., 2018).

2. ADIPONECTIN

Adiponectin is a hormone with anti-inflammatory and antiatherosclerosis properties found abundantly in circulation which is secreted by adipocytes and accepted to increase insulin sensitivity (Brochu-Gaudreau

et al., 2010). The gene that codes adiponectin is found on the 3rd chromosome, and the gene locus where it is found is at a close region to the gene locus held responsible for type 2 diabetes and body adiposity. These are adipoR1 and adipoR2 (Yamauchi et al., 2003). It was stated that, in a normal pregnancy, the maternal adiponectin concentrations in circulation increase in the first half of pregnancy, and they decrease layer in proportion to weight gain and reduction of physiological insulin resistance (Ebihara et al., 2001). It was stated that newborn adiponectin levels are higher than maternal circulation levels during pregnancy (Brochu-Gaudreau et al., 2010). When maternal serum adiponectin levels were examined, no significant change was observed in adiponectin levels despite increasing insulin resistance during pregnancy. It was stated this situation may mean that regulation of adiponectin changes during pregnancy. It was reported that high gestational adiponectin levels are associated with increased "adiponectin resistance" in pregnancy (Mazaki-Tovi et al., 2007).

It was reported that, in patients that were estimated to develop preeclampsia, the first three-month serum adiponectin concentrations were low, while they were high after delivery (D'Anna et al., 2006). It was stated that women with severe preeclampsia have higher median plasma adiponectin concentrations than normal pregnant women (Nien et al., 2007). It was observed that, in pregnancy, maternal adiponectin secretion continuously decreased. It was reported that both plasma adiponectin concentrations and mRNA expression were negatively correlated with adipose mass, and this situation was associated with signals for limiting adiponectin production as a result of adipose tissue accumulation (Catalano et al., 2006).

3. OMENTIN

Omentin, also known as intelectin, was isolated for the first time from intestinal Paneth cells. It was later reported that it is expressed in the heart, lungs, ovaries, adipose tissue and placenta (Shuldiner et al., 2006). It was also stated that omentin is expressed more in visceral adipose tissue in comparison to subcutaneous adipose tissue (Schaffler et al., 2005). Omentin contains 313 amino acids and has a hydrophilic structure. It contains a secretory signal sequence and a fibrinogen-related domain. The negative form of omentin appears as a glycolyzed trimer with a molecular weight of 120 kDa (Fain et al., 2008; Yang et al., 2006). Omentin has two highly homologous isoforms. These are omentin-1 and omentin-2. However, it was reported that omentin-1 is its form that is found in major circulation in human plasma (De Souza Batista et al., 2007). It was determined that omentin levels are high in the umbilical cord blood. It was reported that insulin is required for glucose to be taken into cells in adipose and muscle tissues, and omentin shows an effect that increases insulin sensitivity. For this reason, based on the high levels of omentin in cord blood, it was stated

that omentin may have a stimulating effect on fetal growth (Briana et al., 2011). It was found that previously existing maternal obesity is associated with lower omentin-1 expression in the placenta, adipose tissue and maternal plasma. It was stated that a change in omentin-1 levels in pregnancy may affect development of metabolic disorders in the offspring in the future (Barker et al., 2012). The omentin-1 levels of healthy and diabetic mothers were monitored throughout their pregnancy, and it was reported that there was no significant difference between the groups. Moreover, it was stated that omentin-1 levels were significantly lower in the babies of diabetic mothers, and this situation may pose a risk for insulin resistance development in further life (Franz et al., 2018).

4. RESISTIN

Resistin is a newly identified adipocytokine rich in the substance known as cysteine, and it has a molecular weight of 12.5 kDa. It was discovered during adipose tissue culture studies (Haugen et al., 2001). Another name of resistin is known as "found in inflammatory zone (FIZZ3)". It was reported that it is found in the 19th chromosome in humans (Steppan et al., 2001). Resistin is a member of the protein family known as "resistin-like molecules" (RELM) (Fonseca-Alaniz et al., 2007). It was reported that resistin is expressed mainly in white adipose tissue depending on the adipose tissue among and adipocyte differentiation, and the gene expression of resistin in white adipose tissue is higher than that in brown adipose tissue (Steppan et al., 2001). However, it was stated that resistin is not specific to adipose tissue in humans, and its main source is macrophages (Kusminski, Mcternan & Kumar, 2005). It was reported that, outside adipocytes, resistin is synthesized from pancreas islet cells, mononuclear cells and also the placenta (Kusminski et al., 2005; Minn et al., 2003; Kaser et al., 2003; Haugen et al., 2006). When pregnant women were compared to non-pregnant women, it was shown that their plasma resistin concentrations were higher (Nien et al., 2007; Palik et al., 2007). Furthermore, resistin expression was reported to be higher in the full-term placenta than the first trimester (Lappas et al., 2005). However, another study reported that serum resistin levels showed a significant difference between pregnant and non-pregnant women only in the third trimester (Chen et al., 2005). Plasma resistin levels were examined based on the week of pregnancy, and it was stated that the plasma resistin levels of women in the last week of their pregnancy (full-term) were higher than those in their 1st, 2nd and early 3rd trimesters (Nien et al., 2007). It was determined that plasma resistin levels did not show a significant difference between normally weighted and obese pregnant women with different ages of pregnancy (Nien et al., 2007; Hendler et al., 2005). No difference was found between healthy and diabetic pregnant women in terms of resistin secretion from their adipose

tissues, but it was reported that resistin secretion was higher in the adipose tissues of both groups than their skeletal muscles (Lappas et al., 2005). It was stated that the serum resistin levels of pregnant women with GDM are associated with insulin resistance (Palik et al., 2007).

5. VISFATIN

Visfatin, which is a member of the adipocytokine family, was discovered for the first time in human peripheral blood lymphocytes in DNA studies conducted in 1994 (Samal et al., 1994). The visfatin gene was initially named as the 'pre-B-cell enhancing factor 1 (PBEF1)'. It was reported that the visfatin gene is found on the long arm of the 7th chromosome, its molecular weight is 52 kDa, and it is coded as a polypeptide containing 491 amino acids (Jia et al., 2004). However, in later studies, it was determined that it is secreted from adipose tissue, and a new adipokine known as visfatin was defined. While a strong correlation was determined between plasma visfatin levels and visceral adipose tissue quantity, a weak correlation was reported between subcutaneous adipose tissue and visfatin levels (Fukuhara et al., 2005). It was reported that visfatin is synthesized not only in adipose tissue but also in amniotic epithelial cells, mesenchymal cells and chorionic cytotrophoblasts (Ognjanovic & Bryant-Greenwood, 2002). It was stated that TNF-α stimulates visfatin expression in human placenta cells (Ognjanovic et al., 2001). Plasma visfatin levels were compared in women with GDM in their pregnancy and health pregnant women, and as a result, the plasma visfatin levels in women with GDM were found to be 1.4 times higher than the control group. On the other hand, it was stated that plasma visfatin levels were not related to fasting plasma glucose, fasting plasma insulin, BMI or age (Krzyzanowska et al., 2006). Another study found serum visfatin levels to be higher in pregnant women with GDM than the control group, but a positive correlation was reported between fasting plasma insulin and visfatin levels (Lewandowski et al., 2007). In a study that obtained opposite results, it was determined that the serum visfatin levels in pregnant women with GDM were 25% lower in comparison to the control group. Additionally, a negative correlation was observed between the mother's age and serum visfatin levels and BMI I the 1st trimester. However, while there was an independent relationship between plasma visfatin levels and the mother's age, no correlation was found between the week of pregnancy and visfatin levels (Chan et al., 2006).

CONCLUSION

Consequently, the placenta is a tissue that facilitates all hormonal and metabolic communication between the mother and the baby. While it achieves transfer of several hormones produced in the mother to the baby, it also shows an effect on the mother and the baby through the paracrine

and autocrine routes by producing some hormones itself. It is known that adipocytokines are synthesized mainly in adipose tissue and also in the placenta. Adipocytokines synthesized from the placenta may progress on different levels throughout pregnancy. Age of pregnancy, week of pregnancy or the sex of the fetus may change the levels of some adipocytokines. Excess or insufficient production of adipocytokines synthesized from the placenta may be effective on placental growth, birth weight and several pregnancy complications such as insulin resistance, gestational diabetes and preeclampsia. For this reason, for the pregnancy to continue in a healthy way, the health of the mother and the newborn to be protected and potential pregnancy complications to be prevented in the early period, it is highly important to monitor the levels of these adipocytokines.

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Chapter 26

LAPAROSCOPIC SLEEVE GASTRECTOMY; PRINCIPLES OF SURGICAL TECHNIQUE AND PERIOPERATIVE MANAGEMENT

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Introduction

The vertical sleeve gastrectomy (SG) is a restrictive bariatric operation that was originally described in 1988 as the initial step for supermorbid obese patients to bridge them to a more complex operation (biliopancreatic diversion-duodenal switch procedure (BPD with DS) (1,2). Because of the high morbidity and mortality rate after BPD with DS procedure, Regan and Gagner described the two –stage surgical approach. In this tecnique, initially the supermorbid obese patients underwent SG over a 60F bougie, after 6 to 12 months post surgery the patients underwent a second stage BPD with DS or gastric bypass (3). The aim of the restrictive SG was to initiate surgical weight loss, thereby to reduce the surgical risk of subsequent revision bariatric procedures such as BPD with DS or Roux-en-Y gastric by pass. The majority of patients whom underwent SG as a bridge operation lost their excess weights as well as improved their comorbidities so that the secondary bariatric procedures were no longer required or refused by the patient.

In 1999, the first laparoscopic sleeve gastrectomy (LSG) was reported and Gagner et al published the first report of LSG as a standalone bariatric operation (4). In the last two decades, several studies revelaed that LSG was comparable in short-term and long-term weight loss and also resolution of comorbidites with other laparoscopic bariatric procedures such as laparoscopic adjustable gastric banding (LAGB) and/or bypass procedures (4, 5).

Recently, LSG is the most commonly performed bariatric procedure worldwide among both surgeons and patients. The relative technical ease, a low complication rate, significant remission of comorbidities, and excellent weight loss outcomes has increased the popularity of this operation over other bariatric procedures (6, 7). This chapter aimed to review the patient selection criteria for LSG, surgical technique and tips, perioperative care and a short review of complications after sleeve gastrectomy.

Patient selection and Preoperative work-up

The National Institute of Health (NIH) and the American Society for Metabolic and Bariatric Surgery (ASMBS) stated the criteria for bariatric surgery (5):

(1) Age 18–64 years.

(2) Body mass index (BMI) $\geq$40 kg/m^2.

(3) BMI $\geq$35 kg/m^2 and at least one obesity-related medical comorbidity, such as type-II diabetes mellitus, hypertension, obstructive sleep apnea (OSA), nonalcoholic fatty liver disease, osteoarthritis, lipid abnormalities, gastrointestinal disorders, or heart disease.

(4) Inability to achieve a healthy weight loss sustained for a period of time with prior weight-loss efforts.

The preoperative evaluation and work-up for bariatric surgery should begin several months prior to the procedure for all patients. The majority of patients who undergo bariatric surgery have multiple obesity-related comorbidities. All obese patients require cardiopulmonary work-up and further examinations including psychological, nutritional, and sleep study evaluation (8).

A multidisciplinary team including specialized bariatric surgeon, bariatric nurse, dieticians, nutritionists, and exercise physiologists give preoperative education to all patients. It has been reported that moderate weight loss on a low-calorie diet before bariatric procedures improves visualization during surgery by decreasing intra-abdominal adipose tissue and decreasing liver volume (9). Several bariatric centers give a liquid low-calorie diet prior to surgery for high risk patients to enhance preoperative weight loss (10).

After routine history and physical and surgical risk assessment, standard laboratory tests including complete blood cell count, biochemical panel, liver and thyroid functions, and evaluation for any vitamin deficiencies should be done for all candidates. Routine preoperative chest radiograph is performed on all patients. The patients with sleep apnea receive a sleep study and work-up for pulmonary function tests and are optimized accordingly with bronchodilators and/or positive pressure airway devices. In their study, the authors revealed that smoking cessation prior to bariatric surgery decreased the postoperatively complication rate associated with smoking (11).

Esophagogastroduodenoscopy (EGD) is necessary for all patients before all bariatric surgeries. Any gastric and/or esophageal pathology such as Barrett esophagitis, hiatal hernias found in EGD may change the surgical option in some patients.

Principles Of Surgical Technique

Although there are several surgical techniques regarding LSG procedure; the essentials of LSG surgery technique is widely accepted among bariatric surgeons worldwide. The main principles include:

1) Complete mobilization of the greater curvature of the stomach extending proximally to the angle of His.

2) Visualation of the left crus and base of the right crus.

3) Pyloric preservation with gastrectomy beginning 2 cm to 6 cm proximal to the pylorus.

4) Avoidance of stricture at the gastric incisura.

5) Avoidance a large retained fundic pouch.

In this paper, the author describes the LSG technique in his surgical practice. Prophylactic antibiotics (cefazol® 2g) are given to all patients within 30 mins before skin incision. A prophylactic dose of subcutaneous heparin is also administered preoperatively. Patients are placed in supine position with sequential compression devices as well as pressure points well padded. A urinary catheter is placed after induction of general anesthesia. A Veress needle is inserted at Palmer point in the left upper quadrant to achieve pneumoperitoneum at a pressure of 15 mm Hg. Placement of ports are described in the following position: a 10-mm periumbilical port located approximately 15 cm to 17 cm from the xiphoid process for laparoscopic camera, placement of a 5-mm port in the left upper quadrant and a 15-mm port placed in the right upper quadrant. Finally, a liver retractor is then placed through the subxiphoid 5 mm port (Fig 1). After all ports are placed, the entire abdomen is inspected for any anatomic abnormalities or iatrogenic injury. The stomach is deflated via an orogastric tube. After deflation of the stomach, the orogastric tube is removed.

Before the dissection begins the patient is placed approximately 45 degrees of reverse Trendelenburg position to facilitate better exposure. Initially, the pylorus is identified and the entire greater curvature is mobilized proximal to the pylorus with an vessel sealing device. The greater curvature is mobilized to the angle of His with freeing any attachments to the transverse colon and its mesentery. All peripancreatic attachments to the posterior stomach should be taken down. Identifying and preserving the left gastric artery is critical. As short gastric arteries have high risk for perioperative bleeding, the surgeon should be very careful when dissecting and sealing these vessels. Splenic artery anomalies may be found during surgery so it is also important to take care while identiying the splenic artery.

When the left crus is identified exactly and visualized where it meets the base of right crus, it can be said that dissection of the greater curvature is complete (Fig 2). In case of any hiatal hernias found during dissection those should be repaired, a posterior repair is suggested (12). If a large hiatal hernia is diagnosed, bioabsorbable mesh is used to reinforce repair.

Once dissection is complete, a 36-French bougie tube is placed through the stomach to the level of the pylorus before stapling. The size of the bougie tube is prefarence of the author's institution. Although it is controversial regarding the size of the bougie tube to create gastric sleeve, the majority of surgeons do not prefer to use bougie tube smaller than 32- French. It has been well known that the use of a small-caliber bougie tube increases the post LSG complications such as stricture mostly at the gastric incisura (13).

In many studies there is a consensus regarding the first staple load to start between 2 cm and 6 cm proximal to the pylorus (14, 15). In our experience, creation of the gastric sleeve begins 3 cm proximal to the pylorus. First stapling should be done with the largest staple height because of thicker tissue of antrum. In our practice, we use the Medtronic EndoGIA stapler black load for the first staple firing at the antrum which uses Tri-Staple technology with successively longer staple heights (4.0, 4.5, and 5.0 mm). Of note, during first stapling surgeon should take care to avoid stricture at incisura angularis (Fig 3). Subsequent staplings change based on the thickness of the gastric tissue which may vary between patients. We usually use medium height purple staple loads for the remainder of gastric sleeve. Another critical key point during stapling is lateral traction to oppose the anterior and posterior aspects of the stomach. By this way, corkscrewing effect of the gastric sleeve is prevented and also the risk of leaving a large posterior fundus is minimalized. During stapling, inspecting the posterior aspect of the stomach is required because of the possible injuries regarding with pancreas or big vessels such as the left gastric and splenic arteries. Finally, the surgeon should avoid stapling too close to the gastroesophageal junction (GEJ). As it is well known, the GEJ area has relatively weak regarding vascularity so that stapling less than 1 cm close to GEJ may lead to ischemia which is the most common cause of gastric sleeve leaks (Fig 4).

Some authors prefer to reinforce the staple line by several methods such as a running inverting Lembert suture, simple oversewing sutures of the staple line and/or applying fibrin glue. The aim of these reinforcement methods is to prevent the most common complications including bleeding and leakage. However, the outcomes regarding reinforcement methods in LSG procedure is controversial (15,16). We do not perform any additional reinforcement. As the stapler technology advances, the need for reinforcement methods might not be required in the future.

There are various leak tests such as intraoperative endoscopy, insufflation with air or methylene blue used by surgeons. One of the most common leak test is air- water test. In this method, the surgical field is filled with irrigant mostly distilled water and then the remaining stomach is insufflated. Resulting air bubbles shows the presence of a leak and should be addressed promptly. Another mostly preferred leak test is Methylene blue via the orogastric way which shows spillage of blue contents into the peritoneal cavity. The authors' preference is to make leak test case by case. The ASMBS has stated that leak tests should be performed at the discretion of the surgeon, and has not recommended it as a routine practice (17).

There is no consensus addressing fixation of the sleeve to surrounding omental or retroperitoneal tissues. A few studies focusing on this topic revealed that the results regarding postoperative vomiting, better gastric

emptying, and decreased staple line bleeding or leak are controversial (18, 19). We affix the mobilized gastrocolic omentum to the lateral side of the sleeve with two to four interrupted nonabsorbable sutures in case of there is any concern for the potential for torsion or volvulus,

The resected part of the stomach which is approximately 70%–80%, removed through the 15-mm port site. If there is low risk regarding bleeding or leak, the surgical drains are unnecessary. Drains may be more beneficial when performing revisional surgery, which expectedly has a higher risk of complications. Finally, the 15-mm site is closed laparoscopically using a suture passing device under direct visualization. The liver retractor and all remaining ports are then removed and pneumoperitoneum is released.

See Video 1 showing the key points of LSG.

Early Postoperative Care after LSG

Immediate postoperative care of patients whom underwent LSG depends on the several factors such as demographics of the patients and the course of the surgical operation. In this paper, the authors clinical protocol for postoperative care of LSG is described. In general, the patients are transferred to the floor after surgery and continuous monitoring for vital signs is done in the selected cases. In our clinical practice, adequate intravenous fluids are administered and urine output is closely monitored. Postoperative pain management is essential in patients following LSG. Fort this aim, our prefarance is to use patient-controlled intravenous narcotics continued on postoperative day 1 and thereafter order oral analgesic medication.

Patients are initially kept nothing by mouth until they are awake after surgery. Sips of water and/or a low-sugar phase 1 bariatric diet consisting of clear noncarbonated liquids are initiated on postoperative day 1. After postoperative day 1, the patients are ordered oral intake of 2 L liquids daily. Nausea is one of the most common postoperative complaint of patients after LSG. It has been argued that retching and vomiting may lead stress on the staple line, therefore antiemetics such as metoclopramide should be ordered in the immediate postoperative period (20).

Anesthesia, inadequate use of pain killers, and sleepiness can cause postoperative atelectasis in the LSG patient which can lead to pneumonic processes and hypoxemia. Early aggressive pulmonary toilet, mobilization, and lung expansion maneuvers should be urged to prevent postoperative pulmonary complications (21). Deep breathing and incentive spirometry devices are encouraged for patients in the immediate postoperative period. Obstructive sleep apnea is (OSA) a common comorbidity in the obese

patients. The majority of these patients use noninvasive positive pressure devices for varying severities of obstructive sleep apnea prior to surgery. The patients whom suffer from OSA should be monitored after LSG with continuous pulse oximetry and capnography. In the postoperative period, it is recommened that the patients should continue their continuous positive airway pressure (CPAP) devices after surgery (22).

It has been well known that thromboembolic events such as pulmonary embolism, portomesenteric thrombosis account for a majority of the mortality and morbidity among bariatric surgery patients. The American Society of Metabolic and Bariatric Surgery (ASMBS) has suggested that all patients should be encouraged for early postoperative mobilization and perioperative use of compression devices and recommends that low molecular weight heparin (LMWH) be utilized unless otherwise contraindicated. The authors clinical protocol is to continue LMWH 10 days after surgery. We also suggest an additional 2–4 weeks use of anticoagulation therapy for patients who are at higher risk, such as those with history of DVT, BMI >55 kg/m^2, male gender, prolonged operative time, or patients who are nonambulatory.

In our practice, patients are usually discharged on postoperative day 2. After discharge from the hospital, patients are continued on a phase 1 bariatric clear liquid diet for the first 7-10 days. This is advanced to a bariatric phase 2 pureed highprotein liquid diet for 4 weeks and then finally transitioned to a diet of soft foods. In the rehabilitaion period, we suggest a goal protein intake 1–1.5 g/kg for ideal body weight.

Our bariatric team contacts the patients at home several days after discharge for encouragement, diet reinforcement, and reminders to maintain hydration. They are also advised physical exercise starting 2-3 weeks postoperatively. Postoperative patients require regular and frequent follow-up in clinic. They are seen at 1 week, 1 month, 3 months, 6 months, 12 months, 24 months, and then annually endoscopic examination after the first year. Clinic visits consist of weight and nutritional monitoring, laboratory tests for anemia and/or vitamins deficiency as well as dietary counseling and psychology referral as needed.

Management of Acute complications after LSG

The major acute complications after LSG include bleeding, leak, stenosis, venous thrombosis such as DVT, portomesenteric thrombosis. Late complications include stricture, weight regain, and malnutrition. In this paper, a short review of management for acute complications after LSG will be discussed separately.

Bleeding

It has been reported that the average rate of bleeding after LSG is 2% (23). Main risk factors for haemorrhage are hypertension, anticoagulant therapy, portal hypertension and the surgical technique (24, 25). Based on the ethiology, haemorrhage can be either intraperitoneal or endoluminal. Postoperative melena, tachycardia, pain and significant haemoglobin decrease on blood test is suspection of bleeding. CT scan is helpful to differentiate other diagnoses and also to guide management.

In case of bleeding post LSG, close monitoring is needed and intravenosus fluid resuscitation is essential. Anticoagulation treatment should be discontinued. Conservative treatment is usually enough for haemodynamically stable patients. The patients with haemodynamically unstable may require surgical exploration. Surgical exploration diagnoses the bleeding source as well as allows hemostasis and definitive drainage of the hematoma. If a persisting intraluminal bleeding at the staple line is suspected, endoscopic haemostasis may be required.

Leak

Gastric leak is the life threatening complication of LSG. It has been reported that leak rate is 2.2%–2.4% and nearly 90% of LSG leaks occur at the esophagogastric junction (EGJ) (26). Functional or anatomical stenosis of the gastric tube, poor vascular supply to the EGJ and using inappropriate stapler are the most common risk factors for the gastric leak.

In general, gastric leaks are classified according to the timing of clinical signs: early (within 2 weeks), intermediate (between 2 and 6 weeks) and late (after 6 weeks) (27). Tachycardia, fever tachypnea, leukocytosis on blood tests, signs of peritoneal irritation and left shoulder pain are the most common findings for suspicion of gastric leak following LSG. CT with oral and IV contrast is mandotary for diagnosis of gastric sleeve leak in patients with suggestive symptoms who are clinically stable. The principles of treatment of early gastric sleeve leaks include: adequate drainage of perigastric collections (laparoscopy either percutan drainage) and administration of antibiotics to control abdominal sepsis and nutritional support. Once the leak has been effectively drained, conservative management options such as endoscopic stent placement, endoscopic clip placement, endoscopic placement of a double pigtail stent can be used to help healing of gastric sleeve leaks (28,29).

In case of chronic nonhealing fistulas, surgical treatment options including gastrojejunal anastomosis, conversion to Roux-en-Y gastric bypass, completion of gastrectomy with esophagojejunostomy are described in the literature (30).

Portomesenteric Thrombosis

The incidence of portomesenteric thrombosis (PT) following LSG is approximately 0.3% (31). The etiology has not well known, however post-operative dehydration, change in venous outflow, thrombophilia syndromes, splenic ischaemia, perigastric fluid collection are predisposing factors for PT. Clinical signs such as vague abdominal pain, diffuse abdominal tenderness, fever, nausea and vomiting after LSG are suspicion for PT. Abdominal CT and Doppler ultrasound confirms the diagnosis. The treatment depends on the clinical severity of the patients and main principles include anticoagulation, adequate fluid resuscitation, and bowel rest. Once PT is suspected, anticoagulation therapy must be started as soon as possible either by subcutaneous low molecular weight heparin (1.5–2 mg/kg/day in two injections) or by intravenous continuous infusion of heparin. Oral anticoagulants are given for 3-6 months after the initial treatment of PT. In majority of cases, anticoagulation therapy allows thrombosis regression as well as clinical improvement. Surgery is reserved for patients with complications of thrombosis such as bowel necrosis (32).

Stenosis

Stenosis is a rare complication after LSG that usually result from surgical technique. The rate of clinically significant stenosis occurs nearly in 0.5%–3.5% of cases mostly located at near the incisura (33). The most common complaints of the patients are vomiting and regurgitation.

There are two types of stenosis have been described: anatomical and functional stenosis Anatomical stenosis is usually located at the level of the gastric incisura and generally it occurs due to a wrong placement of the calibration tube. Functional stenosis is also can be identified in two types. Type 1 is described as a localised twist of the gastric tube with the endoscopic appearance of an ‘antireflux valve’. Type 2 is described as a spiral course of gastric stapling that winds around the stomach (34). Endoscopic balloon dilatation is the first treatment option for gastric stenosis with long-term success rates reported at 95%–100% (35). If endoscopic dilation is unsuccesful, endoscopic stenting, longitudinal seromyotomy, median gastrectomy with gastrogastric anastomosis, and conversion to Roux-en-Y gastric bypass are the other surgical options (33, 36).

Conflicts of interest: None

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Figures:

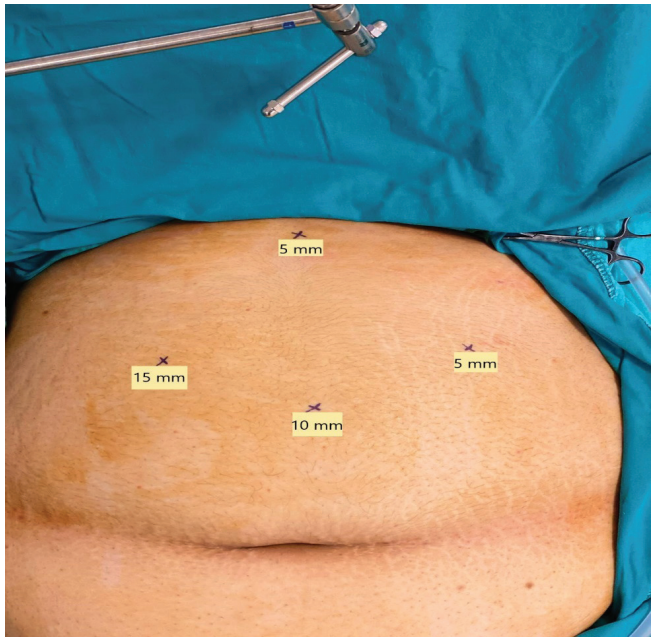

Figure 1: *Placement of ports for laparoscopic sleeve gastrectomy (LSG) technique*

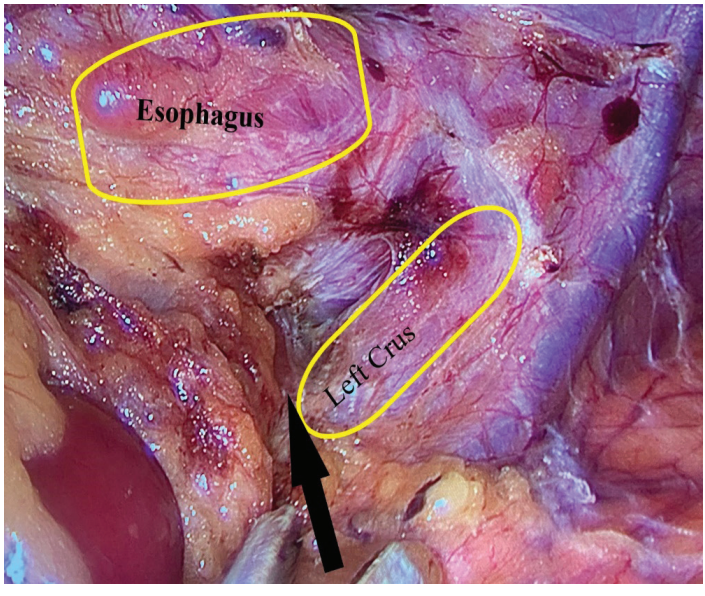

Figure 2: *The black arrow shows the basis of left crus where it meets right crus*

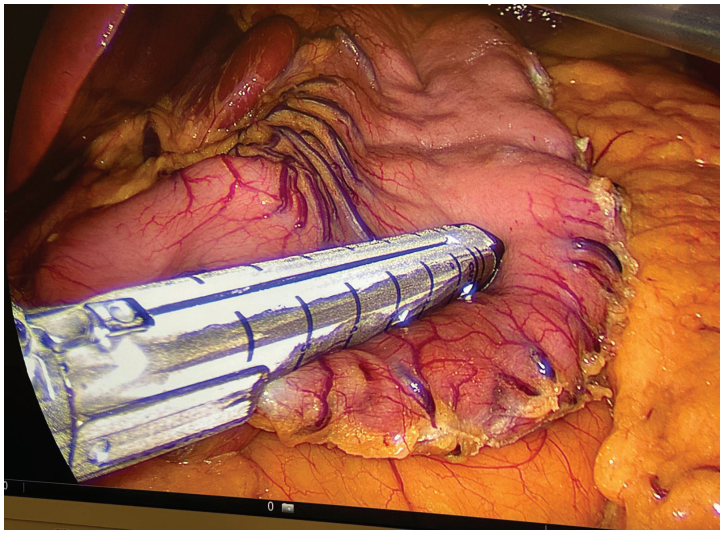

Figure 3: *The position of first stapling to avoid stricture at incisura angularis*

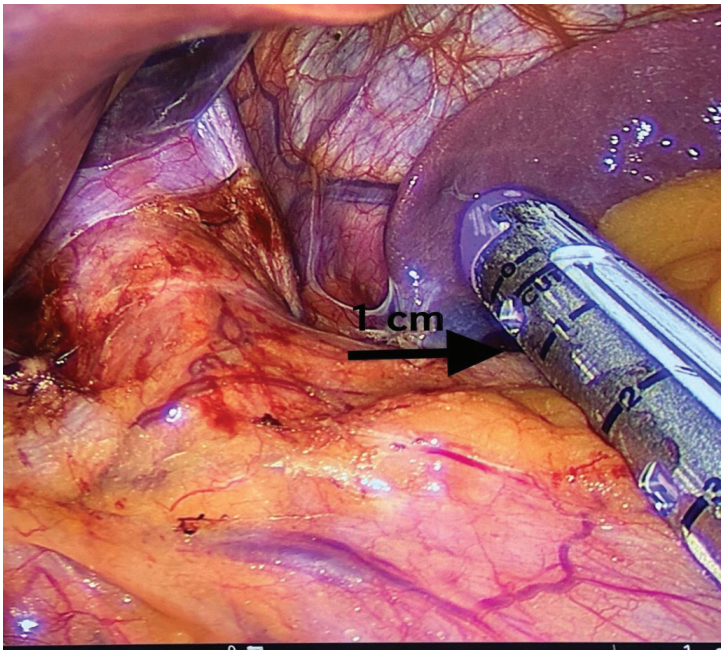

Figure 4: *Last stapling should be at least 1 cm away from the gastroesophageal junction (GEJ).*

Chapter 27

NEW APPROACHES ON SOME NEUROPEPTIDES: KISSPEPTIN AND NEUROPEPTIDE Y

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Neuropeptides

Neuropeptides are described as small protein substances that produced by neurons which regulated secretion and acting on neural substrates (glial cells, neurons or non-neuronal target cells, a muscle or gland e.g.). These short amino acid sequences functions to modulate synaptic activity directly or indirectly (1). From this definition, "neuron" is the key word because the only distinction between neuropeptides and other peptides is the synthesis or use of a neuropeptide by a neuron. To describe a molecule as a neuropeptide, it must have the following properties: (a) a small size protein molecule (usually between 3 and 100 amino acids) (b) produced and secreted by the cells of the nervous system and (c) a specific role in the regulation of neuronal cells (2). Neuropeptides roles as primary neurotransmitters in the central nerve system (CNS), as well as neuromodulator in the peripheral nerves (sensory and motor nerves) (3).

At the neuropeptide synthesis, precursors are usually produced in form as inactive pre-pro-molecules in physiological process. While this process involves specific cleavage, separation and modifications of peptides, formation of bioactive end product occurs via the regulated secretory pathway (RSP) (4).

Processes of posttranslational modifications are including phosphorylation, acylation, glycosylation, and proteolysis conversion (5). Before being stored or released from the cell, the precursors are reduced to the short amino acid chain in the golgi apparatus (via endopeptidases) or directly into the secretory vesicles for transport down the dendrites or axon (6). Release of the small and large vesicles are differently regulated. It has been determined that neuropeptides are released in a calcium-dependent manner to bind to G protein bound receptors (GPCR). Neuropeptides are not immediately reuptaken, recycled or degraded, so that they are bioactive for a long period (7).

The well known functions of neuropeptides in brain include a variety of physiological and behavioral processes such as pain, food intake, body weight regulation, reproduction, anxiety, depression, social behavior, learning and memory (8). Neuropeptides may provide important knowledges as indicators of pathogenic or biological processes. Researches on neuropeptides may give valuable contributions to diagnosis and prognosis in many different disease.

Kisspeptin

Identification of Kisspeptin

In the late 1990's, kiss-1 gene was discovered as a gene that suppres-

ses tumor metastases (human malignant melanoma cell line C8161). This gene were named as “kisspeptin” which dedicated to the famous chocolate brand Hershey Kisses (kiss) in Pennsylvania (USA). The letters “ss” in the kiss gene indicate the suppressive sequence (9). Kiss-1 gene is located at 1q32.1 in humans, which is the long (q) arm of chromosome 1 at position 32.1 (10). The first product of this gene is kisspeptin-145 (a 145 amino acid). Proteolytic cleavage of kisspeptin-145 generates a 54 amino acid amidated peptide (kisspeptin-54) so called as “metastin”. Other forms of kisspeptin-54 (Kp-54) were identified in further studies, and all of them were called as ‘kisspeptins’.

While the longest cleavage product of the kisspeptin precursor protein is Kp-54 , smaller active peptides (i.e., Kp-14, Kp-13 and Kp-10) are present to capable bind to KISS1-R/GPR54 (Kisspeptin receptor/G protein coupled receptor) (11, 12). These fragments have equal potency to bind and activate KISS1-R. Kisspeptin fragments has carboxyl ends with the arginine-phenylalanine (RF) motif and amidated from this terminal (adding NH2). This RF-NH2 motif at the carboxyl terminal is the binding site of the receptor (13).

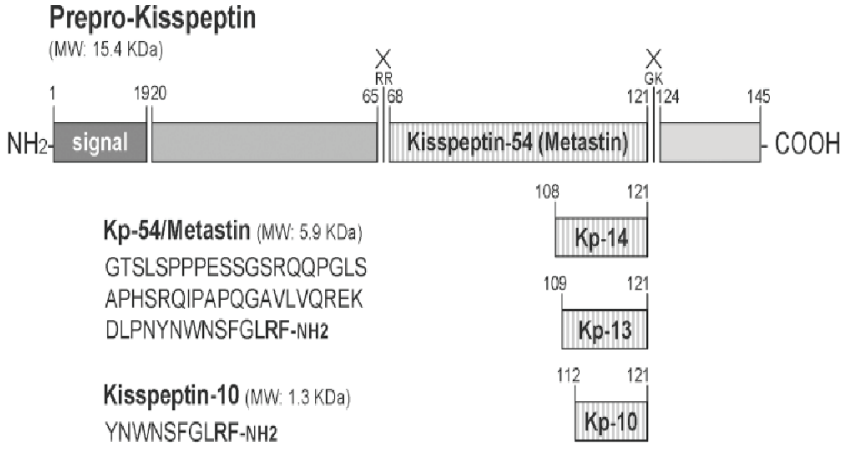

Figure 1: *Human kisspeptins and relative cleavaged fragments (Tena-Sempere M. GPR54 and kisspeptin in reproduction. Human Reproduction Update, 2006; 12: 631-639) (14).*

Kp-54 (54 aa) was nomenclatured as ‘metastin’ because of its preventive effect on cell proliferation in human melanoma and breast cancer cell cultures. Shorter fragments are formed by cleavage the amino terminal (N-terminal) end of Kp-54. While Kp-54 primarily found in tissues and circulation in humans, the most physiologically effective fragment is Kp-10. Kisspeptins (Kp-14, Kp-13 and Kp-10) were determined to be activated by binding to KISS1R with high affinity from the RF sequence (14, 15). Member of RF amides, also kisspeptins has specific roles in repro-

duction, nutrition, blood pressure regulation and pain modulation (mostly reproduction and energy balance) (16). Besides, RF amides and it's related receptors display important physiological effects such as behavioral, sensory and neuroendocrine.

Signalling and receptors of Kisspeptin

GPR54 (G protein coupled receptor) was first identified as an "orphan" receptor in the rat brain (1999), then nomenclatured as KISS1R (AXOR12 or hOT7T175). The human KISSR1 consists of 5 exons and 4 introns, and localised at 19p13.3 chromosomal region. This receptor has a seven transmembrane domains with 398 aa (Gaq/11ecoupled receptor, 75 kDa) which is belonging to the rhodopsin gamma family of GPCR. These receptor has three intracellular (i1, i2 and 13) and extracellular (e1, e2, and e3) structure which binds to these domains. The amino (-NH2) end of the receptor extends out of the cell and the carboxyl (-COOH) into the cell (17). Although KISS1R receptors has approximately 45% similar structure to galanin receptors, galanin or galanin-like peptides cannot bind to the KISS1R (17, 18). KISS1 and KISS1R are mostly expressed in the arcuate (Arc) and anteroventral periventricular (AVPV) nuclei of the forebrain and involved in neuroendocrine regulation of reproduction. While KISS1 and their putative receptors is mostly expressed in the brain (brainstem, cortex and cerebellum), studies have demonstrated the presence of these receptors in central nervous system, pancreas, adipose tissue, ovaries, small intestine, liver and placenta. However, low levels of KISS1R are present in adipose tissue, peripheral blood lymphocytes, lymph nodes and spleen (19, 20). From the studies, it is well known that kisspeptins can exhibit different physiological effects on various tissue types as autocrine/paracrine.

When kisspeptins binds to KISS1R (coupled to Gαq/11), phospholipase C is activated (PLCβ). Activated phospholipase C (PLCβ) induces the hydrolysis of phosphatidylinositol-4,5-bisphosphate. This process leads to the production of intracellular seconder messengers: diacylglycerol and inositol-1,4,5-trisphosphate. While activation of diacylglycerol leads to the activation of p38 phosphorylation, ERK1/2 and protein kinase C, inositol-1,4,5-trisphosphate induces release of intracellular Ca^{++} from the endoplasmic reticulum. So, elevated intracellular Ca^{++} can inhibits cell proliferation and increases apoptosis and differentiation (21).

G protein bound receptor proteins act as signal transducers of extracellular peripheral signals to activate kinases which is stimulate the cellular proliferation and migration associated with metastasis (22). Some studies have proposed that KISS1R may be coupled also with Gaq/15-16, which could contribute to the induction of the release of inositol-1,4,5-trisphosphate and Ca^{++} in some cellular systems, investigations are ongoing on this topics (23).

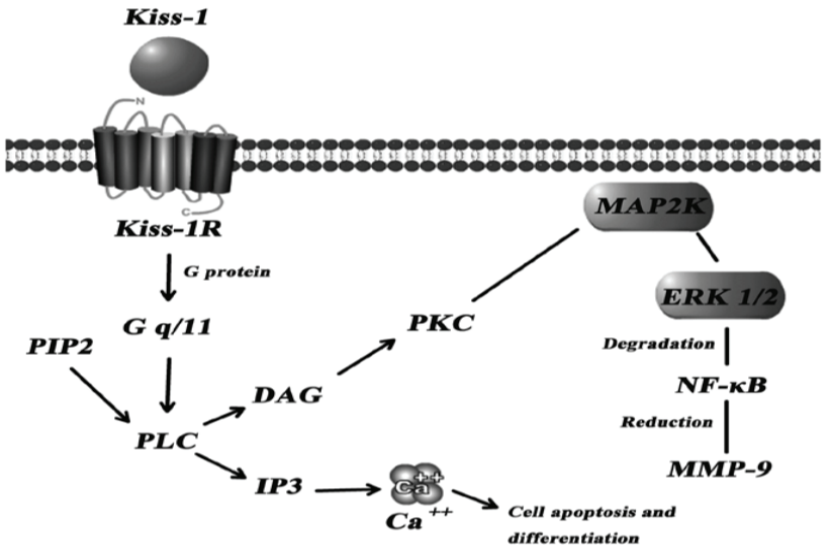

Figure 2: *Signalling mechanisms of KISS1 and KISS1R (Ji et al. The Kiss-1/Kiss1-R complex as a negative regulator of cell motility and cancer metastasis (Review) Int J Mol Med 2013;32:747-754 (21).*

Physiological effects of Kisspeptin

Multiple studies reported that kisspeptins have many physiological effects due to the expression of KISS and KISS1R in different tissues. It has been revealed that KISS1 has effects on the hypothalamus, pituitary, gonadal axis and play physiological roles in cardiovascular control, synaptic conduction, placentation, energy metabolism, locomotor activity and cancer events.

As a neurohormone or neurotransmitter, kisspeptin play a key role in the reproductive system, especially in puberty, sex maturation, fertility and ovulation. The signals which produced by the binding of kisspeptins to the KISS1 receptors lead to the production of gonadotropin-releasing hormone (GnRH) and controls the hypothalamic-pituitary-gonadal axis (24). Induced KISS1R in the GnRH neurons (hypothalamus) provide the release of GnRH from the median eminence by entering the portal hypophyseal circulation. Then, the secretion of GnRH releases the gonadotropins (FSH, LH) from the pituitary (25). As a neuroregulator, in the arcuate region of the hypothalamus KISS 1 neurons act as a sensor to send information about hormonal, nutritional status and environmental changes to GnRH neurons to regulate the secretion of the gonadotropins (26). Moreover, kisspeptins play a remarkable role in cardiac function, glucose homeostasis, body composition, as well as feeding behavior (27).

A study suggested that members of the kisspeptin family may have vasoactive activity. From the study, an inhibitor role of Kp-10 has been

observed in the migration of human umbilical vein endothelial cells and subsequently vascular endothelial growth factor (VEGF) signaling (28). Meat et al. concluded that kisspeptin acts as a potent vasoconstrictor and inhibitor of angiogenesis. According to the study, kisspeptin has an undefined role for the cardiovascular system in humans, because of gene expression of both kisspeptin and its receptor in human aortic, coronary artery and umbilical vein smooth muscles (29).

In a study performed on rats, (exogen administration of kisspeptin as intraperitoneally), it has been determined that coagulation, bleeding and prothrombin periods were significantly longer than the controls. These finding proposed that kisspeptins may have anticoagulant effects in bleeding events (30). In addition, kisspeptin appears to play a role in the regulation of kidney development (31) and insulin secretion (32). In summary, it seems that comprehensive and further studies are needed to fully clarify the different possible physiological effects of kisspeptins.

Kisspeptins in different diseases

As mentioned in the previous section, kisspeptin was initially described as a metastasis suppressor. So, many study has been focused on the role of kisspeptin in cancer events. In a recent study, it has been proposed that KISS1R may regulate tumorigenesis in estrogen receptor-negative breast cancer (33). A study performed by Singh et al. found that KISS1 levels were increased in breast cancer (87 proven cases of breast cancer) compared to the normal tissue. At the study, lower expression of KISS1 were also observed in metastatic cases compared with non-metastatic (34). Levels of KISS1 mRNA and KISS1R were elevated in hepatocellular carcinoma (samples of resected hepatocellular carcinoma), compared with the non cancerous liver (35). In contrast, a study showed that KISS1 protein expression is lost in hepatocellular carcinoma because of the KISS1 tumor suppressing role in aforementioned disease (36). Similarly, reduced levels of KISS1 were observed in bladder cancer (37), colorectal cancer (38) and prostate cancer (39). Besides, Loosen et al. observed increased serum levels of kisspeptin in patients with pancreatic cancer (40).

Taniguchi-Ponciano et al. determined that KISS1 gene has showed 20% genomic gain in 1q32.1 on cervical cancer (CC) cells (41). At the study, it is proprosed that overexpression of KISS gene may serve as a potential molecular marker for CC. According to the literature, controversial findings are seen in the cancer cases. So, further studies are needed to clarify the subject.

A recent study concluded that plasma kisspeptin levels were increased in hypogonadotropic delayed puberty. According to the study, it has been proposed that kisspeptin has a diagnostic value in the evaluation of

this disease (42). Umayal et al. indicated that serum levels of kisspeptin were positively associated with polycystic ovary syndrome (PCOS). At the study, serum kisspeptin concentrations were higher in women with PCOS than the controls (43). Luedde et al. evaluated circulating kisspeptin levels in 133 (75 male and 58 female) critically ill patients during intensive care unit (ICU). Significantly increased levels of kisspeptin were observed in the study (44). A study reported that kisspeptin serum concentrations of acute myocardial infarction (AMI) patients were significantly lower than the control group. Moreover, the study assumed that deficiency of kisspeptin may be a risk factor for AMI (45). In another study, plasma kisspeptin 10 levels were lower in preeclamptic (PE) pregnant women groups than the controls. Additionally, it has been proposed that kisspeptin 10 levels may be a novel marker in PE (46).

Kisspeptin is not only stimulate the hypothalamic-pituitary gonadal axis but also alter the expression of the antioxidant enzyme against oxidative damage. In a study, it is emphasized that kisspeptin has neuroprotective properties against amyloid-□ plus related amyloid proteins (47). Moreover, another study reported the colocalization of kisspeptin, corticotropin releasing hormone, and catalase in amyloid-□ positive plaque-like deposits at the pons in the patient with alzheimer's disease (48).

Based on literature, few study has examined the role of KISS1/KISS1R in different diseases. So, comprehensive investigations on kisspeptin should be ongoing in order to reveal both diagnostic/prognostic biomarker in different diseases.

Neuropeptide Y

Identification of Neuropeptide Y

Neuropeptide Y was firstly discovered by Tatemoto and Mutt in the porcine brain as a novel brain peptide (49). A member of the pancreatic polypeptide family (gut-derived hormone peptide YY/PYY, pancreatic polypeptide/PP), neuropeptide Y consists of 36 amino acid and its primarily found in the central nervous system and brain (cerebral cortex, hippocampus, thalamus, hypothalamus and brainstem). In the periphery, it is found together with noradrenaline in the nerve plexuses around the blood vessels of various organs, adrenergic nerve endings and chromaffin cells of the adrenal medulla. Extraneural tissues such as the urinary tract, spleen, lung, reproductive organs and blood vessels may also express neuropeptide Y (49, 50).

The gene of neuropeptide Y (~ 8 kb) is located on human chromosome 7 at the locus 7p15.1 and consists of 3 introns and 4 exons (51). The biosynthesis of neuropeptide Y include the translation of a precursor neuropeptide Y (prepro-neuropeptide Y) molecule (96 amino acid) which is directly

translocated into the endoplasmic reticulum, where its signal peptide is removed. So, the remaining precursor neuropeptide Y (pro-neuropeptide Y, 69 amino acid) undergoes cleavage by prohormone convertases and neuropeptide Y (39 amino acid) will be formed. This final molecule prevents its degradation by carboxypeptidases with C-terminal amidation (52).

Neuropeptide Y acts as a neurohormone/ neuromodulator and appears to be mostly expressed in the central nervous system and in sympathetic ganglia. Also, these peptide and their relative receptors are found in nonsynaptic neurons in many organs; gastrointestinal tract, salivary gland, retina, thyroid gland, pancreas, urogenital system, heart, spleen, endothelial cells of blood vessels and liver (53).

Signalling and receptors of Neuropeptide Y

The neuropeptide Y molecule is involved in some signal transduction pathways through G protein coupled receptors. As general, receptors of neuropeptide Y are coupled to the G_o or G_i subunit. After binding, these subunits (G_o or G_i) of the G protein komplex are released and in turn the inhibition of adenylate cyclase will be occured. This process prevents the conversion of ATP to cAMP (a second signaling molecule). Then, decreased cAMP levels are modulate K^+ and Ca^{++} channels (54). Also, it has been reported that Y2 and Y4 receptors may bind to the G_q and induce the production of inositol 1,4,5-triphosphate by activating phospholipase C-β (55).

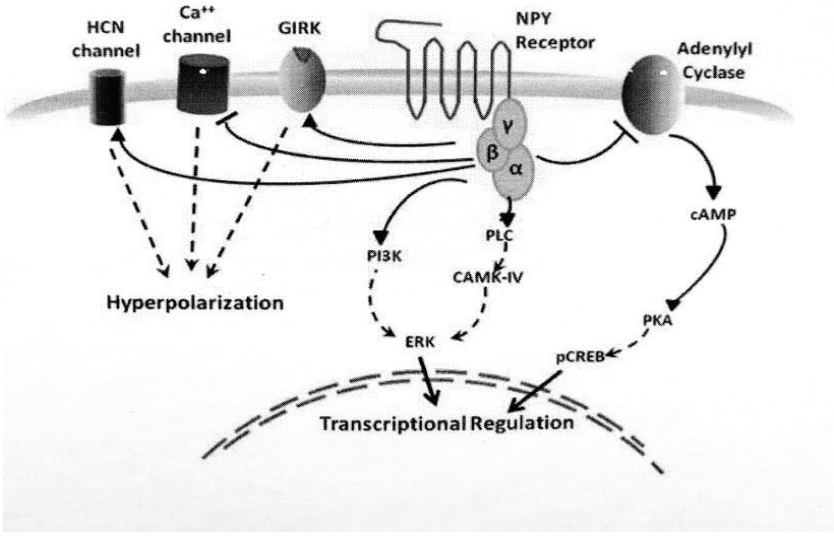

Figure 3: *Signaling pathways of the neuropeptide Y receptors (Sah R, Geracioti TD. Neuropeptide Y and posttraumatic stress disorder. Mol Psychiatry, 2013; 18(6): 646-55 (56).*

To date, seven different neuropeptide Y receptors are identified in vertebrates. While Y1, Y2, Y4, Y5 and Y6 is cloned from mammalians, Y1, Y2, Y4, Y5 are proven in human as functionally (57). Y3 has been characterized as chemokine receptor type 4, so it's included in the chemokine receptor family (58).

The first reported neuropeptide Y receptor is Y1. These receptor is mainly found in blood vessels, central nervous system (thalamus, cerebral cortex) and amygdala. Y2 is primarily located in the pre-synaptic region and inhibits excessive release of neuropeptide Y. Identified formerly as pancreatic polypeptide receptor, Y4 found to be as a neuropeptide Y receptor which is mostly expressed in colon, small intestine and pancreas. Y5 is associated with eating behavior or food intake. Besides, Y6 receptor is cloned, but it is not functional in humans (59, 60). Although these different neuropeptide Y receptors play various roles in human biological processes, their acts is topics of much debate.

Neuropeptide Y has a important roles in many physiological functions such as energy homeostasis, cortical excitability, cardiovascular function, cognition and food intake. Furthermore, the peptide has been suggested to play a role in stress response and anxiolytic properties (61). According to the studies, physiological effects of neuropeptide Y are reported such as daily circadian rhythm (62), regulation of energy balance, memory, regulation of blood pressure (63) and nutrition (64). Moreover, it has been observed that neuropeptide Y play as a regulator in pain modulation (65) and neuronal development (66).

Neuropeptide Y in different diseases

Recent studies are focused on the relation between neuropeptide Y and different diseases. Although neuropeptide Y is mostly distributed in the central and peripheral nervous system, it plays important roles in various physiological functions. Atherosclerosis is still one of the leading cardiovascular diseases and known with high global mortality rates. Endothelial dysfunction, inflammation, matrix modification and proliferation are atherosclerosis initiation factors. A study imply that neuropeptide Y is the most abundant neuropeptide in the heart (67). Zukowska-Grojec et al. reported that neuropeptide Y functions as cardiac regulator with indirect or direct cardiac nerve interactions (68). According to the studies, it has been suggested that elevated neuropeptide Y levels may be a risk factor for cardiovascular diseases. Ullman et al. determined that plasma neuropeptide Y were increased in patients with congestive heart failure and acute myocardial ischaemia (69). Similarly, a study demonstrated that neuropeptide Y concentrations were higher in patients with acute myocardial infarction than the healthy controls. Also, it has been proposed that neuropeptide Y

could be used as a early diagnostic biomarker in patients with acute myocardial infarction (70).

Lung diseases, which take place among the top five diseases that cause mortality, decrease the comfort of life and general health of people. Plasma levels of neuropeptide Y were evaluated in twentyfive elderly asthmatic patients. To the study, it has been observed that neuropeptide Y levels were two fold higher than the controls (71). Besides, serum neuropeptide Y levels were found as significantly elevated in patients with chronic obstructive pulmonary diseases than the healthy group (72). Also, a recent study has disseminated that manipulating neuropeptide Y may represent a new therapeutic target to control allergic airway responses (73).

Based on investigations, neuropeptide Y has a role in neuroprotection, neurogenesis and neuroinflammation due to the paracrine/autocrine effects (74). Martignoni et al. observed a significant reduction at cerebrospinal fluid levels (CSF) of neuropeptide Y in patients with Alzheimer's and Parkinson's than the controls (75). However, another study has determined remarkable increased neuropeptide Y levels in the CSF of migraine patients (76). According to the study, its assumed that neuropeptides may well have a role in the acute and preventive therapy of migraine headache. A study concluded that neuropeptide Y may be a early predictive role in the pathogenesis of subarachnoid hemorrhage-related cerebral vasospasm and ischemia. Elevated levels of neuropeptide Y were observed in subarachnoid hemorrhage and cerebral vasospasm than the healthy groups (77).

The effects of neuropeptide Y on inflammation and pain modulation were also studied in several studies. A contemporary study revealed that increased neuropeptide Y levels may be an independent marker of disease activity in rheumatoid arthritis (RA). Active RA patients had higher neuropeptide Y levels than the controls in the study (78). While its exact mechanisms are unclear, Diaz-delCastillo et al. proposed that intrathecal administration of neuropeptide Y in animal models of postoperative or neuropathic pain has been caused analgesia (79).

Neuropeptide Y and its relative receptors has complex effects on cancer development because of their physiological effects on immune function, tumor biology and energy homeostasis.

A recent study declared that high levels of neuropeptide Y1R expression were observed in breast cancer patients. At the study, its assumed that neuropeptide Y1R may serve as a useful predictor for evaluate the prognosis of breast cancer patients and breast cancer metastasis (80). Interestingly, a study observed that neuropeptide Y levels during chemotherapy were significantly decreased in 23 children diagnosed with cancer (81).

Stress is defined as a multifactorial condition of mental or emotional tension which caused by adverse outcomes. Neuropeptide Y levels may be alter in patients with chronic stress and depressive disorder. A meta-analysis study noted that neuropeptide Y levels were significantly decreased in plasma and cerebrospinal fluid in post-traumatic stress disorder patients than the controls. From the stuy, plasma neuropeptide Y levels were significantly increased in chronic stress patients compared to the healthy group (82).

In this chapter, it is aimed to present the diagnostic values of kisspeptin and neuropeptide Y. As clinical, it is quite important to determine the possible roles of these neuropeptides and relative receptors in different diseases.

to my mother…

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Chapter 28

POLYMERIZATION AND LIGHT CURING UNITS IN RESTORATIVE DENTISTRY

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1. POLYMERIZATION

Polymerization is a chemical process where smaller molecules called monomers are linked together to form large chains of molecules called polymers. The functional groups of monomers possess carbon-carbon double bonds (C=C). These double bonds have 2 pairs of electrons shared by two carbon atoms. While C=C double bonds are the reactive part of the monomer, other atoms and side groups can be linked to one or both of the carbon atoms. The polymerization process basically involves four different reactions. These are activation, initiation, propagation, and termination reactions (1). In the activation step, free radicals that will initiate polymerization are released (2). In the initiation phase, free radicals activate the monomer molecule by opening its unsaturated double bond so that it can be linked to other monomers to form polymer chains. This process continues until there are no more free radicals left in the environment (3). During the propagation stage, new monomers are added to the growing polymer chain. Termination phase takes place when the reaction ends due to reasons such as a decreased amount of monomers available (4). The sum of the molecular weights of a large number of monomers linked together by covalent bonds in a polymer molecule gives the molecular weight of the polymer.

The more the carbon double bond conversion takes place, i.e., the more monomers are added to the chain, the more successful the polymerization is, and better physico-mechanical results are obtained. The unreacted carbon double bonds may either be present as free radicals or added to the polymer chain as pendant monomers (5). Adequate polymerization is an important factor for a successful composite restoration because insufficient polymerization causes increased leakage in restorations, discoloration of the composite, compromised mechanical characteristics due to deterioration of structural properties and decreased connection with the tooth surface (6). Polymerization processes may involve different mechanisms. Synthetic polymers are generally formed by either addition polymerization or condensation polymerization of the monomers.

1.1. Polymerization Steps

- **Initiation:** The free radical required for the initiation of polymerization is formed by the reaction of a chemical activator with heat or light. This process is known as the activation stage. Resulting free radical then binds to the carbon in the methyl methacrylate monomer, opens the double bond, and provides the energy required for addition of other monomers to this bond. Thus, by leaving the carbon atom in unstable state, it turns the bond itself into a free radical. (7). Thereby, the chain of reactions begins.

- **Propagation:** The unstable structure of the monomer molecule, due to the opened double bond as a result of its reaction with the free radical, also opens the double bond of the neighboring monomer molecule. This process propagates continuously, causing the chain to grow. The growth of polymer chains continues until exhaustion of the monomer units. This process may also end up with the monomer going into a reaction with any other material that has a free radical. Thus, this process continues until all monomer molecules become a part of the polymer chain.

- **Termination:** The loss of reactivity of polymer molecules occurs when the energy in the molecule is depleted. This either occurs directly by the double bonds or by the exchange of hydrogen atoms from one enlarged chain to another. Over time, as monomers are added to the chain, the concentration of monomers will decrease and therefore, growth reactions decrease and termination reactions increase. After the depletion of the monomers in the environment, radicals cannot remain stable for a long time, and lose their reactivity by interacting with water or other substances such as oxygen and carbon dioxide in the air (8).

1.2. Polymerization Types

1.2.1. Addition Polymerization

This is a conversion process where multiple molecules of the same structure are repeatedly added to the chain, without any change in their chemical structure, to form a high molecular weight macromolecule with the same chemical composition. Addition polymerization reactions always occur with unsaturated molecules containing double bonds. Opening the double bonds between two carbon atoms of a monomer molecule enables its activation. In return, the monomer reacts with another monomer and transfers its reactivity to another molecule. Then, the double bond in the other molecule opens and this process is repeated for additional monomers. Thus, a chain reaction begins (7). In theory, chain growth can continue until all monomers are depleted. No change in the chemical composition occurs during the addition polymerization. In other words, the structure of the monomer is repeated over and over through the polymer.

Almost all composite resins used in dentistry involve addition polymerization. The addition polymerization may occur in three ways, depending on the initiator in the monomer. Initiators can be cationic, anionic or radical type. Examples are sodium and potassium for anionic initiators, and aluminum trichloride and tin dichloride for the cationic initiators. However, due to the metabolic toxicity of these substances, radical polymerization is most commonly used in dentistry. While ultraviolet light was previously used for radical polymerization, visible light with wavelengths greater than 400 nm is currently used. For visible light, various amines with wavelengths of

400-480 nm such as camphorquinone (a diketone) and reducing agents including N,N-dimethylaminoethyl methacrylate, hydroxyethyl toluene and hydroquinone are used. These radicals turn monomers into the reactive state by interacting with the monomer molecules (7, 9). No by-products are produced during the addition reactions. All monomers have the same molecular weight. In this process, unlike condensation polymerization, larger size giant macromolecules are formed. There is only a difference in the distribution of chemical bonds between the two processes (7).

1.2.2. Condensation Polymerization

It is a chemical reaction that proceeds with a similar mechanism, but between more than one type of monomer species. Condensation polymerization is a chemical reaction where structurally distinct or similar polyfunctional monomers join together, often losing small molecules. In condensation polymerization, the monomers are bifunctional and all become reactive spontaneously. This reaction continues in a chain of sequential reactions until a high-molecular weight polymer is formed. Such polymerizations are also known as "step polymerization" reactions (7).

In a condensation reaction, a small molecule such as water is released as a byproduct and this represents a major difference from addition polymerization. Today, condensation resins are not frequently used in dental restorations or prosthetic applications. Polymerization processes may not always give rise to polymers with all desired characteristics. Monomers affect the physical and chemical properties of the polymers they form. In particular, cross-linked polymers are materials that are physically more stable with less water absorption and solubility. Thus, copolymerization and cross-links are also important in a polymerization process (7).

-Copolymerization-

Copolymerization is a type of polymerization where different monomers at various concentrations are added to the reaction medium to take advantage of different chemical properties of monomers and a polymer thus obtained is called a copolymer. The majority of composite resin materials used in dental practice are of this type. As copolymers add several characteristics to the resulting polymer, copolymerization is frequently used for obtaining resins.

-Cross-Linking-

Monomers with reactive side chains are added to the structure which consists of polymer molecules linked together in a chain. These added side chains allow attachment of one chain to another. As a result, a three dimensional, more physically stable structure is formed. Resulting cross-linked polymers are stronger materials with less water absorption and solubility (7).

1.3. Degree of Polymerization

In composite resins, the degree to which monomers convert into a polymer or the rate of conversion of double carbon bonds (C=C) to single carbon bonds (C-C) along the polymerization is referred to as the degree of conversion or polymerization (10, 11). During polymerization of composite resins, conversion of all monomers into a polymer or a high degree of polymerization is desired. As the degree of polymerization increases, the amount of non-reacting residual monomers in the resin decreases and thus, their physical properties are enhanced. An ideal composite resin should produce low shrinkage while generating the highest degree of polymerization (12, 13).

Not all double bonds of the monomers react in composite resins polymerized by light. The rate of reacting double bonds varies between 55 and 80% (14, 15). The amount of residual monomers is affected by many factors including composition and translucency of the resin, application thickness, sample geometry, concentration of initiators activated by light, light intensity and exposure, and environmental oxygen and temperature (2, 16). Inadequate polymerization can lead to adverse effects of non-polymerized toxic monomers on the pulp, defects in the restoration/tooth bond as well as marginal leakage, postoperative sensitivity, discoloration, erosion and secondary caries due to bonding failure (17). The degree of polymerization has also been associated with hardness values, with lower degree of polymerization causing less surface hardness, reduced wear resistance and lower strength (18, 19).

1.4. Polymerization Methods

1.4.1. Chemical Polymerization

In the chemical polymerization method, materials are polymerized by mixing a catalyst with a base substance. The chemical reaction between benzoyl peroxide and a tertiary amine initiates chemical polymerization of composite resins. The combination of these two materials (the paste with a chemical activator and a paste with a chemical initiator) results in formation of free radicals. Benzoyl peroxide, the initiator activated by a tertiary aromatic amine N, N-bis (2-hydroxyethyl)-p-toluidine is a component of most of the chemically polymerized composites. The multi-step polymerization process begins with the formation of benzoyl radicals that initiate polymerization (20). Currently, the use of chemical polymerization is limited by low color stability of the components used, decreased mechanical properties due to porosity resulting from mixing two components (21), inconsistency of polymerization and insufficient time for using the material for treatment purposes (22).

1.4.2. Light Polymerization

This process is called photopolymerization. UV light was first used for photopolymerization in 1972 but replaced by visible light due to harmful biological effects of UV rays on both the patient and the dentist (1). Light-activated polymerization is the most commonly used method for polymerization of dental composites. Camphorquinone is the photoinitiator in light-cured composites and it is sensitive to blue light at a wavelength of 470 nm. The reactivity of camphorquinone is further enhanced by addition of various amine reducing agents. The concentrations of camphorquinone and amines in commercial composites vary between 0.2 and 1.2% by weight (23). 1-phenyl-1,2-propanedione (PPD), a photoinitiator sensitive to light with a wavelength close to 410 nm, and another photoinitator, Ivocerin, have been recommended as alternatives.

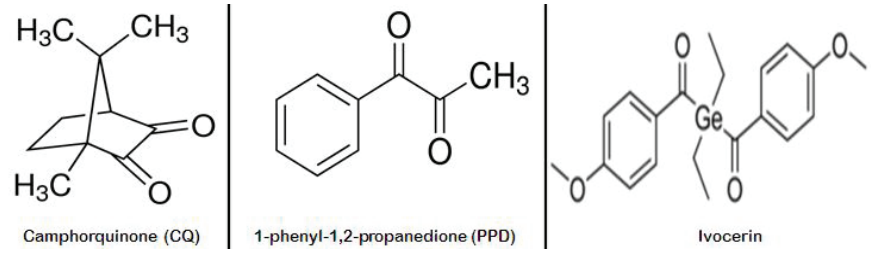

Figure 1. Molecular structure of some photoinitiators

The major advantage of polymerization with visible light over chemical polymerization is that the dentists can have control with the working time. While there are no significant differences between light-cured and chemically polymerized composite resins in the case of complete polymerization, photopolymerized composite resins have greater color stability and lower rates of internal porosity (4).

1.4.3. Both Chemical and Light-Cure (Dual-Cure) Polymerization

The material used for this type of polymerization contains light-sensitive activators as well as chemical catalysts. In this polymerization method, the rate of chemical polymerization is slow. Polymerization starts with light and the process continues and terminates chemically (24). Dual-cured composite resins are used in the case of deep cavities when there are concerns about incomplete polymerization, applications of composites thicker than 2 mm and aproximal areas that are difficult to reach.

1.5. Factors Affecting Polymerization

1.5.1 Material (Composite Resin)-Related Factors

- **Type and amount of filler and other structures:** While the light applied has the highest intensity on the surface of the material, its intensity

decreases due to scattering and reflections as it travels through the material. The type of monomer and filler, filler content and the refractive indices of filler and polymer matrix may affect the ability of light to be transmitted through the layers of resin (25).

- **Shade of the composite resin:** Since darker shades or opaque composite resins absorb more light, they may require longer exposure time for polymerization (26). However, opacity/translucency of the composite resin was reported to have a greater effect on polymerization than its shade (27).

- **Thickness of the composite resin:** Placement of composite resins at a thickness of no more than 2 mm is recommended. When the composite resin is placed as a thicker layer, the light applied on the top surface loses its intensity as it approaches the bottom surface of the composite resin, adversely affecting the properties and longevity of the material due to incomplete polymerization (28). Therefore, a more uniform polymerization is achieved with a composite resin thickness of 2 mm (29).

- **Type of initiator:** The composite resin should have a sufficient concentration of initiator that responds to the wavelength of the light curing unit used for polymerization. Thus, a light curing unit that matches the type of initiator contained in the material should be selected (30).

1.5.2. Effects of Dental Tissue and Cavity

Complete polymerization of the composite resin may not be achieved especially in the aproximal areas as a result of absorption and scattering of the light from the light curing unit while passing through the dental tissues. Since the enamel tissue is translucent, the light transmission is much better in the enamel than in the dentin tissue. For this reason, exposure time is increased to complete polymerization of the restoration throughout the dental tissue (31). The type, width and location of the cavity can affect transmission and scattering of light and thus, are important for polymerization (27).

1.5.3. Factors Related to the Light Curing Unit (LCU)

- **Size of the light curing tip (Tip geometry):** The LCUs currently in use have tips of various diameters. In a LCU with a large diameter tip, the distribution of light is greater than a LCU with a small diameter tip. LCUs with a small diameter tip have a rather high light output intensity. Therefore, the diameter of the light curing tip may affect the quality of polymerization (32).

- **Type of the LCU:** Each LCU has a certain wavelength range, polymerization efficiency and several advantages and disadvantages. There

are studies showing variable results regarding the impact of the type of LCU on polymerization (27).

- **Light exposure time:** Adequate polymerization of the composite resins and bonding agents depends on the exposure time as well as the LCU used. Light exposure time varies depending on the type of LCU and the structure and color of the resin-based restorative material.

- **Angulation of the light curing tip:** When the light curing tip is applied perpendicular to the surface of dental restoration, a circular area with a high light intensity is formed. If the light curing tip is inclined, this circular area assumes an elliptical shape and the light intensity reaching the application area decreases (31).

- **Distance between dental restoration and light curing tip:** The distance between the tip of the LCU and dental restoration is an important factor affecting the polymerization. As increased distance between the light curing tip and the composite resin surface will reduce the energy of light and negatively affect the degree of polymerization (33). For all LCUs, as the distance between the dental restoration and light curing tip increased, the depth of cure and the degree of polymerization decrease (34, 35).

- **Wavelength:** It is the length of the electromagnetic wave emitted by light. Composites are sensitive only to a limited wavelength. The blue light energy between 400-500 nm activates the photoinitiator (camphorquinone) in the composite resin.

- **Power:** It is the total amount of energy emitted by the LCU per unit time. Power is expressed in milliwatts (mW) (36).

- **Intensity:** It is the power of light per unit area of the light applied on and measured in mW/cm^2 (36). Light intensity can be controlled by increasing the output of the device or by reducing the diameter of the light curing tip.

1.6. Photopolymerization Protocols

In recent years, various polymerization "protocols" were introduced by manufacturers to increase the amount of polymerization, reduce internal stresses, maintain marginal integrity and decrease polymerization shrinkage (37).

- **Standard (continuous) polymerization:** The light power, which depends on the LCU used, remains constant at the same intensity from the beginning to the end of polymerization and is applied continuously throughout the process in different intervals such as 10, 20, 30 or 40 seconds.

- **Soft-start polymerization:** In the soft-start polymerization protocol, low light intensity is used initially and then increased gradually. This allows for a slow rate of polymerization with formation of less free radicals and extends the time for the composite to flow to the surface. Viscoelastic phase of the composite is prolonged and shrinkage stress of the composite resin can be controlled by slowing the hardening rate (38).

The soft-start protocol can be applied using four different regimens including Step-cure polymerization, Ramped-curing, Exponential polymerization, Pulse delay polymerization and Oscillating polymerization. The lamp units designed for application of the soft-start protocol automatically generates low intensity light, which is followed by high intensity light.

2. LIGHT CURING UNITS

Composite materials polymerized with light have been introduced in 1970s (39). At the beginning, dental materials were polymerized using Ultraviolet (UV) light which was subsequently replaced by visible light due to concerns that UV light might be harmful for both the patient and the dentist (40). Currently, light curing units (LCUs) are used for initiating polymerization reaction of composite resins (41). However, the mechanism in the polymerization reaction using a LCU is mainly based on activation of polymerization initiators found in the resin matrix to generate free radicals and to achieve this, the wavelength of the light used must be close to that of polymerization initiators (42). Polymerization with light activation starts at electromagnetic wavelengths between 400 and 500 nm. The absorbance spectrum of camphorquinone, the most widely used photoinitiator, is in this range and peaks at 470 nm.

Choosing a LCU may not be as simple as it seems because many factors such as the type of LCU, tip geometry of the curing unit and the light intensity output significantly affect the polymerization of the material used (43). Visible LCUs are used for polymerization of composite resins, resin-modified glass ionomers, polyacid-modified resin composites, fissure sealants, binding agents, periodontal materials, adhesive agents and temporary restorative materials (44). Owing to advances in the field of LCUs, four visible curing lights, each functioning through a different system, are available for use in dental practice (45, 46). These include:

1. Quartz-tungsten halogen (QTH) LCUs
2. Plasma-arc curing (PAC) LCUs
3. Argon-Ion Laser LCUs
4. Light Emitting Diode (LED) LCUs

2.1. Quartz-Tungsten Halogen (QTH) LCUs

These LCUs developed in 1970s after UV systems are still in use. Also known as conventional LCUs, these units use a halogen bulb with a tungsten filament as the LCU (47). QTH lamps produce light energy when the tungsten filament is heated in a halogen gas such as iodine or bromine as the electricity flows through it (48). These units produce visible blue light at a wavelength ranging from 380 to 520 nm and emit light at an intensity of 300-850 mW/cm^2; they can polymerize composite resins up to a depth of 2 mm in 40 seconds (49). Halogen LCUs are systems that irradiate blue light needed for polymerization by absorbing white light and filtering out the light at different wavelengths (47).

The most expensive component of halogen LCUs is the filter mechanism. Excessive heat may be produced when these filters lose their properties and fail to filter the light and this may cause adverse effects on the dental tissues (50). For this reason, light efficiency of these devices should be checked and tested periodically. During the production of light by the device, heat is released by the halogen lamp and shortens the lifespan of the lamp. This heat generation requires a cooling fan which may be noisy. If the power unit is shut down immediately after use due to noise, evaporated tungsten atoms cannot return to the surface of the filament, resulting in a short bulb life (51). Additionally, since the resulting heat leads to extra energy requirement, it can lead to a reduction in both the light output and the operating time of the device. The lifetime of the bulb is approximately 100 hours and the light output decreases with continuous use (24). Also, it has been observed that many QTH LCUs fail to achieve the minimum light intensity specified by the manufacturers. All of these shortcomings may cause inadequate polymerization of the dental restoration (52). It was reported that the QTH bulb converts 70% of electrical energy to heat and only 10% produces visible light and only 0.5-2% of the energy input is emitted as blue light (53, 54).

Figure 2.A halogen LCU and its technical features (Hilux 250-Benlioğlu Dental Ankara, TURKEY)

In order to eliminate the long curing time of conventional halogen LCUs, high-power halogen LCUs have been developed which are less expensive than plasma arc curing light and argon lasers and provide faster polymerization (55). These devices have a higher light intensity, which is greater than 800-1000 mW/cm². Increased light intensity is achieved using high-power lamps. Another method is to use turbo tips that collect the light and concentrate it on a smaller area (56, 57). Although polymerization is achieved in a shorter time due to increased power, there are also studies reporting increased polymerization shrinkage forces of the composite (58).

The polymerization efficacy of QTH LCUs have been evaluated by several studies in terms of polymerization depth and surface hardness. In one study, the effects of a QTH and second-generation LEDs on the depth of polymerization and surface hardness of three different composites were assessed. Second-generation LEDs were found to achieve greater depth of cure than the QTH in all composites but no difference was observed between QTH and LEDs with respect to surface hardness in two composites (59). Another study compared the effectiveness of LED LCU (light curing unit) and halogen LCU on the degree of conversion of 7 different composite resins and LED LCU was found to have a superior effect on the degree of conversion than halogen LCU in all study groups (60). In a study by Ceballos et al., two composites were polymerized by LED and QTH LCUs for 20 seconds and LED LCU was shown to have a better polymerization efficacy than QTH LCU (61). In contrast to aforementioned data, there are some studies reporting comparable or better results using QTH LCUs (62, 63).

Halogen light curing units are larger than other LCUs and the production of blue light is more complex. Another drawback is that there is a risk of blowing microorganisms into the mouth by the cooling fan (31, 61). New LCUs have been developed due to several disadvantages of QTH curing lights such as excessive heat generation, lamp lifetime limited to hours, difficulty of disinfecting the filters, deterioration of the reflector and filters over time, noisy operation of the ventilating fan and reduced light intensity (64).

2.2. Plasma Arc Curing (PAC) Lights

Plasma arc curing lights are LCUs that can deliver a considerably high light intensity output (≈ 2400 mW/cm^2). These units produce light from hot plasma that forms between two tungsten electrodes that are surrounded by xenon gas (38). The polymerization time of composite resins can be shortened to a great extent owing to this high light power intensity (65). PAC lights have a broader range of wavelengths (31). An exposure of 10 seconds from a PAC light is equivalent to 40 seconds from a halogen light curing unit (24). Polymerization performed in 3 seconds with these LCUs showed similar conversion rates with a polymerization using halogen LCUs in 30-40 seconds (47). Currently, they are mostly used for bonding orthodontic brackets and bands.

Plasma arc curing lights work at wavelengths between 370 nm and 450 nm or 430 nm and 500 nm (43). Thus, polymerization of systems with both camphorquinone and photoinitiators other than camphorquinone can be achieved. As with halogen light curing units, PAC units also have filters that filter out other lights and allow emission of blue light. Although PAC units are more powerful LCUs than halogen LCUs, their use is not recommended for polymerization of composites. The hardness values obtained by polymerization with plasma arc curing lights have been shown to be lower than LED and QTH devices (66). PAC units were reported to cause more polymerization shrinkage of composite resins and increased microleakage at enamel and dentin margins due to their high light intensity (67, 68). In addition, excess heat released during the production of high intensity light was reported to cause pulpal damage (38).

2.3. Argon-Ion Laser LCU

Argon lasers, one of the lasers available for use in dental practice, have a wavelength range of 450-502 nm and are used for polymerization of composite resins (38, 69). They require shorter exposure times for curing composite resins in comparison to conventional visible light curing units (69). Since these devices do not emit infrared light waves, not much heat is produced that can potentially harm tissues (38). Composite resins polymerized using laser were reported to have better mechanical and

physical properties, with a lower rate of residual monomers than conventional LCUs (70, 71). No loss of energy occurs in these systems as seen in halogen light curing units. These laser systems do not require a separate filtration process.

Argon-ion laser LCUs produce a very narrow beam of light and work at specific wavelengths in the range of 454-466 nm, 472-497 nm and 514 nm (43). A number of disadvantages have been reported including the failure to cure composites with photoinitiators other than camphorquinone due to the narrow bandwidth of these systems, and their high cost (38). Additionally, the use of these units is limited because the device is large and not portable and not regarded much practical for composite polymerization by clinicians.

2.4. Light-Emitting Diode (LED) LCU

LED LCUs have been developed to address the shortcomings of halogen LCUs and overcome their disadvantages (63). These LCUs transmit a visible blue light with a wavelength spectrum of 440-485 nm that is sufficient for activation of camphorquinone, a polymerization initiator contained in many composite resins (47, 72). No heat dissipation occurs since the light is generated during the passage of electrons through gallium nitride semiconductors in LED units and filtration is not required in these systems because they emit light only at a specific wavelength (73). LED light-curing units are portable systems with low power consumption and operate quietly because they do not need a cooling fan (74). 95% of the light generated can be used for polymerization, resulting in high energy performance of the curing light. These devices produce light at a low voltage (1–4 volts) and therefore, are not dependent on the mains power (75). Compared to halogen LCUs, these lamps have a long lifetime (≈ 10,000 hours) and no fluctuations are observed in the light intensity since the system does not produce heat and contain a fan (76).

2.4.1. First-Generation LED LCUs

The first LED LCUs manufactured were experimental prototype models that produced light at the correct wavelength and deliver a sufficient number of photons required for the polymerizations of light-cured resin-based dental materials (77). This first LED-based devices contained arrays of many individual LED emitters (cans) (57). In fact, although these LCUs had a low irradiant output power, they have attracted great attention because they were cordless, lightweight devices that required low maintenance and the LED light could last for thousands of hours (51). Despite their low light power, they could provide a comparable level of polymerization with QTH LCUs by extending the polymerization time (78). However, since the first-generation LED lights had a narrow emission spectrum,

they may not be fully absorbed by composite resins with polymerization initiators sensitive to a different wavelength than that of camphorquinone (79). In addition, first-generation LCUs introduced by manufacturers used a large number of low-energy diodes (38) producing a light output of approximately 400 mw/cm^2 (59) resulting in inadequate polymerization (80).

2.4.2. Second-Generation LED LCUs

Second-generation LED LCUs have been developed because first-generation had insufficient light output and required a long time for adequate polymerization. Initially, thermal pads were developed that emitted smaller and more efficient light, instead of LED cans as separate units (51). In this way, new generation LED LCUs were manufactured using a smaller number of powerful diodes and had a broader wavelength spectrum between 430 nm and 490 nm. They produced an irradiance output of approximately 1000 mw/cm^2 and allowed a short polymerization time with sufficient performance (31, 81). A shorter polymerization time achieved by 2nd generation LED lights compared with QTH LCUs boosted the demand for these LCUs. However, cooling fans or metal heat sinks were placed in some of these devices to aid dissipation of heat because of potential overheating and malfunction of the device due to higher light output (51).

Figure 3. Examples of second-generation LED LCUs (Elipar S10, Elipar DeepCure-S -3M ESPE, MN, USA)

In a study investigating the effect of a halogen LCU and a LED curing light on the depth of polymerization and microhardness of 8 different restorative materials, the LED device showed better performance than the halogen lamp in both of the tested parameters (53). In another study, the wear and microhardness of a composite resin cured with a halogen light curing unit and LED LCU were compared and greater microhardness and

less wear were reported with the LED LCU compared to the halogen LCU (82). In a study by Antonson et al., 10 different LED light-curing units including 1st and 2nd generation devices were compared with a halogen LCU (Optilux 501) for depth of cure using the etch test and found that the greatest depth of cure was achieved in samples cured with a LED LCU for 10 seconds and 20 seconds. The authors also found that an exposure time of 20 seconds or longer provided a better depth of cure and a polymerization time of 40 seconds was needed for optimal polymerization (83). In contrast to the aforementioned studies, there are some studies in the literature that demonstrated better results in certain parameters with halogen LCUs (62, 63).

2.4.3. Third-Generation LED LCUs

With the increased popularity of tooth whitening, the bright yellow color of camphorquinone contained in the composite resin made shade selection difficult after bleaching. New photoinitiators have been developed both for this reason and to improve polymerization efficiency in parallel with the advances in the formulation of the materials over time (57, 78). The new 3rd generation (polyvawe, multiwave, multipeak LED LCUs) LCUs can generate light at two or more wavelengths and activate photoinitiators such as mono acyl phosphine oxide (Lucirin TPO), Ivocerin or 1-Phenyl-1,2-Propanedione (PPD) which are found in the structure of some composites (84). These LCUs produce both violet light with a shorter wavelength and blue light with a longer wavelength (27). While violet light activates photoinitiators that are sensitive to a light within the range of 350-420 nm wavelength, blue light generally activates camphorquinone which is sensitive to a wavelength of 468 nm (85, 86).

Figure 4. An example of a third-generation LCU and its technical features (Valo Cordless – Ultradent, USA)

In these LCUs, the light emitting diodes generating light at diverse wavelengths are arranged on different planes at the tip of the device, which can hinder homogeneous polymerization of the resin-based composite materials (87, 88). The type of photoinitiator found in the composite resin should be taken into account when using these devices. Using these LCUs for composites containing only camphorquinone as the photoinitia-

tor might adversely affect polymerization quality. In addition, if the product range of the manufacturer consists of LCUs that emit light at a single wavelength (as in the case of 3M products), the composites are manufactured accordingly and there is no need to use 3rd generation LCUs for such products (51).

CONCLUSION

A good polymerization of resin composites is essential to achieve a long-lasting dental restoration with the desired mechanical properties. LCUs used for the polymerization of resin-based composites which hold an important place in restorative dentistry are among indispensable equipment of a dental clinic. In this context, recognition of the polymerization properties of the composite resins and the features of LCUs is crucial. An important step in the polymerization involves choosing the right LCU and using it for the intended purpose. With these considerations in mind, the current study discussed polymerization, features of polymerization and LCUs in detail.

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