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CONTENTS

Chapter 1

SCROTAL EMERGENCIES AND MANAGEMENT

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INTRODUCTION

Acute scrotum is a urological emergency occurring clinically with suddenly emerging scrotal swelling, rash, pain, or sensitivity. Symptoms can start in minutes depending on etiology or they can be defined as symptoms continuing for 1-2 days. The rapid evaluation of acute scrotum for diagnosis and treatment has vital importance. Testicle torsion should be treated within six hours to prevent irreversible events which may occur as testicle and organ loss and also deaths due to epididymal-orchitis secondary sepsis in patients with advanced age and immune deficiency (1-3).

ETIOLOGY AND EPIDEMIOLOGY

Ischemic, traumatic, infectious, inflammatory, neuropathic and idiopathic causes are among the cause etiologies of acute scrotum. Acute epididymo-orchitis, testicle torsion, appendix testicle torsion, varicocele, hydrocele and inguinal hernia are among the most common causes. Prevalence of these diseases may differ based on age. While the most common acute testicle cause is appendix testicle or testicle torsion in children, it is epididymitis in males over 25 years of age.

Even though almost all of possible diagnoses in acute scrotum are severe and cause severe organ failure results, testicle torsion, strangulated hernia, fournier gangrene leading infections, traumas occurring with hemorrhage and testicular rupture are conditions requiring urgent surgery (1-4).

Although the incidence of patients referring to emergency room with acute scrotum complaints is not completely known, the ratio of patients referring with male genitourinary complaints is between 0.5-2%. In a study on 238 child cases, acute testicle torsion, appendix testicle torsion and epididymitis ratios of patients referring to emergency room with acute scrotum were reported as 16%, 46% and 35%, respectively (2,3).

Even though testicle torsion can be observed in any age, its prevalence decreases dramatically with advanced age. On the other hand, prevalence of appendix testicle torsion as the result of acute scrotal pain in childhood is higher than testicle torsion prevalence (4,5).

Epididymitis is the most common cause of acute scrotum in adult patients. The estimated number of patients diagnosed with epididymitis in emergency room per year in USA is 600 000.

TESTICULAR TORSION

Testicular torsion, in other words spermatic cord torsion is formed by the turning of spermatic cord around itself together with its vascular structures. It is a condition requiring urgent surgery to protect the testicle from ischemia.

Its prevalence up to 25 years of age is 1/1500-4000. Testicular torsion can be observed as intra vaginal and extra vaginal. Extra vaginal torsion is generally observed in neonatal period in which the testicle is hypermobile as it cannot complete its descend to scrotum with tunica vaginalis. Invaginal torsion generally occurs with infarct and ischemia caused by the decreasing of arterial blood flow to testicle because of spermatic cord turning around itself due to Bell-Clapper deformity (6,7).

Testicle torsion can be both complete and incomplete. Thus, early ultrasonographic image of a patient referring with testicle torsion symptoms can be reported normal.

Although patients generally refer to the emergency room with pain, nausea or vomiting, swelling or rash in the testicle, abnormal testicle position, cremasteric reflex loss starting suddenly or lasting 24 hours, it should be kept in mind that these findings are not only for testicular torsion findings.

High resolution scrotal doppler ultrasonography is the golden standard of testicle torsion diagnosis. It has 85-100% sensitivity and 75-100% specificity. Urine analysis and culture in addition to scrotal doppler ultrasonography are examinations which should be demanded for eliminating other acute scrotum causes like epididymitis.

In recent years, Testicular Workup for Ischemia and Suspected Torsion (TWIST) which is a clinical evaluation score has been defined in testicle torsion predictivity and testicular swelling was scored 2 points, hard testicle 2 points, cremasteric reflex loss 1 point, nausea or vomit 1 point and testicle ascending 1 point in this scoring. Based on the total of these scores, 0-2 points was accepted to be low, 3-4 points as average and 5-7 points as high risk for testicle torsion. In the studies made, TWIST sensitivity was reported as 76%, specificity as 100% and positive predictivity value as 100% for testicle torsion (8,9).

Testicle torsion in a condition requiring urgent surgical intervention. Delayed cases result in organ loss. Benefiting from surgical treatment is directly related to torsion duration and torsion degree. Although torsion degree may change, 720 degree turn may be required for complete torsion. Clinical presentation generally starts with venous occlusion related congestion. Heterogeneous and hyperechoic ultrasonographic image occurs with arterial occlusion and ischemia. While hemorrhage and infarct findings are observed in the first two hours, irreversible ischemic findings appear after six hours. Spermatic cord torsion related infarct occurs due to testicle turning 720 degrees. Although ischemia starts as early as 4 hours, it may cause irreversible damage after 24 hours. While testicle saving ratio is 90-100% in patients referring to emergency room with testicle torsion when surgery is applied in first six hours, testicle saving ratios in patients applied surgery after 24 hours decrease below 10% in order (6,7).

APPENDIX TESTICLE TORSION

Appendix testicle torsion is the most common cause of acute scrotum in emergency room referral with scrotum complaint in childhood and adolescence with a ratio of 40-60%. Its distinction from testicle torsion is important in distinctive diagnosis in addition to its benign course. It has clinical presentation similar to testical torsion. Early appendix testicle torsion can be distinguished easily from other acute scrotum cases with mass finding as little as a rice that is quite sensitive at the epididymis head in the physical examination. The blue spot seen as the marker of appendix testicle necrosis and infarct on scrotum on the spot corresponding to the spot where appendix testicle is located is pathogonomic for appendix testicle torsion. Ultrasonographic findings are bleeding increase around appendix testicle and enlargement of these structures (5-8).

EPIDIDYMITIS/ EPIDIDYMO-ORCHITIS

Epididymis located on the posterosuperior of the testicle and the sperms maturize here before ejaculation. Epididymitis is defined as the infection or inflammation of epididymis. It is generally unilateral although it can be bilateral rarely. Its etiology and treatment differ according to age and active agent. It is the most common cause of acute scrotum in adult patients. Its inflammation occurring with the testicle is called epididymo-orchitis. The concurrence ratio of epididymitis and orchitis is over 50%. Epididymitis or epididymo-orchitis generally occur as the reflux of bladder and urethral infections on ejaculator channels and spreading on epididymis. While the cause of epididymitis is generally sexually transmitted chlamydial or gonore infection with a lower ratio in males between 15-30 years of age, gram negative urinary pathogens such as E.coli which is the prostatitis cause in males over 35 years of age and don't have sex partner are dominant. Mumps virus is the most common viral cause. It should be kept in mind that atypical agents may also be epididymitis causes in patients with immune deficiency (9,10).

Gradually increasing scrotal pain seen rather than suddenly starting scrotal pain and patients also define complaints such as dysuria, frequent urination and urgency.

Patients should be questioned carefully for any trauma, previous sexually transmitted disease, urinary tract infection, prostatitis, previous surgery and urinary catheterization.

A complete genital examination should be done, scrotal swelling degree, hyperemia, scrotal temperature increase, sensitivity in posterosuperior of testicle and epididymitis should be evaluated carefully. Decreasing of pain with testicle examination is evaluated in favor of epididymitis (Prehn finding). Contrary to testicle torsion, cremasteric reflex loss doesn't disappear in

epididymitis. Testicle is in normal position contrary to the testicle torsion in epididymitis or epididymo-orchitis. Again penis and urethral meatus evaluation for urethral discharge and rectal evaluation should certainly be done to search the focus of infection rather than diagnosing epididymitis directly.

Although seen rarely, child epididymitis requires research on urogenital anomaly presence. The studies made demonstrated that urogenital anomaly was detected in more than 30% of the children under 14 years of age referring with epididymitis. Thus child patients referring with epididymis should certainly be investigated for structural and functional urological anomalies. The examination should be started with urine analysis and culture. Presence of white blood cell or erythrocyte in the urine according to urine analysis will give us an idea on acute infection or inflammation. In case of suspicion, urethral swab must also be done for detecting sexually transmitted diseases. Children under 14 years of age should be examined for urogenital anomaly and men over 50 years of age should be examined for infravesical obstruction (6-10).

Ultrasonography should be used both to evaluate genital organs and testicular blood flow.

Although the treatment should be planned against infectious agents, the principle is to start oral levofloxacin (Levaquin; 500 mg once daily for 10 days) or oral ofloxacin (300 mg twice daily for 10 days) treatment against most common infection agents (C. trachomatis, N. gonorrhea, E. coli). If sexually transmitted disease is suspected, ceftriaxone (single 250-mg dose) and doxycycline (100 mg twice daily for 10 days) or azithromycin instead of these two can be used for treatment. Scrotal elevation and ice application would provide dramatic recovery for decreasing pain and inflammation in epididymitis or epididymo-orchitis treatment (11).

Success is achieved through oral treatment in almost all patients but, if systemic symptoms, abscess formation or fournier gangrene leading findings are present in a few patients, they should be hospitalized and intravenous (IV) treatment should be started and surgical exploration should also be performed if required.

In addition to medication, the patients should be informed for sexual protection against sexually transmitted diseases, concurrent partner examination and for adequate fluid intake and not delaying urology control for recurrent urinary infection if urinary tract infection is present as an etiological cause.

STRANGULATED INGUINAL HERNIAS

Strangulated hernias are one of the inguinoscrotal pathologies which should be known in distinctive diagnosis especially in patients in childhood

in acute scrotum. The prevalence ratio among patients referring to the hospital with acute scrotum was reported in a wide range as 7-49% in different studies (4,5). In acute scrotum cases, it was observed that more than half of strangulated inguinal hernia cases were children and 80% of these were on the right side. Especially in neonatal cases referring with acute scrotum, strangulated inguinal hernia must be kept in mind in distinctive diagnosis. 80% of hernias are of indirect type and are herniated towards inguinal channel. Through auscultation inside the strangulated hernia testicle, intestine peristaltic movements can be heard.

Treatment is urgent surgery.

OTHER ACUTE SCROTUM CAUSES

Varicocele

It is the dilatation of pampiniform plexus veins inside spermatic cord. It is observed most commonly on the left side, as left gonadal vein is drained with right angle to renal vein. Advanced level of varicoceles may occur as the cause of acute scrotum with scrotal swelling and pain. Surgery is indicated generally due to varicocele caused infertility cases. Although surgical treatment is suggested for pain, the patient must be told that these pains may not end after the operation (12).

Hydrocele

Hyrocele is the accumulation of an abnormal level of fluid between tunica vaginalis layers. Although the patients refer with painless swelling complaint in scrotum, they may also refer with large hydrocele, complicated infected hydrocele cases can also refer with acute scrotal pain. Physical examination and ultrasonography are used for diagnosis. Its treatment is surgical (13).

Testicular microlithiasis

It is the presence of a large number of milimetrical calcifications in the testicle. It is more common in oligospermic patients. Although the etiology is not completely known, it can be considered to be related to Sertoli cell dysfunction. It commonly occurs together with some diseases such as undescended testicle and Klinefelter syndrome. Diagnosis is made randomly through ultrasonography in patients referring with scrotal pain. Due to suspicions on increasing germ celled testicle tumor, annual ultrasonography control and palpation are recommended. Symptomatic treatment for pain is recommended (12, 13).

Testicle tumors

Although testicle tumor patients refer to the doctor with painless masses, they can also refer with tumor-secondary hydrocele and hemorrhage-related acute scrotal pain.

Although ultrasonographic examination is 100% successful in diagnosis, testicle MR should be done in suspicion.

Surgery for testicle tumor is inguinal orchiectomy which should be done as quickly as possible (11-15).

Testicular rupture and hemorrhage

It generally occurs due to trauma occurring during sports, motorcycle accidents, attacks, falling in horse riding position or other kind of traumas. Trauma can be in different dimension such as complete rupture, hematoma, hematocele or testicle dislocation. Blood flow to testicle should be evaluated both for urgent sonographic imaging and parenchymal evaluation as the trauma may cause torsion of testicle. Urgent surgery should be applied both for preventing permanent organ damage or loss in testicular rupture or testicle parenchymal damage (4-6).

Fournier gangrene

Fournier gangrene is a polymicrobial necrotizing fasciitis with an aggressive clinical course. Although it is not observed commonly as an acute scrotum cause, fournier gangrene is important due to high morbidity and mortality. Immune suppressive, diabetic patients with scrotal pain and infection suspicion in perineum or genital area are risky patients for Fournier gangrene. Although computed tomography (CT) is the first choice in suspicion, ultrasound can also be evaluated as an urgent option in patients not suitable for CT. Treatment for fournier gangrene is urgent surgery and debridement followed by reconstruction and suitable antibiotherapy (12-15).

CONCLUSION

As there is no consensus on urgent approach in many clinics on diagnosis and treatment of patients referring to emergency room with acute scrotal pain, intervention duration may take time especially in testicle torsion cases. Thus urgent scrotal pain of urology should be considered as the acute abdomen of general surgery. It shouldn't be forgotten that we are racing against time in torsion suspicion and urological consultation should be demanded as soon as possible. As distinctive diagnoses have a wide spectrum, priority should be given to find out if urgent surgery is required or not considering prediagnoses based on physical examination, age and complaint starting of the patient.

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Chapter 2

EVALUATION OF THE MICROAESTHETICS (DENTAL DIMENSIONS AND PROPORTIONS) IN ORTHODONTIC FINISHING PROCEDURES - A LITERATURE REVIEW

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1. Introduction

Contemporary orthodontics efforts to achieve functional and aesthetic modifications within the limits of anatomic and physiological constraints through the examination of patients' three-dimensional dynamic relationships during both rest and functional states.(Brisman, 1980) The process of problem-based treatment planning entails two primary steps. Firstly, a diagnosis must be made to identify the specific aspects of the patient's smile that require correction, improvement, or enhancement. Secondly, a treatment strategy must be visualized and implemented to address these concerns and alleviate any worries the patient may have. The proposed strategy ought to encompass not only one side problems such as Class II skeletal malocclusion or open bite, but also encompass facial symmetry and smile esthetics.(Brisman, 1980)

Historically, it was posited that attainment of optimal dental alignment would result in compatible positioning of soft tissues, thereby resulting in an aesthetically pleasing facial contour. In contemporary times, advancements in computer imaging technology have enabled the examination of patients in frontal and vertical planes, facilitating static and dynamic evaluations in three dimensions of space.(Romsics et al., 2020) According to Sarver and Ackerman, it is recommended that the orthodontist include time as the fourth dimension in their evaluation process.(David M Sarver & Ackerman, 2003a, 2003b)

In contemporary times, the significance of facial aesthetics has been increasingly recognized, and possessing an attractive facial appearance provides certain benefits in every aspect of existence. Research indicates that individuals who are physically attractive, irrespective of their gender, are often perceived as more intelligent than those who are not. Additionally, their personal characteristics tend to be more highly valued, and they are more likely to attain successful and fulfilling careers, social relationships, and marital unions.(Dion, Berscheid, & Walster, 1972; Langlois et al., 2000) This phenomenon provides evidence for the concept of 'physical attractiveness stereotype', which suggests that individuals who are perceived as physically attractive are also perceived as possessing positive traits and characteristics. (Alomari, Alhaija, AlWahadni, & Al-Tawachi, 2022)

Following an orthodontic treatment, an apparent enhancement in an individual's self-assurance and overall quality of life can be noted. The enhancement of a patient's smile aesthetics has been shown to result in increased attractiveness, leading to following psychological benefits.(Machado, 2014) Rubin outlines three smiling models based on the main muscle movements of the lips and cheeks.(Rubin, 1974)

The Commissure Smile: Also referred to as the Mona Lisa Smile, is a facial expression that is frequently observed in social situations, such as when greeting someone in an elevator. The upward movement of the corners of the

mouth is a result of the straining of the zygomaticus major muscle during a commissural smile.

The Pose Smile: The facial expression commonly referred to as "Pose Smile" is also known as the "cuspid" or "social smile." The significance of the posed smile in the fields of dentistry and orthodontics lies in its temporal reproducibility. The present model of the smile involves the upward movement of the upper lip towards the superior region, thereby exposing the teeth.(Ker, Chan, Fields, Beck, & Rosenstiel, 2008; V. O. Kokich, Kiyak, & Shapiro, 1999) The movement of the lips bears resemblance to that of a window shade. This model is commonly observed in self-portrait photographs.

The Complex Smile: The smile in question is commonly referred to as the "real smile," "Duchenne smile," "spontaneous smile," or "sophisticated smile." The term "Duchenne smile" is derived from the research conducted by the French neurologist Duchenne de Boulogne. This type of smile is commonly referred to as the "heartfelt" smile.(Kiyak, 2008; Shaw, Rees, Dawe, & Charles, 1985) The act of smiling spontaneously is typically a reflexive response that is triggered by a sensation of happiness, and it is characterized by its dynamic nature. The phenomenon of a spontaneous smile, which involves the activation of all facial muscles, is consistently associated with a greater degree of lip elevation.(D M Sarver, 2001) The activation of the anterior temporal lobe, responsible for regulating the sensation of happiness in the brain, is triggered by the maximum smile accompanied by squinted eyes.

This review will focus on the finishing processes as a constituent of the microaesthetics concept, with the aim of achieving optimal outcomes in orthodontic treatment and enhancing the patient's smile. Finishing procedure is a comprehensive issue in orthodontic treatment. Given the increasing competitiveness of the field of orthodontics, achieving precise and optimal finishing has become increasingly crucial for both patients and practitioners.

The finishing protocols in orthodontic treatment can be categorized into four primary headings:

1) Fundamental concepts

2) Aesthetic orthodontic procedures refer to dental treatments that aim to improve the appearance of the teeth and smile

3) The utilization of multidisciplinary methodologies

4) Corrections of occlusion

Scholarly literature related to the finishing protocols in orthodontics highlights the significance of comprehensive planning that includes all stages, starting from the initial bonding of brackets to the ultimate finishing procedures. Incomplete delineation of steps in a plan may lead to an extension

of the duration of treatment and a possible decrease in the quality of outcomes. Thus, to provide effective treatment, it is essential to create a carefully designed treatment plan and to select appropriate orthodontic appliances based on the patient's case.

1.1. The Impact of Dental Dimensions and Proportions on The Perception of Aesthetics

It is important to acknowledge that the assessment of facial attractiveness is naturally subjective. Whilst the perception of facial beauty may be subjective, it is possible to objectively evaluate facial proportions.(Alomari et al., 2022; Brandão & Brandão, 2013; Romsics et al., 2020) The perception of aesthetics undergoes transformation in line with societal progression. However, the fundamental inquiry that arises pertains to the delineation of optimal standards.(Naini, Moss, & Gill, 2006)

Orce-Romero et al. conducted a study with the objective of identifying shared characteristics of aesthetic perception across diverse global populations. The study was conducted wherein the smiles of 500 renowned individuals, whose photographs were featured in Time Magazine, were analyzed. The researchers arrived at a conclusion that the vertical height of the upper lip, width of the smile, appearance of the upper incisor teeth, dental symmetry, and facial harmony are the key determinants of an attractive smile.(Orce-Romero et al., 2013a)

Additional research indicates a change in aesthetic perception, including that of dental professionals and other individuals.(Al-Johany, Alqahtani, Alqahtani, & Alzahrani, 2011; David M Sarver, 2011) To attain a pleasing smile, it is crucial to consider the microaesthetic factors. To achieve successful treatment outcomes, it is essential that orthodontists collaborate with dental professionals from various disciplines to establish a comprehensive and interdisciplinary approach.

According to Kokich, Kıyak, and Shapiro, individuals within social situations tend to initially evaluate the relationship between an individual's facial expression and smile, followed by a more detailed analysis of their dental features.(Kiyak, 2008; V. O. Kokich et al., 1999) Therefore; microaesthetics should be analysed together with miniaesthetics (smile) and macroaesthetics (facial harmony).

1.2. The Impact of The Chromaticity of Dental Enamel on Aesthetic Perception

The shade of teeth tends to be brighter and translucent in younger individuals, while it becomes progressively darker as age advances. This is attributed to the constriction of the pulp chamber, the development of secondary dentin, and the erosion of tooth enamel. The process of enamel

thinning results in a reduction in translucency, thereby leading to the increased visibility of the underlying yellow dentin layer.(Brandão & Brandão, 2013; King, Evans, Viana, BeGole, & Obrez, 2008)

The brightness of maxillary central incisors plays a crucial role in achieving a smile that is both aesthetically pleasing and natural. In the hierarchy of brightness and whiteness assessment, lateral incisors assume a position of second ranking, with canines following suit as the third-ranked tooth type. The hue of the premolar teeth is closer to the laterals, and it is lighter and brighter.(Brandão & Brandão, 2013; King et al., 2008)

1.3. The Dimensions of Crowns in Terms of Their Width and Height

While dental dimensions may vary among individuals and facial models, it is generally accepted that the height of central incisors depends within the range of 10.4-11.2 mm and the width depends within the range of 8.73-9.3 mm.(David M Sarver, 2004; Shillingburg, Kaplan, & Grace, 1972a) The main factor to be taken into account is the correlation among the dental proportions. (David M Sarver, 2004) The assessment of dental proportions needs the consideration of both height and width ratios of each tooth, as well as the overall proportion of the teeth.

Gillen et al conducted a study wherein they formulated the proportion of the width of upper anterior teeth:(Gillen, Schwartz, Hilton, & Evans, 1994)

a) The width of the lateral tooth is equivalent to 78% of the width of the central tooth (lateral tooth = central tooth x 0.78)

b) The width of lateral teeth is equivalent to 87% of the width of canine teeth (lateral tooth = canine tooth x 0.87)

c) The width of the canine tooth is equivalent to 90% of the width of the central tooth (canine tooth = central tooth x 0.90)

On average, the proportion of height to width of upper incisor teeth is approximately 75-80% for centrals, 66-70% for laterals, and 80-85% for canines. While there is no significant statistical difference in these averages between genders, females tend to exhibit wider teeth than males.(Orce-Romero et al., 2013b)

According to Gillen et al, it is necessary that orthodontic brackets be attached at an equivalent height on both central and canine teeth. The authors noted that to achieve an optimal aesthetic result, the height of the upper lateral clinical crown should be equivalent to 82% of the heights of the central and canine clinical crowns.(Gillen et al., 1994)

Prior to starting orthodontic treatment, it is necessary to identify any incisal

edge corrosion resulting from abrasion or attrition, as well as any inadequate restorations that require correction. During the duration of treatment, it is crucial to maintain these proportions and avoid any alterations while applying Air-Rotor Stripping (ARS).(V. G. Kokich & Spear, 1997) In cases where there is a loss of proportion of the upper anterior teeth, it is essential to consider treatment plan alternatives such as extraction, distalization, or maxillary expansion to facilitate the application of necessary restorations.

To ascertain the width of anterior teeth, it is recommended to employ formulas that consider the mean tooth width across various populations. The optimal dimensions of lateral incisors and canines have been established based on the dimensions of upper and lower central incisors, as clarified in the following discourse:

The variable Y represents the width of the upper central tooth, while the variable X denotes the width of the lower central tooth. As per established guidelines, the upper lateral width should be reduced by 2 mm in comparison to the central width, while the canine width should be decreased by 1 mm in comparison to the central width.(Chu, 2007b, 2007a) The upper central incisor exhibits a width that is 3 mm greater than that of the lower central incisor. Similarly, the lower lateral incisor shows a width that exceeds that of the lower central incisor by 0.5 mm, while the lower canine incisor is 1 mm wider than the lower central incisor. (Fig. 1)

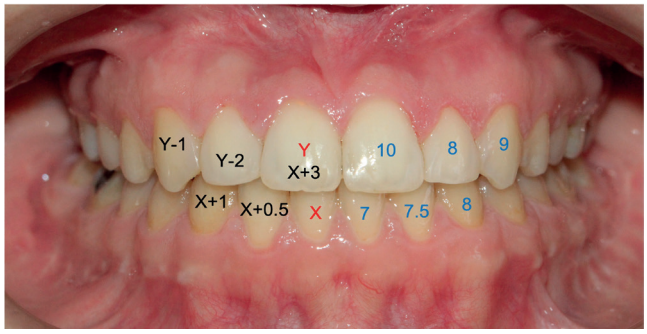

Figure 1. *Ideal proportions of anterior teeth for upper and lower arches*

The specifications have been formulated utilizing the measurements of the researcher, Chu, who additionally developed a gauge to quantify this proportion.(Chu, 2007b, 2007a) The gauge was brought to market by the company Hu-Friedy®.

The gauge is comprised of both a vertical and horizontal line, each of which exhibits distinct chromatic properties. To ascertain the optimal ratio of a tooth, it is necessary that the hue present on the horizontal axis, which indicates the width of the tooth, corresponds to that on the vertical axis, which

represents the length of the tooth. By utilizing this gauge, it is possible to ascertain tooth proportions without resorting to mathematical computations. This can ,be accomplished by examining the colour scale on the pre-established formula. (Fig. 2)

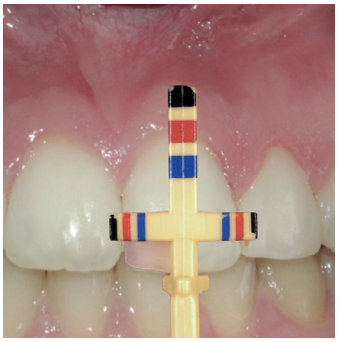

Figure 2. *Gauge for measuring ideal proportion of tooth designed by Chu and using of Chu's gauge*

1.4. The Impact of Virtual Crown Width on Aesthetic Perception

The precise dimensions of width and height are acquired via direct anthropometric measurements on teeth, providing the data reliant on absolute values. Virtual reality is an optical illusion that is created through the perception of visual beams.(Mayekar, 2001) An object's perception is facilitated by the reflection of light from its surface onto our retina at a 90° angle. Certain components of an object may not be perceptible to the human eye because of the refraction of light in a different direction or absorption. Shadows are a sign of the presence of curved surfaces that cause light to deviate from its original path. In contrast to the physical width of a tooth, the virtual dimension of teeth is an image that is subject to the influence of reflected light. The virtual dimension of a tooth is influenced by various factors, including the positioning of adjacent teeth, the morphology of the dental arch, and the anatomical characteristics of the individual tooth.(Mayekar, 2001)

The anterior part of the dental arch tends to be the primary focus of an individual's visual attention. The significance of the Golden Proportion lies in its ability to improve the aesthetic perception of the anterior teeth. (Lombardi, 1973) The prevalent perspective is that a structure or a work of art that adheres closely to the Golden Ratio may be considered aesthetically pleasing. Lombardi asserts that this ratio is similarly relevant within the field of dentistry.(Lombardi, 1973) According to the his statement, the display of the lateral teeth should constitute 62% of the central teeth while smiling, and the display of the canines should be equivalent to 62% of the lateral teeth. (Lombardi, 1973) Despite the similar width of the central and canine teeth,

the canine appears relatively small in a smile owing to its placement within the dental arch, the curvature of its surface, the sharpness of its incisal edge, and the distinct optical properties of its reflective qualities.(Lombardi, 1973) (Fig. 3)

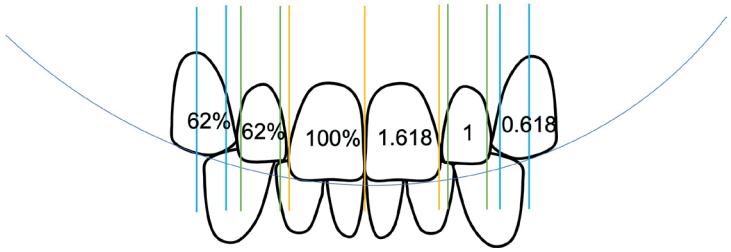

Figure 3. *Ideal appearance of upper anterior teeth while smiling*

A frequently utilized technique in prosthetic therapy involves the utilization of optical reflection of light to achieve distinct anatomical contours of central and lateral teeth during the restoration procedure. Through using an optical illusion, it is possible to cover up the actual dimension of a tooth and manipulate the perceived dimension to either expand or contract. Patients with a narrow maxillary arch may exhibit significant buccal corridors resulting from palatinal torques of posterior teeth, which can negatively impact the aesthetic outcome of their smile from an orthodontic perspective. Orthodontic treatment often involves the use of dental arches to achieve dental expansion, which can effectively modify the apparent width of teeth through optical illusion. The degree of this expansion is dependent on the buccal crown torque of the posterior teeth, which can result in the appearance of wider crowns. (Al-Johany et al., 2011; Janson et al., 2011; Mayekar, 2001) The observed outcome is an increase in the width of the patient's smile and the elimination of dark buccal corridors. The establishment of adequate torque movements in the canine and posterior teeth has a positive impact on the patient's aesthetic perception.(Al-Johany et al., 2011; Alomari et al., 2022; Brandão & Brandão, 2013; Rubin, 1974)

An additional factor that enhances the perception of aesthetics involves the proper alignment and levelling of teeth in a harmonious anatomical configuration on a basis, together with the appropriate placement of contact points. The presence of distal rotation in teeth may result in an appearance of microdontia due to the unavailability of the buccal surface for observation. Upon achieving a favourable occlusion in patients exhibiting varying smile models, the resulting light response will be optimal, thereby facilitating the creation of a highly authentic expression for the patient, according to the orthodontist's evaluation.(David M Sarver, 2004)

Duthie et al's study reveals that mandibular laterognathism patients

exhibit asymmetry not only in their mandibular region, but also in their basal base.(Duthie, Bharwani, Tallents, Bellohusen, & Fishman, 2007) Patients with significant facial asymmetries are typically managed through orthognathic surgery. In milder instances, asymmetry can be concealed using camouflage treatment.(Duthie et al., 2007) In situations of asymmetry, the maxillary tooth crowns result in buccal tipping after mandibular laterognathism.(Duthie et al., 2007) When fixing the buccal tipping of upper canines in these patients, it is crucial to consider the thickness of the bone in that area and apply the appropriate root torque for optimal results. Karring et al have reported that dehiscence and fenestration can occur in patients who did not receive orthodontic treatment. (Karring, Nyman, Thilander, & Magnusson, 1982) Before the application of thick and rectangular arches in orthodontic treatment, it is advisable to conduct a thorough periodontal assessment.(Bollen, Cunha-Cruz, Bakko, Huang, & Hujoel, 2008; Gkantidis, Christou, & Topouzelis, 2010; V. G. Kokich, 1996; Rubin, 1974) In situations where patients exhibit asymmetry and there is insufficient buccal bone, in addition to the inability to apply root torque of the canine, it may be necessary to employ techniques that create optical illusions. The canine tooth's crown is commonly divided into two parts, and a dental procedure known as enameloplasty is performed by utilizing finishing burrs to reduce the distal surface. Due to the absence of light reflection in the region, the distal aspect of the canine tooth will be hidden during the patient's smiling motion. After undergoing enameloplasty, it is necessary to apply polishing to the buccal surface of the canine and recommend the daily use of 0.05% sodium fluoride mouthwash.

1.5. The Impact of Virtual Crown Height on Aesthetic Perception

The utilization of optical illusion to modify the virtual width of teeth can also be applied to adjust the virtual height of crowns. The amount of reflected light can exhibit variability based on the degree of anterior teeth torque and the inclination of the crowns in an upward or downward direction when viewed from a frontal perspective. Hence, a notable difference exists between the actual and virtual height. Torque control is an important factor to consider, particularly in orthodontic procedures that involve the extraction of the first premolar tooth. To address the needs of these patients, it is necessary to apply third order bend in the retraction arches or utilize high torque brackets. The inclination of the occlusal plane has an impact on the virtual height of crowns.(David M Sarver, 2004) Appropriately adjusting the torques of anterior teeth can improve the overall aesthetic outcome.(David M Sarver & Ackerman, 2003a)

Cephalometric radiography is utilized for the assessment of anterior teeth torque, and the determination of orthodontic treatment is based on cephalometric measurements. Photographs captured at appropriate angles can provide valuable

insights into the inclination of anterior teeth. To ensure accuracy, the center of a frontal intraoral photograph must align with the point of intersection between the midsagittal plane and the occlusal plane.(Hardan & Moussa, 2020; Kalpana, Rao, Joseph, & Kurapati, 2018; Shagam & Kleiman, 2011; Wagner, 2020) Ideally, it is recommended that the occlusal plane be oriented parallel to Camper's plane, which is defined as the line connecting the ala of the nose to the tragus of the ear. When the incisor teeth are situated in the natural head position, the clinical crown of the teeth reflects the maximum amount of flashlight. Insufficient palatinal root torque in centrals can be observed when the flashlight is directed towards the cervical. Moreover, if the flashlight is directed towards the incisal edge, an excessive amount of palatal root torque becomes readily apparent. The improvement of anterior teeth torques can lead to an observable enhancement in aesthetic appeal, particularly in patients with retroclined incisors in Class II Div 2. Upon completion of the treatment, the flashlight is observed to shift from the cervical region of the incisors towards the central region of the crowns, resulting in a perceived increase in crown length.(Kalpana et al., 2018; D M Sarver, 2001; Wagner, 2020)

Orthodontic treatment can also enhance the aesthetic appearance of individuals with upper and lower teeth that protrude. By avoiding treatments that involve tooth extraction to maintain the soft tissue profile, teeth retrusion can be accomplished using ARS. As a result, an increased virtual height becomes apparent at the end of the treatment due to the wider surface area of the clinical crowns reflecting light.(Kalpana et al., 2018; D M Sarver, 2001; Shagam & Kleiman, 2011; Wagner, 2020)

1.6. The Impact of The Upper Central Teeth on Aesthetic Perception

Kina and Bruguera emphasized that in the process of prosthetic restorations, it is essential to prioritize the central teeth in a smile to achieve a harmonious, strong, and attractive appearance.[38] Research indicates that an individual's sense of aesthetics may be influenced by the appearance of their upper central teeth. Specifically, individuals with prominent central incisors in their smile are often perceived as more attractive and youthful.(Alomari et al., 2022; Anderson, Behrents, McKinney, & Buschang, 2005; King et al., 2008; Shillingburg et al., 1972a; Shillingburg, Kaplan, & Grace, 1972b)

According to King et al's findings, the aesthetic appeal of the upper central incisal edge can be enhanced by lowering it 0.5-1 mm below the laterals. This finding should be taken into consideration during orthodontic treatments. It is essential that the incisal edges of the upper incisors follow to the curvature of the lower lip when smiling.(Anderson et al., 2005; V. O. Kokich et al., 1999; David M Sarver, 2004)

Prosthetic specialists can create novel aesthetic trends by modifying the curvature of a smile through prosthetic restorations.(V. G. Kokich & Spear, 1997; David M Sarver, 2004; Shillingburg et al., 1972b, 1972a) In some cases, orthodontic practitioners may collaborate with prosthetic treatment experts after orthodontic intervention, resulting in significantly improved aesthetic treatment results. Research on prosthetic interventions indicates that the enhancement of the prominence of central incisors in facial dynamics is dependent on the flatness of their surfaces, which facilitates greater light reflection.(Al-Johany et al., 2011; Gillen et al., 1994; Lombardi, 1973)

1.7. The Impact of The Height of the Contact Points on Aesthetic Perception

Ensuring the accurate placement of contact points and achieving a natural and deep appearance is of equal significance to achieving symmetry and width in a smile.

It is necessary for the height of the contact point of centrals to be equivalent to 50% of the crown height. The vertical dimension decreases gradually towards the distal aspect, with a reduction of 40% between the central and lateral incisors, and a decrease of 30% between the lateral incisor and canine.

Kina and Bruguera suggest that the alignment of the hypothetical line that connects the edges of contract points should be in line with the smile and adhere to the curvature of the lower lip.(Brandão & Brandão, 2013; V. G. Kokich & Spear, 1997; Orce-Romero et al., 2013b; David M Sarver, 2004) The term 'six horizontal smile lines' has been determined to refer to a set of lines that are often used in the analysis of smiles.(Brandão & Brandão, 2013; Naini et al., 2006)

There exist three methods to acquire the proportionality of contact points:

1) Crown height of anterior teeth can be increased through prosthetic restorations.(Alomari et al., 2022; Brandão & Brandão, 2013; V. G. Kokich & Spear, 1997)

2) The correction of gingival contours can be achieved through the surgical procedure of gingivectomy, which can result in the attainment of the optimal height and width proportions of incisors. During gingivectomy, it is necessary to ensure the adequate removal of tissue between the contact point and alveolar bone crest while also preventing the formation of black triangles. (Tarnow, Magner, & Fletcher, 1992)

3) Enameloplasty and interproximal enamel reduction are techniques that can be employed to improve the contact area, while embrasures can be created in the incisal edges.(David M Sarver, 2011) Interincisal embrasures are shaped as an inverted 'V' and they are narrow and symmetric between

centrals, asymmetric between centrals and laterals and larger and asymmetric between laterals and canines.(Alomari et al., 2022; Bollen et al., 2008; Brandão & Brandão, 2013; Gkantidis et al., 2010; V. G. Kokich, 1996)

Especially in female patients, sharp and marked interincisal angles making the tooth to appear masculine can be made rounder and wider through enameloplasty, and a feminine appearance can be achieved. Before applying this procedure, the patient must be informed and provided with the simulation of future teeth, drawn with a black dermographic pen.(David M Sarver & Ackerman, 2003b)

1.8. The Impact of The Teeth and Periodontium Relation on Aesthetic Perception

1.8.1. The Importance of Gingival Contour

The utilization of 'Pink Aesthetics' is recognized as a significant factor in dental procedures, whereby optimal outcomes can only be attained by modifying the periodontium and dental anatomy. This approach is essential for dentists to achieve the desired results.(V. G. Kokich, 1996; V. O. Kokich et al., 1999; David M Sarver & Ackerman, 2003a) The parameters for achieving a healthy gingival contour have been identified by Kokich et al.(V. O. Kokich et al., 1999) It is necessary that the margin of the central and canine gingival contours be placed at an equivalent level, while that of the lateral must be positioned 1mm lower. The gingival contour is of greater significance in instances where a high smile line and excessive gingival display are observed. There exist three potential approaches that can be utilized in cases where there is asymmetry in the gingival contour:

1) If the incisal edges are correctly levelled: Gingival hyperplasia can occur in patients with thick keratinized gingiva and inadequate oral hygiene. In these cases, orthodontic specialists may recommend that patients seek the expertise of a periodontal specialist to facilitate an increase in the clinical crown height. The measurement of sulcus depth is conducted using a periodontal explorer, while the removal of gingival tissue necessitates consideration of the average 2 mm biological width. Approximately three months post-surgery, the gingival tissue undergoes a restorative process and attains its ultimate morphology.(V. O. Kokich et al., 1999)

2) If there is an abrasion or attrition in the incisal edges and asymmetry is observed in gingival margin: The application of brackets ought to apply to the gingival levels rather than the incisals.(Gkantidis et al., 2010; V. G. Kokich, 1996; Tarnow et al., 1992) The process of collagen and elastic fiber readaptation following a gingival levelling intrusion requires a minimum of six months. Composite and prosthetic restorations are utilized during the finishing procedure to achieve a balance between the optimal dental proportions and

the proportions of the teeth.(D M Sarver, 2001; David M Sarver, 2011) After attaining the optimal gingival contours via teeth intrusion and, if required, gingivectomy, it is necessary to allow a minimum of three months for the gingival tissue to recover.(V. G. Kokich, 1996)

3) If there is a bone defect: Apical gingival recession, whether general or local, may be observed based on the patient's medical history. In these cases, a gradual dental extrusion technique is applied, followed by the wearing down of the incisal edges.(Gkantidis et al., 2010) The limit of the extrusion: The process of extrusion can be utilized until the point at which the proportions of the crown and root become equal. In situations, such as planned tooth extraction or think bone and soft tissue augmentation for implantation purposes, it is possible to exceed the established limit.(V. G. Kokich & Spear, 1997)

Gingival contours are especially important in the determination of the optimal timing for implant placement. It is known that the growth ends at age 16 for females and 18 for males. However, it is important to consider that dentoalveolar growth continues from 17 to 23 years of age.(Thilander, 2008) In contrast to teeth, implants do not exhibit the same degree of adaptability to the movement of surrounding bone. Consequently, the implant may become infra-positioned, leading to recession of the gingival tissue. To attain favourable results, it is imperative to consider the patient's age and smile line when selecting an implant for the anterior region. The removal of the implant is a difficult procedure resulting in huge bone defects.(Thilander, 2008)

The appearance of dehiscence-fenestration and gingival recession is commonly observed in patients with thin gingival biotypes, particularly during the application of buccal root torque.(Gkantidis et al., 2010; V. G. Kokich, 1996) The systematic review conducted by Bollen et al has demonstrated that even in optimal orthodontic treatments, a minimum of 0.03 mm gingival recession and 0.13 mm alveolar bone loss can be observed.(Bollen et al., 2008) In the patients, it is important to be careful in applying force in orthodontic treatments, and light and intermittent forces must be applied. (Fig. 4)

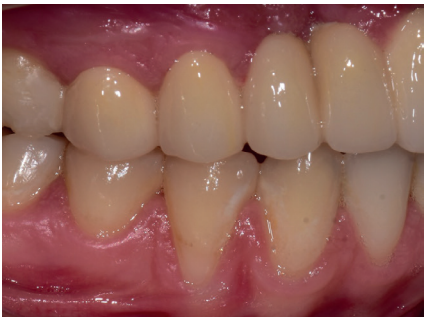

Figure 4. *Dehiscence in the lower first premolar*

1.8.2. The Importance of Gingival Zenith

The highest point of the gingival curvature in anterior teeth is referred to as the zenith point, which exhibits variability.(González-Martín, Lee, Weisgold, Veltri, & Su, 2020) According to Sarver, the highest point of the lateral tooth will be positioned at the midpoint of the crowns in alignment with the tooth's longitudinal axis. The zenith is displaced distally for the central and canine. (David M Sarver, 2004)

The gingival zenith of upper anterior teeth in healthy populations has been determined by Chu et al in a recent study:(Chu, 2007b)

- The zenith located on the central teeth is positioned 1 millimeter distal to the line that divides the middle of the crown along the long axis of the tooth.
- The zenith located on the lateral teeth is positioned 4 millimeter distal to the same line.
- The zenith located on the canine teeth is positioned at the center of the same line.

The gingival papilla located between the central teeth occupies approximately half of the height of the crowns, with a gradual reduction in height towards the distal region.

The location of the zenith is influenced by the characteristics of root morphology, enamel-cement structure, and periodontal tissues; therefore, the positions must be watched in placing the brackets on upper anterior tooth. Furthermore, the second order bends, commonly referred to as artistic bends that determine the mesiodistal tippings of the teeth, have an impact on the location of the roots and the zenith. The mesiodistal angulation of the anterior teeth is characterized by a greater degree of tipping on the lateral and canine teeth compared to the central teeth. Zenith deviation of central crown is 2 mm in a mesial tipping of 10°, which is the acceptable aesthetical limit.(Janson et al., 2011) (Fig. 5)

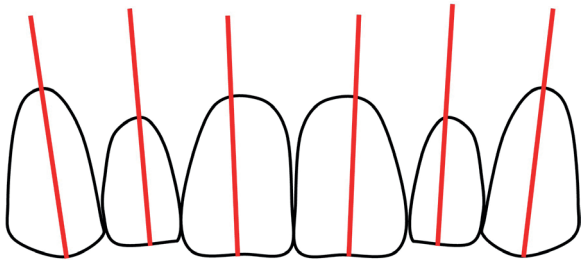

Figure 5. *Second order bends and zenith points of upper anterior teeth*

1.8.3. The Height of Papilla and Black Triangles

As previously stated, it is necessary that the interdental papilla is in the space between the teeth and expands apically towards the distal region. When the space between teeth is less than 0.3 mm, the interradicular bone is affected and papilla is not observed in this space. On the other hand, in large interradicular spaces as in diastema, papilla is shortened and flattened. (Fig. 6)

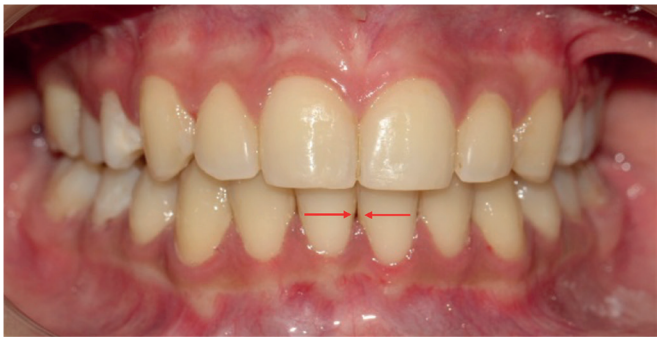

Figure 6. *Black triangle due to shorten and flattened papilla*

According to Tarnow, Magner, and Fletcher, the papilla is in 5mm from the alveolar bone crest, towards the contact point, in 98% of cases. When the distance from the contact point to bone crest is 6 mm, the possibility of existing black triangles is 50% and when it is 7 mm, the possibility increases to 73%.(Tarnow et al., 1992) The occurrence of black triangles is a common outcome following the correction of severe crowding in patients who have experienced bone loss due to periodontitis, as well as in cases where the teeth have a triangular shape. After aligning the teeth and closing the diastema, the dental arch form is corrected and the contact point is approximated by using interproximal enamel reduction in these patients.(Bollen et al., 2008; V. G. Kokich, 1996) In cases where teeth are excessively long and narrow, prosthetic restorations may be thought a viable option after to orthodontic treatment.

1.9. The Impact of The Proportions of Dental Arches (Bolton Discrepancy) on Aesthetic Perception

The Bolton ratio is a mathematical computation used to determine the correlation between the mesiodistal width of the mandibular and maxillary teeth. Measurement errors can happen in cases of severe dental crowding, as accurately measuring tooth width can be challenging. Additionally, if the desired occlusion cannot be achieved during the finishing procedure, it may be necessary to repeat the measurements to account for any Bolton discrepancy. (Shellhart, Lange, Kluemper, Hicks, & Kaplan, 1995)

Interproximal reduction or diastema opening between teeth is a common method used to eliminate dental volume variance between lower and upper teeth in patients with Bolton discrepancy. In cases where tooth extraction is required, particularly in the case of a lower incisor tooth, it is necessary to carry out a set-up procedure at the beginning of the treatment. This involves establishing the optimal overjet and overbite, and if necessary, applying torques to the incisor teeth towards the end of the treatment.(V. G. Kokich, 1996)

The main aim of orthodontic treatment is to achieve Class I molar relationship. Once the molar relationship has been established, various clinical cases may arise in relation to the Bolton discrepancy:

1) After establishing the Class I molar relationship, the presence of a Class II relationship in canines indicates an excess of material in the upper posterior teeth. In such cases, it is recommended to apply an ARS from the mesial of the upper molar to the distal of the canine.

2) After establishing the Class I molar relationship, the presence of a Class III relationship in the canines indicates an excess of lower posterior teeth material. In such cases, it is recommended to apply an ARS from the mesial of the lower molar to the distal of the canine.

3) After establishing the Class I molar and canine relationships, the presence of an anterior crossbite indicates an excess of lower anterior teeth material. In such cases, it is recommended to apply an ARS from the mesial sides of the lower canines to the entire incisor area.

4) After the Class I molar and canine relationships have been established, the presence of excessive overjet may indicate an excess of upper anterior teeth material. In such cases, it is recommended to apply ARS from the mesial sides of the upper canines to the entire incisor area. However, if the dimensions of the upper incisors are at risk of being affected by ARS, an alternative option could be to increase the clinical crown width of the lower anterior teeth.

2. Results

Microaesthetics involve therapeutic methodologies that are specifically associated with orthodontics and other dental professions involving aesthetic concerns. Comprehensive knowledge of all dental disciplines is necessary for the optimal success of orthodontic treatment. Excellence is not solely determined by a singular detail, but rather by a combination of numerous factors. Studies show that if ideal outcome cannot be achieved although the required procedure in finishing is performed, it is advisable to have the patient evaluated by a team of aesthetic professionals from diverse fields, to avoid expending time and unnecessary procedures.

The American Board of Orthodontics (ABO) stated that on the evaluation

of the presented cases, 'All cases orthodontically treated have some defects, and the cases herein exposed represent the effort of the professionals to achieve the certification of excellence.' The orthodontist must perform at his best in all cases and complete excellence must be sought, utopic though it is. It is recommended that microaesthetics be considered as an essential component of attaining both miniaesthetics and macroaesthetics. We should be patient to increase the life quality and self-esteem of the patient, which may be referred as 'hyperaesthetics' and we should keep being motivated by focusing on the strong effects of the millimetric alterations on people's lives.

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Figures Legends:

Figure 1: Ideal proportions of anterior teeth for upper and lower arches

Figure 2: Gauge for measuring ideal proportion of tooth designed by Chu and using of Chu‘s gauge

Chapter 3

ATRAUMATIC RESTORATIVE TREATMENT (ART) APPROACH IN MINIMALLY INTERVENTION DENTISTRY: BACKGROUND AND RECENT DEVELOPMENTS

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Introduction

Dental caries is a multifactorial disease characterized by demineralization of enamel. It is caused by biochemical changes in the hard tissues of the teeth as a result of microorganisms originating from the oral flora that settle on the tooth surface metabolizing the sugars taken with food and producing acid. The hard tissues of the teeth are perpetually affected by the dynamic alterations in pH levels within the oral cavity. When factors that maintain the pH at a critical level are not mitigated, the enamel structure experiences excessive mineral loss, resulting in enamel tissue loss and the typical cavitation characteristic of dental caries. Consequently, dental caries is distinguishable from other forms of progressive destruction such as mechanical abrasion on the tooth crown or chemical erosion.

Dental caries is acknowledged as the most prevalent bacterial infection globally. Carbohydrate-rich food ingestion, bacterial fermentation of these foods, and subsequent acid production are the primary factors contributing to the development of caries. In other words, caries is an event influenced by multiple factors, and its occurrence is unlikely in the absence of any one of these factors (Keyes, 1960). It is suggested that cariogenic bacteria, specifically Mutans streptococci, lactobacilli, and actinomycetes, have a role in caries formation. Mutans streptococci are believed to be responsible for caries initiation, lactobacilli for dentin caries, and actinomycetes for root caries lesions. The flow rate and buffering capacity of saliva, fluoride intake, carbohydrate consumption, bacterial levels, and oral care habits are all factors that can affect this condition.

Dental caries has the potential to be reversed in its early stages and can be halted up to a certain point. However, if the balance between demineralization and remineralization in the oral environment continues to shift in favor of demineralization, cavitation occurs (Fejerskov, 2004; Zero et al., 2001). The International Caries Detection and Assessment System (ICDAS) scores can be used to assess the progression of caries (Ekstrand et al., 2018; Frencken et al., 2021). Preventative measures may reverse caries when it is limited to enamel (ICDAS codes 1, 2, and 3). However, restorative treatment is required once caries reaches outer dentin (ICDAS code 4). Careful clinical and radiographic evaluation is crucial to ensure that no pulpal involvement is present (Ekstrand et al., 2018). Caries that has progressed to this extent can only be treated through interventional methods. To manage the issue of dental caries in the community, it is essential to first raise awareness of preventative measures and then ensure that appropriate treatment is administered.

Prevalence of dental caries

Since the mid-20th century, various studies have been conducted worldwide to measure the prevalence and severity of dental caries. Recently,

there has been a shift towards studies measuring the need for treatment. The key criteria for evaluating the oral dental health of a population include the prevalence of caries experience, the percentage of individuals with untreated caries, the average number of teeth in the dentition, the DMFT index (caries, missing, filled teeth index), and the percentage of edentulism (Saydam et al., 1990). The ages of 5, 12, and 15 are considered critical for assessing the distribution of dental caries.

Developing countries are faced with significant challenges in reducing the burden of dental caries, particularly with regards to permanent teeth. While the prevalence of caries in deciduous teeth is low in countries with high-income countries, the rate of increase in caries burden remains relatively high in some developed countries (Wen et al., 2022). This highlights the need for continued efforts to monitor and manage caries burden in these countries. In Spain, individuals have to pay for their dental treatments themselves rather than having them covered by an insurance plan or the government (Patel, 2012), which may be a contributing factor to the fast increase in caries burden. The rapid rise in the prevalence of caries highlights the need for a review of the current insurance system and for stronger efforts in preventing caries (Wen et al., 2022).

The prevalence of dental caries in permanent teeth is generally lower in high-income countries. This finding is consistent with the trend of decreasing lifetime prevalence of dental caries in high-income countries observed over the past 30 years (Frencken et al., 2017). Countries with higher income tend to have lower caries burden, which can be attributed to the implementation of robust dental public health programs. These programs involve significant investments and focus on various strategies such as reducing sugar consumption, promoting the use of fluoridated toothpaste, and improving oral health behaviors. These efforts contribute to better oral health outcomes and help prevent dental caries in populations (Wen et al., 2022).

The first assessment of oral and dental health in Turkey was carried out in 1988, following the criteria set by the World Health Organisation (WHO) in 1987 (Saydam et al., 1990). Subsequently, in 2004, the Turkey Oral and Dental Health Profile survey was conducted across the nation. Furthermore, the prevalence of caries in the age group of five years was higher compared to eight European countries, based on the WHO criteria (Gökalp, 2006). In Turkey, the prevalence of caries has been reported to be high across different age groups. The prevalence of decayed deciduous teeth was found to be 69.8% in the nationwide survey conducted in 2004. Caries prevalence was reported to be 61.1% at the age of 12 years, 61.2% at the age of 15 years, 75.8% in the 35-44 age group and 59.3% in the 65-74 age group (Gökalp, 2006). In a more recent survey conducted in 2018, the prevalence of decayed deciduous teeth decreased to 64.4%, but caries prevalence remained high, with rates of 46.6%,

58.5%, 63.8%, and 44.4% in the 12-year, 15-year, 35-44-year, and 65-74-year age groups, respectively (Tezel et al., 2021). This indicates that preventive programmes are not being adequately implemented in our country during the transition period from infancy to childhood to prevent dental caries. The high caries risk in Turkey can be attributed to factors such as inadequate oral care, infrequent dental visits, unhealthy dietary habits, and other risk factors. Thus, there is a pressing need for nationwide programs and initiatives to address this issue.

Burden of dental caries on society

The treatment of dental diseases is one of the most expensive medical interventions globally, resulting in significant loss of work and education hours each year due to the need for health services. This is because time spent in health institutions for treatment disrupts the workforce and education of children (Çubukçu, 2003). In developing countries, the high cost of dental treatment is mainly due to the lack of effective caries prevention programs and repetitive restorative procedures, which are linked to the treatment or restorative cycle. Preventive interventions aim to maintain the overall health of the community and protect public health by providing better quality services and reducing unnecessary payments and loss of labor force due to these diseases. A cost-benefit analysis was conducted in the report "Strategy Evaluation of Dental Health Services in Turkey" between therapeutic and preventive dental health services. The findings of the report suggest that investing one dollar in preventive dental health practices could save future expenditures of 8 to 50 dollars on restorative, emergency, and other medical treatments (Akar, 2014).

Dental caries is a significant burden on public health. In order to reduce this burden, not only preventive health services but also the treatment of existing caries is necessary. In response to this need, various approaches have emerged, but the most prominent of these is the atraumatic restorative treatment (ART) approach, which is one of the most effective methods. The ART was developed in the 1980s and encompasses all the principles of an alternative dental treatment philosophy known as minimal intervention dentistry (Dawson & Makinson, 1992a, 1992b). In minimal intervention dentistry, importance is given to caries diagnosis and assessment of caries risk, and the approach emphasizes the prevention, arrest, and remineralization of early lesions while selectively removing the diseased tissue to the maximum extent possible to preserve healthy tissues, followed by minimal invasive restorative treatment for dentin lesions. Although it was initially developed to meet the need for effective restorative and preventive treatment in underserved communities, the ART approach has become a global phenomenon in the last two decades (Holmgren et al., 2013).

After studies conducted in low- and middle-income countries showed

that ART provided successful results as traditional restorations, the idea emerged that ART could be an approach used in developed countries as well. In countries with higher income, there is often a recognition of special population groups that may benefit from specific dental treatments. These groups can include children, disabled individuals, anxious patients, or elderly patients, who may have unique dental needs and require tailored approaches to treatment. Providing appropriate dental care and addressing the specific concerns of these population groups can contribute to improving their oral health outcomes and overall well-being (Schmalz, 2012). Originally developed for communities with inadequate access to dental care, ART has since found its place in dental practices in developed countries, and several studies have been conducted (Burke et al., 2005; Honkala et al., 2003; Seale & Casamassimo, 2003; Ziraps & Honkala, 2002).

Atraumatic restorative treatment: The concept

The technique known as ART is a part of minimal intervention dentistry for managing dental caries. It involves removing the decay with hand instruments and then restoring the cavity with an adhesive material (Ercan et al., 2009; Roshan & Sakeenabi, 2011; van't Hof et al., 2006). This technique is particularly useful for patients who may not have access to traditional clinic settings, such as those in rural areas without electricity, and for individuals who have difficulty cooperating with treatment. ART provides a viable alternative to traditional dental treatments and can be performed in a variety of settings.

ART is not limited to low- and middle-income countries and has been adopted by many countries worldwide, including those in the BRIC (Brazil, Russia, India, China, and South Africa) and Western regions. Evidence shows that ART is now part of the dental curriculum in many countries and is used by private practitioners to supplement other treatment approaches. The ART method is gaining recognition globally (Navarro et al., 2009; Ruiz & Frencken, 2009). The use of rotating instruments to open the cavity, followed by manual cleaning and adhesive restoration, is not considered ART. The term "modified ART" is not appropriate for this technique (Frencken & Leal, 2010). The ART approach is gaining attention in many parts of the world and is used by private practitioners in various countries, including Brazil, Japan, Netherlands, UK, and the USA, as a complementary treatment approach (Frencken et al., 2012).

Atraumatic restorative treatment: The technique

In the context of the ART approach, the organization of instruments and materials follows a sequential order corresponding to their intended usage. To streamline the process and save time, it is beneficial to prepare an adequate quantity of cotton pellets in advance, considering their frequent utilization at various stages of the ART procedure (Holmgren et al., 2013). This practice

ensures that the appropriate size of cotton pellets is readily available when needed.

Effective isolation is crucial during restorative procedures to prevent contamination of the operating area by saliva or blood, which can compromise the adhesion of glass ionomer cement to the tooth surface. Isolation of the target tooth is achieved using cotton rolls, which should be replaced when they become saturated with saliva. The tooth's surface is then cleansed of plaque using damp cotton pellets, ensuring meticulous removal of any plaque or food debris present in the pits and fissures. Subsequently, the tooth surface is gently dried using either dry cotton pellets or air spray. Adequate isolation enables a clearer assessment of the extent of the carious lesion and the integrity of the enamel. Discoloration or translucency of the enamel often signifies demineralization, indicating potential weakness and potential lateral spread of the caries process along the enamel-dentin junction, beneath the enamel (Frencken et al., 1996; Holmgren et al., 2013).

In most cases, the administration of local anesthesia is not required when the ART technique is conducted properly. This is due to the reduced anxiety and discomfort associated with ART compared to conventional restorative treatments, as well as the fact that only necrotic (non-vital) dentin tissue is removed during the procedure (Holmgren et al., 2013; van't Hof et al., 2006).

In cases where carious lesions have a small cavity opening, it is frequently necessary to enlarge the entrance of the cavity. To achieve proper instrument stabilization, a dental chisel is employed along with a suitable finger rest. The corner of the instrument is positioned at the cavity entrance, typically at the deepest point of the pit or fissure on the occlusal surface. By applying gentle pressure, the instrument tip is rotated forwards and backwards. This technique effectively breaks the weak unsupported enamel surrounding the cavity entrance, enabling sufficient access to the carious lesion using the smallest excavator available (Holmgren et al., 2013; Leal & Takeshita, 2018).

Excavators are utilized in the ART procedure to eliminate soft and infected dentin. The process of removing carious tissue commences from the margin of the dentin-enamel junction. Thorough cleaning of the dentin-enamel junction is crucial to impede the progression of caries and to ensure optimal coverage of the restoration's coronal part. The smallest excavator is employed with circular scooping motions beneath the enamel. The excavated soft and infected carious tissue can be placed on a cotton roll positioned adjacent to the tooth. Removal of unsupported enamel is only necessary if it is thin, weak, or to facilitate additional access for the complete elimination of soft dentin around the dentin-enamel junction. A portion of the enamel can be gently fractured along the line of the enamel prisms using an enamel chisel. The cavity floor necessitates meticulous excavation, removing only soft tissue to

prevent exposure of the pulp (Frencken et al., 1996; Holmgren et al., 2013; Leal & Takeshita, 2018).

Numerous studies have extensively examined the condition of demineralized dentin beneath adequately sealed restorations, and the findings indicate that complete removal of all demineralized dentin is not necessary (Frencken & Leal, 2010). Removing all demineralized dentin hinders its potential for remineralization and needlessly compromises the integrity of the tooth structure. The extent of carious tissue removal and the amount left behind depend on factors such as the depth and size of the lesion and the risk of pulp exposure. According to the International Caries Consensus Collaboration, soft, decayed dentin should be eliminated while retaining hard, demineralized dentin. "Soft" refers to tissue that deforms when subjected to pressure from a hard instrument and can be effortlessly removed with minimal force, whereas "firm" denotes tissue that is physically resistant to manual scraping, requiring some instrument pressure for removal (Schwendicke et al., 2016). In deep cavities, it is advisable to retain some soft dentin at the cavity base to prevent pulp exposure, as suggested by the International Caries Consensus Collaboration (Frencken, 2017; Whitworth et al., 2005).

Following the complete removal of carious tissue, the cavity is thoroughly cleansed using wet cotton pellets and subsequently dried with a dry cotton pellet. As local anesthetics are not typically administered during the procedure, warm water is preferred during this stage to minimize tooth sensitivity. It is advised to refrain from using an air-water spray at this particular stage (Holmgren et al., 2013).

The utilization of manual instruments during the procedure results in the formation of a smear layer on the dentin surface. In order to enhance the chemical and mechanical bonding between the glass ionomer cement and the tooth tissues, it is imperative to eliminate the smear layer by employing a dentin conditioner. When employing conventional glass ionomer cement or encapsulated glass ionomer cement with a liquid component consisting solely of water, a dedicated dentin conditioner specifically formulated for this purpose should be utilized. Typically, a 10% polyacrylic acid is employed for this task. In cases where the liquid of the hand-mixed conventional glass ionomer cement contains the same acid as the dentin conditioner, it can be used as a dentin conditioner; however, the concentration is often too high and necessitates dilution. A cotton pellet is slightly moistened, dipped in a small amount of liquid, and applied to the cavity, pits, and fissures for a duration of 15-20 seconds. Inadequate or excessive surface conditioning may adversely affect the bond strength. The cavity, pits, and fissures are then rinsed and dried using cotton pellets. It is crucial to avoid contamination of the tooth surface with saliva or blood at this stage, as it can negatively impact the bonding of the glass ionomer cement to the dentin and enamel. If surface contamination

occurs, thorough rinsing and drying should be performed, followed by repeating the surface conditioning process (Holmgren et al., 2013; Frencken, 1997).

Just prior to the placement of the high-viscosity glass ionomer cement (HVGIC) in the cavity, it should be prepared by following the recommended powder-liquid ratio provided by the manufacturer. It was demonstrated that when HVGIC was mixed at a lower powder-to-liquid ratio than recommended by the manufacturer, there was a reduction in compressive strength and load-bearing capacity of the glass ionomer cement restoration (Fleming et al., 2003). Conventional glass ionomer cement utilized in the ART approach typically requires a higher powder-to-liquid ratio and may present greater challenges during the mixing process, thus necessitating careful attention. The mixed glass ionomer cement is introduced into the cavity using the round-ended hand instrument, starting from beneath the enamel ridges at the edges of the cavity. This technique helps prevent the entry of air bubbles into the restoration. In the case of encapsulated glass ionomer cement, it should be promptly placed into the cavity following capsule activation and mixing. The applicator tip of the encapsulated glass ionomer cement assists in delivering the material beneath the cavity while preventing the ingress of air bubbles. The glass ionomer cement is slightly overfilled into the cavity, and additional glass ionomer cement is placed in the pits and fissures adjacent to the cavity (Holmgren et al., 2013; Leal & Takeshita, 2018).

A small quantity of petroleum jelly, such as Vaseline, is applied to the gloved index finger and placed on the occlusal surface, allowing firm pressure to be exerted while introducing the glass ionomer cement into the cavity, pits, and fissures. The application of Vaseline serves to prevent adhesion of the glass ionomer cement to the glove, as well as to minimize the absorption and loss of excess water by the glass ionomer cement. The fingertip is gently rolled in both the buccal-lingual and mesio-distal directions, ensuring the even distribution of the material across the entire occlusal surface. After a minimum duration of 10 seconds, the finger is moved laterally to prevent the material from protruding out of the cavities, pits, and fissures, allowing any excess material to overflow and be readily eliminated. The surplus material is then removed using a scraper tool or a large excavator, all while avoiding displacement of the restoration (Holmgren et al., 2013; Leal & Takeshita, 2018).

Prior to the glass ionomer cement reaching an advanced state of hardness, the occlusion is assessed using articulating paper. Any areas of the restoration that are determined to be excessively high can be adjusted using a scraper tool or a large excavator. The occlusion is further evaluated by reapplying Vaseline onto the restoration, making necessary adjustments until the patient experiences comfort. Vaseline is applied one final time over the completed restoration. It is important to inform the patient not to consume any food for a

minimum of 1 hour following the procedure (Leal & Takeshita, 2018).

Clinical procedures can be customized based on the available equipment and the operator's typical working technique. Depending on the circumstances, local anesthesia, a rubber dam, or a rotary instrument may be employed instead of a dental chisel to achieve minimal access to the lesion site. However, it should be noted that the use of a rotary instrument deviates from the traditional ART approach. Given that the ART technique consistently yields satisfactory clinical outcomes, there is no requirement for methods or equipment that may heighten patient anxiety (Holmgren et al., 2013). Furthermore, it is inaccurate to categorize the utilization of rotary instruments in performing the ART procedure as modified ART. In cases involving proximal lesions and cavities, the utilization of matrix band and wedges is recommended to achieve the appropriate tooth contour and proximal contact point.

Cavity disinfection during ART procedure

Chlorhexidine is known for its wide-ranging antibacterial properties, effectively targeting different types of bacteria, including mutans streptococci (Gram-positive bacteria), Gram-negative bacteria, aerobic bacteria, facultative anaerobic bacteria, and fungi (Emilson, 1977). Studies have investigated the incorporation of chlorhexidine salts into glass ionomer cements and have demonstrated that this addition can enhance the antimicrobial activity of the cements while preserving their physical and chemical properties (Hoszek & Ericson, 2008; Takahashi et al., 2006; Türkün et al., 2008). However, conflicting results have been reported regarding the effects of chlorhexidine inclusion on the biocompatibility and mechanical properties of the restorative material. Certain studies have reported potential negative impacts on the biocompatibility and mechanical properties of glass ionomer cements when chlorhexidine is incorporated into their formulations. These effects may include compromised biocompatibility, reduced strength or hardness, altered setting characteristics, or changes in other physical properties (Jedrychowski et al., 1983). It is important to consider these findings when evaluating the suitability of chlorhexidine-modified glass ionomer cement for specific applications in clinical practice. Further research is needed to fully understand and address these concerns.

In a recent study, the use of chlorhexidine as a cavity disinfectant or its incorporation into glass ionomer cement has been investigated both in vitro and in vivo (Duque et al., 2017). The study concluded that glass ionomer cement containing chlorhexidine could be considered as an alternative to traditional glass ionomer cement used in ART due to its additional antimicrobial effect, which is particularly beneficial for children with high counts of mutans streptococci during the initial phase of treatment. The inclusion of chlorhexidine in glass ionomer cement enhances its antimicrobial

and antibiofilm properties in vitro, without causing any detrimental effects on cytotoxicity and mechanical properties. Follow-up evaluations demonstrated that ART restorations with chlorhexidine-modified glass ionomer cement exhibited a similar survival rate and superior antimicrobial performance compared to conventional glass ionomer cement at the 7th day (Duque et al., 2017).

Restorative component of ART

The choice of an appropriate restoration material with suitable biological and physical properties is a crucial factor in the success of ART. The material selected should possess properties that support restorative and protective functions, ensuring compatibility with the tooth structure and promoting favorable biological responses. Both the biological and physical properties of the material play significant roles in determining its effectiveness in the ART approach. While various restorative materials have been utilized in conjunction with ART, including resin composite (Eden et al., 2006; Topaloglu-Ak et al., 2008), compomer (Louw et al., 2002), and resin-modified glass ionomer (Ercan et al., 2009; Faccin et al., 2009; Cefaly et al., 2005), HVGIC has emerged as the most suitable adhesive material for ART.

In the ART approach, glass ionomer cements are commonly used as restorative materials due to their biocompatibility with oral tissues, chemical bonding to tooth tissue, and ability to prevent secondary caries formation through high fluoride release. Traditional glass ionomer cements have low fluoride release and a fragile structure, but the development of stronger HVGICs has addressed these limitations. Meta-analyses have shown that single-surface ART cavities restored with HVGICs have higher success rates compared to medium hardness glass ionomer cements (van't Hof et al., 2006). Multiple studies have emphasized the importance of viscosity in the success of ART, specifically in relation to glass ionomer cement. HVGIC products like Fuji IX (GC Dental Industrial Corp., Tokyo, Japan) and Ketac Molar Easymix (3M Dental Products, St. Paul, MN, U.S.A.) have been found to be more durable compared to low- or medium-viscosity glass ionomer cements such as GC Fuji lining LC (GC Dental Industrial Corp., Tokyo, Japan) and Ionofil Plus (Voco, Cuxhaven, Germany) (Bonifácio et al., 2009; Frencken, 2014; Shivanna et al., 2020; van't Hof et al., 2006). Initial formulations of glass ionomer cements with reduced viscosity were found to have lower wear resistance and inadequate compressive strength, especially in areas subjected to high stress (Bali et al., 2015; Mandari et al., 2003). In response to these limitations, manufacturers have developed advanced HVGIC formulations with a higher ratio of powder to liquid, resulting in enhanced physical characteristics including better resistance to wear, increased ability to withstand compression, and improved fit at the restoration margins (Yilmaz et al., 2006; Gok Baba et al., 2021; van

Duinen et al., 2005). These advancements have made HVGICs more suitable for use in ART procedures.

Studies have reported increasingly positive results in terms of the ART technique and outcomes. Long-term follow-up studies have demonstrated high success rates for ART restorations using HVGICs in single-surface cavities of both primary and permanent dentitions. Meta-analyses have revealed that the annual failure percentages for ART restorations are approximately 5% in primary molars during the first three years and 4.1% in posterior permanent teeth over the first five years (de Amorim et al., 2018). Furthermore, a recent systematic review indicated that there were no significant differences in survival percentages between ART restorations and traditionally placed multiple-surface restorations for single-surface restorations in both primary and permanent molars (Frencken et al., 2021).

A study comparing ART applications performed in school and clinical settings reported similar results at the end of one year, suggesting that ART may offer advantages in the school setting (Roshan & Sakeenabi, 2011). The success rates of class I cavity restorations in permanent teeth treated with ART and glass ionomer cement have been reported as 90-100% at one year and 59-88% at three years (Ercan et al., 2009; Roshan & Sakeenabi, 2011). Various factors such as the choice of material, operator experience, patient age, and oral care level have been found to influence the success rate in ART studies (Ercan et al., 2009; van't Hof et al., 2006). ART is considered a promising and alternative treatment method, particularly for individuals living in developing countries with low socioeconomic status, limited access to dental care, and no social security coverage, as it helps ensure the maintenance of oral health in such populations.

Silver diamine fluoride

Silver diamine fluoride (SDF) is an advantageous substance that can be incorporated into the ART procedure as a supplementary treatment (AAPD, 2018). SDF combines silver nitrate and sodium fluoride and, upon application to carious tissues, hinders the progression of the caries lesion by interacting with bacteria (Knight et al., 2007). SDF usage is indicated for individuals, particularly children, who may face difficulties in accessing dental clinics. SDF offers a minimally invasive approach wherein the material is applied to carious dentin without the need for tooth structure removal. It is important to note that one potential drawback of SDF is the possibility of tooth discoloration resulting from the presence of silver. Nevertheless, considering the overall benefits, the utilization of SDF in conjunction with ART is highly recommended (AAPD, 2018).

Modified Atraumatic Restorative Treatment with SDF (SMART) is an

approach that integrates the caries arresting properties of SDF with the ability of glass ionomer cement to seal the carious lesion. In SMART, SDF is applied to the affected tooth, followed immediately by the placement of a conventional glass ionomer cement restoration. Conventional glass ionomer cement is considered an ideal restorative material for SMART due to its ability to tolerate moisture and its compatibility with moist surfaces. SMART aims to combine three well-established clinical procedures, each supported by a strong body of evidence, while respecting the individual principles of each procedure. These three procedures are as follows: caries arrest using SDF (Gao et al., 2016), partial or incomplete caries removal (van Thompson et al., 2008), and placement of a conventional glass ionomer cement restoration (Mickenautsch & Yengopal, 2016).

SDF has been widely utilized in numerous countries as a means to halt or decelerate the advancement of caries in cases where conventional treatment methods are indicated (Gotjamanos, 1996). Its efficacy has been established since the 1960s, particularly in Asian countries, with minimal reported complications (Gotjamanos, 1996; Llodra et al., 2005). Clinical trials conducted in China and Cuba have demonstrated the effectiveness of SDF in preventing and arresting caries in primary teeth among preschool children (Gotjamanos, 1996; Llodra et al., 2005; Lo et al., 2001). Recent studies have elucidated three key mechanisms by which SDF contributes to the prevention and treatment of caries. These mechanisms include: a bactericidal effect on cariogenic bacteria such as *Streptococcus mutans* and others (Chu et al., 2012; Mei et al., 2013a, 2013b), promotion of remineralization and inhibition of demineralization in enamel and dentin (Chu et al., 2012; Mei et al., 2014), and reduction of collagen matrix degradation in dentin through the inhibition of collagenase (Mei, et al., 2013a, 2013b). Furthermore, the fluoride content of SDF plays a significant role in its efficacy (Buzalaf et al., 2011). Upon application to the tooth surface, SDF reacts with hydroxyapatite, forming silver phosphate and calcium fluoride compounds that act as reservoirs for fluoride and phosphate ions, facilitating remineralization (Buzalaf et al., 2011; Lou et al., 2011). Moreover, in the presence of microbial acid attacks, fluoride interacts with hydroxyapatite crystals on the tooth surface, preventing demineralization (Buzalaf et al., 2011).

The primary reported side effect of SDF is the black discoloration of carious enamel and dentin. (Gotjamanos, 1996; Llodra et al., 2005). This dark coloration is attributed to the formation of silver phosphate (Yee et al., 2009). Consequently, this side effect may limit the use of SDF in the anterior region, particularly for patients with early childhood caries who have high aesthetic expectations (Yamaga et al., 1972). Parents are more accepting of SDF applications in posterior teeth, while the black discoloration associated with its use raises aesthetic concerns for anterior teeth. However, in cases where

behavioral management techniques are ineffective, parents may be more willing to accept anterior tooth staining if it provides an alternative to dental treatment under general anesthesia (Crystal et al., 2017).

Chemo-mechanical caries removal

Minimal intervention dentistry, a philosophy that advocates for conservative approaches in dental care, has given rise to various concepts such as ART. In line with this philosophy, other conservative techniques have been proposed with the belief that their combination with ART could potentially enhance the outcomes of ART procedures. One such approach involves the use of Carisolv™ (RLS Global AB, Gothenburg, Sweden) gel, a chemo-mechanical caries removal system, to assist in achieving caries-free cavities before restoration (Kirzioglu et al., 2007). Chemo-mechanical caries removal, which comprises sodium hypochlorite and three amino acids, works by selectively softening dentin, making it easier to remove using hand instruments (Gil-Montoya et al., 2014). Despite early promising results, it has been observed that the addition of chemo-mechanical caries removal gel to ART does not significantly alter the prognosis of ART restorations (Seifo et al., 2019).

Selective caries removal

In contemporary dental practice, the management of carious lesions has evolved based on a better understanding of the caries process and an increased body of clinical evidence in operative dentistry. Traditional cavity designs, such as G.V. Black's designs, were primarily developed for dental amalgam restorations and involved the surgical removal of both carious infected dentin and additional tooth structure (Mount, 2008). However, it is now recognized that the removal of caries-affected dentin is not always necessary, particularly with the advent of adhesive bioactive and bio interactive restorative materials. Moreover, there is growing awareness of the potential for dentin remineralization, which has reduced the reliance on extensive tooth structure removal as required by the traditional cavity designs (Banerjee et al., 2017; Schwendicke et al., 2019).

The non-selective caries excavation approach, which involves excessive removal of tooth structure, can have negative short-term and long-term consequences. In the short-term, it can damage the dentin-pulp complex unnecessarily (Banerjee & Watson, 2015). In the long-term, it compromises the mechanical integrity of the tooth, increasing the risk of cracks, fractures, and related complications (Mackenzie & Banerjee, 2017; Schwendicke et al., 2016). Aggressive removal of healthy tooth structure in deeper cavities increases the likelihood of exposing the dental pulp and causing irreversible damage to the odontoblastic palisade, which can result in the loss of primary odontoblasts and their function (Banerjee & Watson, 2015; Bjørndal et al.,

2019). In contrast, selective caries removal arrests carious lesion activity, reduces the risk of pulpal exposure, and preserves the odontoblastic palisade, which promotes the deposition of reactionary dentin (Banerjee & Watson, 2015; Bjørndal et al., 2019; Schwendicke et al., 2016). It also minimizes the risk of bacterial ingress into the pulp, thus maintaining pulp vitality. This approach maximizes the tooth's prognosis and can reduce long-term management costs and burdens associated with dental care (Banerjee et al., 2017; Mackenzie & Banerjee, 2017; Schwendicke et al., 2016).

In deep cavities where there is a risk of pulp exposure, a calcium hydroxide lining material may be applied selectively, targeting the area closest to the pulp. However, in most cases of ART restoration, there is no need for a lining material unless the cavity is extremely deep. Excessive use of lining materials can reduce the available surface area for the adhesion of the glass ionomer cement (Holmgren et al., 2013). It is worth noting that calcium hydroxide has been found to stimulate dentin repair, and glass ionomers are considered biocompatible. A study by Weerheijm et al. (1993) demonstrated the hardening of soft dentin underneath glass ionomer fillings after a seven-month period. These findings suggest that glass ionomers can contribute to dentin repair and remineralization.

ART prioritizes the selective removal of carious lesions while preserving unaffected tooth structure. This approach minimizes the unnecessary loss of tooth tissue, thereby reducing the potential harm to the dental pulp and maintaining its vitality. Compared to traditional techniques involving local anesthesia and high-speed equipment, ART is regarded as a minimally invasive procedure in the treatment of dental caries. The objective of selective caries removal is to safeguard the mechanical integrity and resilience of the tooth while effectively managing the progression of caries, thereby enhancing the likelihood of long-term favorable outcomes. Consequently, the ART approach has gained prominence as a preferred method within contemporary dental practice.

Survival rates of ART restorations and sealants

An outcome measure refers to a specific parameter or criterion used to assess the effectiveness or success of a particular intervention, treatment, or procedure. In the context of ART restorations, outcome measures are used to evaluate the results and determine the overall performance of the restorations. These measures can include various factors such as symptomatology, clinical findings, radiographic evidence, functional outcomes, patient satisfaction, or the need for additional treatment or intervention. Outcome measures provide objective and measurable data that help in determining the success or failure of ART restorations and guide further decision-making in patient care.

Outcome measures used to assess the success of ART restorations can be categorized as primary or secondary (Molina et al., 2019). Primary outcomes focus on the absence of symptoms and the absence of clinical or radiographic evidence of caries progression in the treated tooth. Secondary outcome measures in ART restorations indicate the occurrence of failure, which could involve the need for retreatment due to secondary caries, replacement of a restoration that has failed or been lost, or more advanced procedures like root canal treatment or extraction. These outcomes reflect the compromised condition of the ART restoration and the need for additional interventions to address the underlying issues. These measures provide valuable insights into the effectiveness and longevity of ART restorations (Molina et al., 2019).

The survival rates of ART restorations can be influenced by the complexity of the treated tooth surfaces. Generally, single-surface ART restorations using HVGIC exhibit higher survival rates compared to multi-surface restorations (Smales et al., 2000; van't Hof et al., 2006). For example, a study found that after 12 months, Class I and V ART restorations had success rates of approximately 80-90%, while Class III and IV restorations had lower rates of 55-75% and 35-55% respectively (Mjör & Gordan, 1999). Similar investigations have shown that single-surface ART restorations in both primary and permanent teeth have one-year survival rates of 95% and 97% respectively. However, these rates decrease to 86% after 3 years and 72% after 6 years. These findings highlight the importance of considering the complexity of the restoration when evaluating the long-term success of ART restorations (van't Hof et al., 2006).

Retention rates of ART restorations have shown variation over time. One study reported higher retention rates in the first year (81%) with a minor decrease to 66% in the following year (Sharma et al., 2021). Other studies have reported relatively stable retention rates over a 3-year period, ranging from approximately 92% in the first year to 82% and 71% in the second and third years, respectively (de Amorim et al., 2012). Overall, there is growing evidence to support that HVGIC ART restorations have comparable or even longer survival rates compared to conventional restorative treatments and composite restorations (de Amorim et al., 2012; Smales et al., 2000). The survival rates of ART sealants were evaluated based on the extent of partial or complete dislodgement over a three-year period (van't Hof et al., 2006). Studies have shown that HVGIC ART sealants had higher survival rates compared to low- or medium viscosity glass ionomer cement and resin-based sealants when applied under the same conditions. The one- and three-year follow-up research indicated the superior performance of HVGIC ART sealants in terms of survival rates (Goldberg, 2020; van't Hof et al., 2006). These findings provide additional research evidence supporting the effectiveness of the ART approach in the treatment of dental caries.

Factors affecting success of ART

Despite the simplified nature of ART compared to conventional restorative approaches, the success of ART restorations can still be compromised or even fail due to disruptions in the procedural sequence. Various factors related to the operator, materials utilized, or deviations in application procedure have the potential to undermine the effectiveness of ART restorations (Mickenautsch & Grossman, 2006). In the field of clinical dentistry, the precise evaluation and justification of treatment approaches are essential for achieving favorable outcomes. Therefore, ensuring the suitability of patients for ART procedures assumes paramount importance. Additional operator-related factors encompass the attainment of adequate moisture control, adherence to recommended caries removal techniques as outlined in previous sections, and proficiency in handling HVGIC during material mixing and placement (Franca et al., 2011). Operator experience and training have consistently emerged as crucial factors influencing the triumph of ART restorations (Jiang et al., 2021; Luengas-Quintero et al., 2013). There is conflicting literature regarding the specific significance of moisture control, caries removal, and material handling in ART restorations. Different studies present varying perspectives and findings on the importance of these factors in the overall success of the procedure. The role of moisture control, caries removal techniques, and proper material handling techniques in achieving optimal outcomes in ART restorations remains a subject of debate and further investigation (Jiang et al., 2021), a consensus exists regarding the pivotal role of ART-specific training in augmenting the success rates of the procedure (Frencken & Leal, 2010; Jiang et al., 2021; Luengas-Quintero et al., 2013; Molina & Kultje, 2003; van't Hof et al., 2006).

Materials and technique are two additional factors that have been found to influence the long-term success of ART restorations (van't Hof et al., 2006). The choice and proper handling of HVGIC is crucial, including achieving the correct consistency through mixing. The technique involves appropriate excavation of soft dentin, proper cavity conditioning, and careful incremental placement of the mixed HVGIC into the cavity. Moreover, specific techniques such as the finger technique, involving initial pressure followed by sideways movement during removal, as well as thorough restoration finishing, as discussed in previous research, are important considerations to ensure the durability of the final restorations (van't Hof et al., 2006).

ART in pandemics

Due to the COVID-19 pandemic, dental professionals are required to wear full personal protective equipment during aerosol-generating procedures or when present in the room. However, this can be uncomfortable and affect performance, and there is a recommended fallow time of 15-30 minutes after the procedure, causing delays in seeing the next patient (Bahia et al., 2021). In

contrast, the ART technique is not considered an aerosol-generating procedure, and therefore, it does not require a fallow time or full personal protective equipment, including FFP3 masks or equivalent. This can potentially alleviate the burden on dental practices and enable them to address more patients' dental needs within a working day during the COVID-19 pandemic (Bahia et al., 2021).

The COVID-19 pandemic has exacerbated the need to address the existing inequality in caries burden. Even before the pandemic, studies have quantified the disparities in caries prevalence. The closure of schools during the COVID-19 pandemic has further disrupted school-based oral health programs, which have been instrumental in promoting oral health equity. These programs have proven highly effective in addressing oral health disparities (Gargano et al., 2019). Unfortunately, the World Health Organization (WHO) predicts that the ongoing pandemic is likely to widen existing oral health inequalities. It underscores the importance of addressing these disparities and implementing measures to mitigate the impact of COVID-19 on oral health outcomes (WHO, 2023).

The COVID-19 pandemic has brought about significant transformations in healthcare systems worldwide, prompting countries to implement urgent measures to control the outbreak. This unprecedented situation has highlighted the importance of early detection, prompt isolation of infected individuals, contact tracing, and widespread testing to mitigate the rapid transmission of the disease. It has also underscored the need for health systems to prioritize preparedness for emergency situations, including pandemics. Moreover, the pandemic has emphasized the value of digital health applications such as telemedicine, remote healthcare services, and online consultations. These technologies have played a crucial role in ensuring continuity of care and reducing the risk of viral transmission. Integrating digital health into healthcare systems is vital for future readiness and resilience.

In the field of dentistry, where aerosol-generating procedures are commonly performed, alternative approaches like ART can be instrumental in minimizing the need for extensive dental treatments during similar pandemic conditions in the future. By adopting ART techniques, dental professionals can provide essential oral healthcare while minimizing the generation of aerosols and the associated risks. Therefore, it is crucial to incorporate ART into dental education programs globally, enabling dentists to effectively respond to future pandemics or similar crisis situations while ensuring the well-being of both patients and healthcare providers.

ART for early childhood caries

According to the American Academy of Pediatric Dentistry, the presence of one or more primary teeth with caries (cavitated or non-cavitated) in a child

who is 71 months of age or younger is defined as early childhood caries (ECC) (AAPD, 2020; Dashper et al., 2019).

In highly developed countries, the lower prevalence of dental caries, particularly ECC, can be attributed to increased awareness and attention given to this issue by parents, policy makers, and legislators. The emphasis on ECC prevention and management has led to improved oral health outcomes in these countries. Research supports the observation of a lower prevalence of ECC in developed countries compared to developing countries (Anil & Anand, 2017). However, it is important to note that ECC remains a neglected health concern in many developing countries. Limited resources, inadequate oral healthcare infrastructure, and a lack of awareness and prioritization of ECC contribute to its higher prevalence in these regions. Additionally, there is a correlation between the socio-demographic development status of a country and the prevalence of caries in deciduous teeth, with developing countries experiencing higher rates of caries. This trend aligns with the association between increased sugar consumption and higher gross domestic product per capita observed in developing countries (Siervo et al., 2014). To address these disparities, it is essential to prioritize ECC prevention and management in developing countries through comprehensive oral health programs, public health initiatives, and education targeting parents and caregivers. By raising awareness, improving access to oral healthcare services, and implementing policies that promote oral health, it is possible to reduce the burden of ECC in these regions and improve the overall oral health of children.

ART can serve as an appropriate interim step for children with ECC who are unable to undergo general anesthesia or sedation. It allows for rapid infection control and promotes cooperation between the patient, parent, and dentist until definitive treatment can be provided (Mota et al., 2013). Analysis of dentin samples from teeth restored using ART has shown a significant reduction in bacterial count and increased mineralization in dentin (Massara et al., 2002). The ART procedure, which is minimally invasive and performed quickly using hand instruments, is also valuable for accurate pulp diagnosis. In the American Academy of Pediatric Dentistry (AAPD) guidelines, this method is referred to as "interim therapeutic restoration" (ITR) (AAPD, 2004). The use of the ART approach is preferred for both children and parents due to the absence of local anesthesia and the use of rotating instruments, which can cause anxiety. This method is considered favorable when appropriate indications are present.

Conclusion

Despite its initial development for use in underdeveloped countries with limited resources, ART has demonstrated its effectiveness as a treatment option in developed countries, particularly among specific populations. These

populations include children, the elderly, individuals in nursing homes with limited self-care abilities, patients with physical, medical, or mental disabilities, and those with dental anxiety or phobia. ART has shown high success rates in both primary and permanent teeth, particularly in single surface cavities. The application of ART sealants has also proven to be highly effective in preventing caries. One of the advantages of ART is that it does not require local anesthesia or the use of rotary instruments, making it a comfortable treatment option preferred by patients, especially children and those with dental anxiety.

Furthermore, ART can be considered as an alternative treatment method alongside oral hygiene education and preventive measures in public health services, as well as in potential future pandemic conditions like the recently experienced COVID-19 pandemic. Given these favorable attributes, ART should no longer be viewed as a temporary and alternative treatment limited to underdeveloped countries. Instead, it should be considered as one of the primary treatment options in developed countries, particularly for special populations. The integration of ART into dental education programs and further clinical trials are necessary to enhance its acceptance and implementation.

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Chapter 4

FACE MASK USE IN COVID-19 PANDEMIC

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A face mask is a physical barrier that provides protection against infections which are mainly transmitted by infectious respiratory droplets that can enter through the mouth and nose. Face masks and respirators are frequently worn by healthcare workers (HCWs) for hours on regular basis. The COVID-19 pandemic has caused one of the most serious catastrophes in modern human history. It is known that both symptomatic and asymptomatic individuals infected with SARS-Cov-2 may be able to spread the virus. Thus, the use of a face mask on a daily and extended basis has evolved into a crucial personal and public health practice in COVID-19 (Chua M 2020).

It has been shown that use of face mask may trigger face dermatoses like acne, rosacea, seborrheic dermatitis, and contact dermatitis (Damiani G 2021, Park SR 2019, Rudd E 2021).The cutaneous reactions that are caused by face masks may be related to significant changes in the skin such as increasing humidity, temperature, and hydration and decreased respiration of the mask-wearing area. In addition, a face mask may block sebaceous glands and alter sebum production which changes skin pH and thus the inflammatory response of the skin (Park SR 2019). Moreover, some chemicals such as formaldehyde in N-95 and surgical masks may have an irritant effect on the skin (Yaqoob S 2021).

1.Face Masks and Their Properties

It has been strongly advised to wear a mask to prevent the transmission of infected respiratory droplets. Masks serve as physical barriers that stop mucosalivary droplets from entering the mouth and nose (Chua M 2020). Respiratory droplets that cause respiratory infection are divided into two groups according to their aerodynamic diameter. Those smaller than 5 micrometer that remain suspended in the air are called “fine particle aerosols” and those that are larger than 5 micrometer and fall rapidly from infected individuals are called “coarse particle aerosols”. Fine particle aerosols spread the disease easily as they are carried to long distances by air. Close contact is required for coarse particle aerosols to cause disease. SARS-CoV-2, which remains viable for hours in fine droplets, can be transported to long distances with the air and cause disease by inhalation of healthy people (Karmacharya M 2021).

1.1 Types of Face Masks

The effectiveness of masks in preventing the transmission of infectious agents is affected by various issues such as the type of mask, the material used, the fit on the face, leakage and mask-wearing technique. Masks are divided into two categories as certified and homemade. Certified masks meet the criteria of institutions such as U.S. Centers for Disease Control and Prevention (CDC), U.S. National Institute for Occupational Safety and Health (NOISH), andU.S. Food and Drug Administration (FDA).The certified mask class includes

respirators and medical masks. Respirators are equipment that significantly reduces the risk of inhalation of viruses, bacteria, dust, gases and vapors. There are different types of filter facepiece respirators (FFRs) such as N95, P100, FFP2, FFP3, P2, KN95 and DS. The most frequently preferred respirator by healthcare professionals are disposable N95 filter face masks, which can filter 95% of very small particles such as 0.3 micrometer. N95 respirators, which have a 4-layer structure, can not be used widely due to their high cost (AndrewAS 2022, Tcharkhtchi A 2021). In addition, medical masks with a 3-layer structure and a looser fit than respirators are widely preferred to prevent contamination in the clinical environment. They are considered as regulated medical devices by the FDA. Studies have shown that medical masks, that are believed to hold only coarse particle aerosols, can provide protection against Corona and Influenza viruses, although they fit loosely on the face (Karmacharya M 2021).

WHO has reported that 3-layer non-certified homemade masks (basic cloth face masks) made up of woven or non-woven fabric can reduce viral load, although it is not a guarantee. It has been shown that these masks hold respiratory droplets up to 1-10 micrometers due to their multi-layered texture. Although respirators and medical masks provide good protection, homemade masks are good alternative in cases where widespread use is required such as pandemic period (Karmacharya M 2021, LiT 2020).

1.2. Structure of Masks

Masks are made from a variety of polymer fibers, including polyester, polyethylene, polypropylene (PP), polyamide, polycarbonate, and polyphenylene oxide. These substances are sufficiently slippery to display hydrophobic and nonabsorbent qualities (Karmacharya M 2021). To stop leakage or particle penetration (sub-micrometer aerosols) from gaps that arise between the face and the mask, PP has been used to seal the edges of standard masks. Cotton, silk, linen, tissue paper, and common household items like towels and pillowcases are used to make handmade masks, however, these materials lack structural strength and effective particle filtration. Fitting and filtration capacity are the foundations for material effectiveness. A loosely fitting mask increases the risk of infection because small particles can easily enter through cracks. Proper fit and filtration efficacy must coexist, and enhancing just one of these factors will not boost effectiveness (Hill WC 2020).

The production of fabric masks has become part of the garment industry where controls such as fabric safety and skin tolerability are not under control. Dyes used in textiles can cause contact dermatitis in the face areas that the mask comes into contact.Textile contaminants create a potential health risk by diffusing from the skin and mixing into the systemic circulation. The structure of the fabric type and the dyes are important because of the proximity of the mask to the nose and mouth.

Textile, which is a woven material, is grouped as synthetic (ie, plastic-derived) or natural materials (ie, cotton, linen, silk) by means of structure. Natural fibers absorb moisture from the skin and provide higher breathability than synthetic textiles. They are helpful in keeping the skin surface dry and thus preventing the growth of microorganisms. However, the increased saturation level of humidity in this type of fabric creates a feeling of discomfort and weight for the mask users. Biofunctional textiles or synthetic fabrics are designed for air permeability, moisturizing, and cooling effect. They have a high evaporation coefficient without putting on weight, which is crucial for comfort.

It is known that friction between textile and skin triggers dermatological conditions such as acne, dermatitis and post-inflammatory hyperpigmentation, and worsens inflammatory conditions such as seborrheic dermatitis, perioral dermatitis, and rosacea. The fabric should have a soft surface, a tight woven and a regular surface. Tight-fitting fabrics have high ultraviolet protection. However, it may cause an increaserelated with heat sensitivity skin problems (AD, cholinergic urticaria, hyperhidrosis, miliaria rubra and rosacea).

There is no protocol on how to store reusable masks in situations such as exercising and eating (putting them down to jaw-line level, storing them in a bag, etc.). After daily use, it is recommended to wash at high temperatures to kill microorganisms in order to remove respiratory droplets, saliva and odor. In cases where daily washing is not possible, high temperature ironing will both kill viruses and minimize bacterial growth.

Biofunctional textile, on the other hand, is preferred for reusable masks with its self-cleaning feature. Nanoparticles that are used for textile modification, type of textile and feature of textile such as antimicrobial activity and UV protection are reviewed in some studies (Teo WL 2021). It was found that after two days of use without washing, silver impregnated textiles showed considerably lower levels of *S. aureus* and overall bacterial colonization than a placebo textile (Daeschlein G 2010). Given the pathophysiologic parallels between bacterial growth and chronic inflammation in hidradenitis suppurativa and acne, a case of hidradenitis suppurativa was successfully treated with silver-coated textiles (Morand M 2019). When atopic dermatitis patients wore zinc oxide textiles, their symptoms of pruritus, itchiness, and subjective sleep quality improved quickly. This is thought to be a result of the zinc oxide textile's high antioxidative capacity, potent antibacterial activity and favorable biocompatibility (Wiegand C 2013). Self-sterilizing copper oxide-impregnated biocidal fabrics are effective against antibiotic-resistant bacteria, including methicillin-resistant S. aureus and vancomycin-resistant enterococci when used to reduce hospital infections and the spread of antibiotic-resistant bacteria (Borkow, Gabbay 2004). Copper oxide impregnation in protective face masks have additional biocidal properties, preventing hand and environmental

contamination due to improper use of masks and may reduce the risk of infection (Borkow,Gabbay 2010).

Due to the extensive usage of fabric masks and their impact on both newly emerging and preexisting dermatological diseases, dermatologists should be aware of changes in the skin microbiota. The widespread use of masks due to the pandemic and the antibacterial properties of biofunctional fabrics may offer therapeutic prospects for treating chronic skin diseases and microbiome dysbiosis in masks while it might be lowering the prevalence of antibiotic resistance (Teo WL 2021).

2.Physiological changes in the skin due to the use of masks

It was shown in a study that conditions such as increased pH, sebum secretion, skin hydration, transepidermal water loss, and erythema during the use of N95 and surgical masks decreased to the pre-mask level after discontinuing the use of the mask. It has been stated that this return to initial values may take longer in N95 type of masks. Although erythema with cutaneous blood vessel enlargement and increased blood flow appear to be a temporary reaction on the skin as a result of exposure to heat and pressure, longer-lasting erythema may promote inflammation (Hua W 2020).

Wearing a mask for a long time causes high humidity in the skin area under the mask. Irritation and discomfort caused by prolonged use of masks have been reported not only in HCWs but also in the general public.In humans, the dermis is a dynamic system that maintains the balance of differentiation and proliferation of keratinocytes and protective homeostasis. The upper epidermal layers exert their protective properties by rapidly sensing environmental changes and regulating their response. In a study, although there was no visible change in the skin characteristics (erythema, dryness, and pore formation) in the evaluation made by the expert 15 minutes after the prolonged mask use, significant changes (increased sebum production, decreased hydration, and weakened stratum corneum integration) were detected by instrumental measurements (Feng L, 2022).

Itching is described as a feeling that causes scratching and may be a sign of both systemic and dermatological illness. To the best of our knowledge, there is not enough data to completely describe this however studies imply that itching may accompany COVID-19 associated dermatoses such as urticaria, erythematous rash, or varioliform eruptions. Other pandemic-related variables such as the use of cleaning agents, psychosocial stress, and prolonged usage of masks may also contribute to itching. It is known that the general public has increased anxiety during the COVID-19 pandemic. It is well-recognized that anxiety and stress may worsen itching. Although not specifically investigated skin dryness which is a major contributor to itching was reported in up to 68.6%

of HCWs. In one study skin reactivity to masks was assessed among Chinese HCWs. According to the study, wearing face masks for an extended period of time may exacerbate dermatosis and cause more itching. The incidence of itching varied between HCWs and students who had active facial dermatosis. HCWs group reported itching at a higher frequency despite the fact that more students had reported suffering from facial skin issues at the time the survey was performed. The face masks may become scratched or touched as a result of the itching which might reduce the protection ability. More investigations are required to determine the potential treatment for face-mask-associated itching because of the ongoing COVID-19 pandemic and the requirement tomasks (Scarano A, 2020; Krajewski PK, 2020, Zuo Y 2020).

Long-term use of a mask, regardless of whether it is N95 or surgical mask, rises in skin temperature in skin area particularly beneath the mask. It is shown that wearing a mask decreases heat loss from the body by evaporation, conduction, convection, and radiation. Thermal effects cause discomfort, which can lead to improper use of face masks resulting in a lower protection effect. A study was conducted in which the skin temperature, and irritation by using thermal infrared imaging (IR) assessed while wearing surgical masks or N95 respirators. The results of the study show that wearing a surgical mask or respirator for 1 hour caused an increase in the temperature (>34.5°C) of the skin beneath the face mask which causes notable thermal discomfort. Taking the mask off tended to cause the temperature to drop quickly after 1 minute and then to the baseline after 5 minutes (Scarano A, 2020).

A rise in skin temperature in the area covered by the mask contributes to skin drying. The skin'stendencyto growmorerough as a consequence of friction and there stricte drange of motion of the musclessurrounding the lips may contribute to the development of wrinkles. The expansion of skin pores may be caused by an increase in sebum production in the area around the mask, which reduces skin suppleness, or by skin dryness, which makes pores appear larger. In a study investigating the effect of long-term mask use on skin pores and wrinkles, 20 women who took part in the trial were told to wear a mask for at least 6 hours each day for 4 weeks. Before and after wearing the mask for 4 weeks, measurements of skin pores and wrinkles were taken. To assess the effects of usingmoisturizer, some are asaremoisturized. Portions of facial skin without moisturizer application showed an increase in measurement of skin pores and wrinkles. On the other hand, measures of skin pores and wrinkles reduced in places where moisturizer was applied. This is likely because the moisturizer stopped the skin from becoming less elastic by reducing the dryness brought on by wearing a mask (Park M, 2021).

According to an international consumer analysis of the COVID-19 effect, the mask influences the perception of cosmetics and beauty routines. Makeup can migrate to the mask, leaving a filthy piece of cloth on the skin

for several hours. Consumers reported that cosmetic deterioration inside the mask, as well as around the eyes may be seen as humidity from the mask rises. Cosmetic practices have been adapted attendantly, with a reduction in lipstick application, a transition from high coverage face items to thinner coverage products to reduce color transfer and more. Concurrently, consumers are experiencing more skin problems, boosting the requirement for products that address acne, pimples, and blackheads. These skin issues also resulted in less face makeup in an endeavour to reduce the strain on inflamed skin caused by the mask, as well as a rise in attention in cosmetics with skin protective and relaxing effects.

Even long before the COVID-19 pandemic, the longevity of facial cosmetics was a crucial aspect in product satisfaction. Past research has revealed the connections between face cosmetics and skin physiology. Without a face mask, foundation cream would deteriorate after interacting with sebum and perspiration, leading to color alteration and reduction of covering capabilities. When wearing a face mask, the interacting system is complicated, and makeup foundation is subject to an entirely new set of stresses due to the altered sebum and perspiration production as well as the altered microclimate beneath the mask and the abrasion of the cloth on the contact area. Indeed, it was demonstrated that using a face mask raises skin temperature on the cheeks. Together with skin alterations, the microclimate inside a surgical mask revealed temperatures up to 4 degrees celcius higher than the outside and humidity levels were very near to 100% (Yokoyama E 2021). Additionally, face masks make skin more red and this effect lasts after the mask is removed. Future research should consider this fact while assessing the color progression and also by extending the interval between mask extraction and assessment timing. The assessment of mask-friendly face cosmetics should include a skin-calming component in addition to the durability and mask stain. Consequently, some kind of skin advantages through cosmetics with adapted evaluation methodology such as decreasing the amount of makeup might also aid in the creation of products that are favorable to masks.

3.Flora of the Skin

3.1. Healthy Skin Flora

The innate and adaptive immune systems of the human body produce complex signals that enable microbial populations to communicate with the host continuously across the complicated barrier organ which is the human skin. Microbiota is the name given to the community of microorganisms found in some regions (gut, nose, oral mucosa, pulmonary mucosa, scalp and the skin) of human body. Skin microbiota may be divided into two groups as resident and transient microorganisms. The resident microorganisms (core

microbiota) is a stable group of microbes that is regularly present in the skin and re-establishes itself following disruption. The core skin microbiota is generally harmless and benefits the host.This reciprocal relationship is defined as a commensal relationship. Transient microorganisms (tourists) don't create a permanent home; instead, they emerge from the environment and last for a few hours to a few days before disappearing. According to recent studies, the healthy human skin microbiome remains constant over time despite exposure to the environment.

The 3 most common bacterial genera observed in the skin flora are Staphylococcus, Propionibacteria and Corynebacteria. Moreover, the presence of yeast (Malezzia furfur), parasite (Demodex) and viruses in the flora are demonstrated due to new identification techniques. The balance between microorganism communities is constantly under the influence of exogenous (environmental) and endogenous (host dependent) factors. Disruptions in this balance are called dysbiosis and cause detorioration of the skin barrier function. There are specific microorganism communities according to the skin type of our body areas. For example, lipophilic bacterial species such as Propionibacteria are frequently observed in the sebaceous forehead, the alar crease (side of nostril) and retro auricular crease (behind the ear) regions, while Staphylococcus, Propionibacterium, Micrococcus, Corynebacterium, Enhydrobacter and Streptococcus species are predominantly observed in drier skin regions (Dreno B 2016).

3.2. Skin Problems and Microbiota

It has been shown that changes in the number of microorganisms such as *Cutibacterium acnes*, *Staphylococcus epidermidis*, *Bacillus oleronius*, *Demodex folliculorum* on the skin trigger rosacea, takes advantage from sebum overproduction producing/amplyfing inflammation (papules, pustules, and erythema) and play a role in its pathogenesis (Daou H 2020, Diamini G 2021).

Furthermore, dehydration, transepidermal water loss, and sebum dysregulation are procomedogenic factors, capable to promote *Cutibacterium acnes* multiplication and hence innate immune response and leading to inflammatory lesions (papules and pustules) (Yusuf NK 2020).

When using a face mask, bacteria from the upper respiratory tract and skin may be transmitted to the mask. Bacterial cells may find the surface to reproduce in the face mask, as well as the required temperature, humidity and nutrient-rich environment for their proliferation. Overgrowth of some bacteria would theoretically upset the natural balance in the microbiome, increase the risk of inflammation and infection. For example, it has been shown that *S.aureus*, a member of the natural flora, causes skin infections due to an increase in number. *S. aureus* is known as a commensal bacteria

and an important pathogen, producing respiratory tract infections. It also colonizes the airways because it expresses surface adhesins. In some studies, it has been shown that *S.aureus* plays a role in acne as the number of colonies increase.

In the study evaluating the bacterial load and bacterial combination accumulated on the mask after

4 hours of use of cotton and surgical masks, 1.48×10^5 CFU/mask in cotton masks while 1.98×10^4 CFU/mask in surgical masks were observed. When comparing surgical and cotton masks that must be worn for at least 4 hours, surgical masks seem to be the better choice in terms of bacterial load accumulation. This might be as a result of surgical masks' greater ventilation capabilities and lesser water retention when compared to cotton masks (Delanghe L 2021). In another study, it was shown that the bacterial load accumulated on the surgical mask surface was directly correlated with the operation time (Zhiqing L 2018).

Both cotton and cloth masks are recognized as excellent substrates for microbial growth and to retain moisture effectively. Not only microbial contamination, but also the class of bacteriais also important. Roseomonas, Paracoccus, and Enhydrobacter taxa prevailed the microbiome on cotton face masks after 4 hours of usage, while Streptococcus and Staphylococcus taxa prevailed in the microbiome on surgical masks, which were also present on the cotton masks. Bacteria that cause inflammatory skin diseases, including atopic dermatitis and acnevulgaris, include *S. epidermidis* and *S. aureus* (Delanghe L 2021).

4.Effects of Face Masks on Skin Health

It is known that mask use causes changes in some conditions such as skin hydration, transepidermal water loss, pH and sebum production. It is known that these changes are procomodegenic and play a role in the pathogenesis of both acne and roscea by triggering inflammatory reactions (pustule, papule, erythema) (Diamini G 2021, Yusuf N 2020, Daou H 2020). Moreover, masks are typically used in conjunction with medical dressings and lotions to avoid dehydration and pressure ulcers. However, topical treatments can be comedogenic and detrimental to underlying inflammatory dermatoses of the face, particularly acne.

4.1. Acne

Acne vulgaris is a chronic inflammatory disease of pilosebaceous follicles with symptoms such as comedones, pustules, nodules and scars. The common primary lesion in acne is comedo. It can be in the form of open (blackhead) or closed comedo (whitehead). In an open comedo, a

papule with a large central opening filled with darkened keratin, is observed. In closed comedones called white spots, they are usually 1 mm yellowish papules. Macrocomedons may reach 3-4 mm. Papules and pustules formed as a result of inflammation may cause erythema and edema. It is most common on the cheeks, but also on the nose, forehead and chin. It begins to form when the follicles are blocked with a keratinous plug as a result of hyperproliferation and abnormal differentiation of keratinocytes. Due to the narrowing of the follicular opening, the lower part of the follicle enlarges with sebum and allows the contents to be emptied into the dermis due to damage to the follicular epithelium. The accumulation of keratin, sebum and microorganism (especially C. acnes) triggers proinflammatory mediators. Depending on this situation, papules, pustules and nodulocystic lesions occur, respectively. Mask-associated acne is a subtype of acne mechanica, caused by the obstruction of the pilosebaceous outlet due to repetitive mechanical and frictional effects. The hot and humid microenvironment in the mask increases the number of colonized bacteria on the skin. Morever the chronic irritation of the follicular ostia due to friction facilitates acne formation (Acne book chapter=Kaynak 30, Rudd E2021).

Acne is rapidly rising as a result of the increased use of different types of face masks such as; cotton masks, surgical masks, and N95. Although it is stated that FFP2/KN95 type masks are more risky for acne development due to high humidity, occlusive effect and temperature, it has been determined that the same problems can be seen in all mask types. This rapid increase in acne formation in the use of masks was so common that a new name, Maskne, was invented to describe it. Maskne is an abbreviation for mask-related acne, which defines a kind of acne in the O-zone (Figure 1), caused by the extended use of face masks. With more than 200.000 hashtags on social media, this term has been successfully incorporated into everyday speech. It is known that long-term use of mask changes the skin surface microclimate, the production of sebum and therefore microflora of skin by changing the temperature and humidity beneath the mask. Maskne is a documented comorbidity brought on by personal protective equipment use, especially during the COVID-19 pandemic, more so in the general public than in healthcare personnel (Diamini G 2021, Spigariolo CB 2022). In a prospective cross-sectional research the clinico-epidemiological spectrum of maskne and its risk variables are investigated. There was a statistically remarkable relationship between the number of lesions and the length of mask usage, with the quantity of lesions rising dramatically with the number of hours of mask usage. The study indicated that the chin was the most commonly affected region, followed by the cheeks and jawline, and the N95 mask is commonly rassociated with acne production (Arora A, 2023).

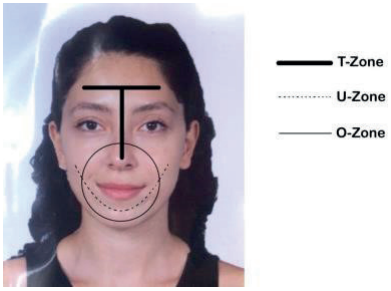

Figure 1. *The T zone of biological acne, the U zone of adult acne, and the O zone of maskne all exhibit different acne patterns.*

A study was performed with individuals who were diagnosed with rosacea or acne and recently received topical therapy. While in lockdown, these patients were unable to receive dermatological face to face consultations, which caused them to stop using their topical therapy. The patients were stable and agreed to receive solely clinical evaluations during their regular four-week teledermatological sessions. Dermatology Life Quality Index (DLQI) and Global Acne Grading Scale ratings for the six regions were used to determine the severity of the acne. They found that age, gender, and the amount of hours per day spent using a mask had no discernible effects on these ratings (Diamini G 2021).

4.2. Rosacea

Rosacea is a condition that may suddenly worsen with facial erythema (redness), papules and pustules, telangiectasias, and hypersensitivity reactions such as itching, stinging, and burning of the convexities (chin, cheeks, nose, forehead). The areas of skin where the symptoms are observed match the areas covered by the mask. Rosacea is divided into 4 different clinical types depending on the main clinical features: erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea and ocular rosacea. Aberrant innate immune response, neuro-vascular dysregulation, skin barrier destruction, and an increase in the number of arthropods called Demodex have been associated with rosacea pathology. Patients with rosacea often report a dry skin that can be easily irritated.Rosacea may be triggered or worsened by prolonged use of masks. (Huang YX 2020, Rudd E 2021).

4.3. Other Facial Dermatoses

4.3.1. Contact Dermatitis

Irritant contact dermatitis (ICD) constitutes a major group of mask-related dermatoses. ICD is an exogenous type of eczema and occurs as a result

of direct physical or chemical injury. Cheeks and nasal bridge are also the most affected areas in cases of prolonged mask use. Prolonged use of masks (≥6 hours) worsens symptoms. ICD shows a broad clinical profile from a dry, scaly appearance to edema, erosion, and ulceration (Rudd E 2021).

Allergic contact dermatitis (ACD) is a delayed type IV hypersensitivity reaction to an external allergen, and is much less common than ICD. It is known that preservatives such as formaldehyde and dibromodicyanobutane found in N95 and surgical masks may cause ACD. In addition, nickel and cobalt, which are used as metal wires to fit the mask, are shown to cause ACD. Contact and pressure urticaria due to mask use is notably rare. It occurs immediately or delayed (4-6 hours) following the use of mask. A properly fitting mask, neither too tight nor too loose, is advised. Also replacement of personal protective equipments with new ones whenever there is a long use of the same equipments may be recommended (Rudd E 2021, Al Badri FM 2017).

4.3.2. Eczema

Since using a mask will have an irritating and covering effect for the skin of the patient with atopic eczema, the moist environment under the mask will worsen the eczema. Seborrhoeic eczema is characterized by greasy yellow scale areas which are common on the scalp, eyebrows, interbrow and nasolabial folds. As in atopic eczema, the moist, hot and occlusive effect of the mask may aggravate eczema in this type of eczema (Rudd E 2021).

4.3.3. Urticaria

Urticaria due to mask use (pressure or contact urticaria) is an uncommon condition. However, immediate or delayed (4-6 hours) lesion formation are reported on the face areas where the mask is pressurized. Masks that are not too tight to fit the face and regular replacement of the mask are important in preventing the formation of urticaria (Rudd E 2021).

5. Conclusion

Understanding the alterations in skin features brought on by mask use is crucial due to extensive mask use. Many investigations have verified that the mask causes a variety of skin problems such as acne, and dermatitis. These studies showed that the mask causes various physiological changes in the area it comes into contact. Since the mask did not enter everyone's lives during the COVID-19 pandemic and has been used by health workers for many years, the effects on the skin during the pandemic could be predicted. However, the poor material choice and prolonged use of the mask made it more devastating for the skin.The alteration brought about by the mask was also microscopically evaluated by examining the skin flora. The fact that the mask causes skin

issues is undeniable, but whether it is a significant factor on its own should be demonstrated by more studies.

It is anticipated that there will be a rise in the occurrence of mask rosacea, maskne, and other skin lesions. Some suggestions can be made to dermatologists and primary care physicians in terms of alternative treatment and diagnosis by using data in the research. In order to assess the most effective therapeutic strategies and prevent the pro-inflammatory impact of using masks, further research on mask related skin disorders is required to better comprehend the pathophysiologic process of facemask use.

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Chapter 5

GOVERNMENT SUPPORTS AND INCENTIVES IN TURKISH HEALTH TOURISM

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Objectives of the Chapter

- The scope of health tourism government supports and incentives
- Health tourism government supports and types of incentives
- Introduction of the innovation activities and process of health tourism together with government supports and incentives

Introduction

Until recently, the main reasons for tourists to travel were to see new places, explore and take a vacation. However, by the 2000s, many factors such as finding a cure for some diseases, healing by using the underground resources of the targeted region, and living a fit and healthy life were added to these goals. Therefore, one of the service sectors that has experienced an increase in service exports in recent years has been health tourism. Health tourism covers the types of tourism that are the primary motivation of individuals that contribute to physical, mental and/or mental health through medical and health-based activities that increase their capacity to meet their own needs and increase their capacity to function better as individuals in their environment and society.

Health tourism has a wide range of travel types made for health and fitness services covering areas such as plastic surgery, beauty, curing, treatment, rehabilitation, visiting, entertainment and cultural activities. Nowadays, with the spread of the Internet and other communication technologies, it has become much easier to access all kinds of information, and with these facilities, people have found ways to try to visit health institutions in these countries and treatment facilities for various diseases in institutions with international accreditation, less waiting time and cheaper treatment facilities. Due to the expenses arising from these trips, health tourism had a market value of more than 100 billion US dollars in 2014 all over the world (SATURK, 2022). This situation has attracted the attention of many countries as well as Turkey. Due to the high economic values created by this sector, health tourism has become a sector in which developed countries have made huge investments, which means that competition is increasing every day. When the positive economic effects of health service exports on a country's current account deficit and employment rates are taken into account, it turns out that the health tourism sector should have an important place among macro policies.

Health tourism, which has become one of the important issues of tourism policies in our country, adds surplus value to the country's income in an economic sense and provides opportunities that include not only "health" but also other tourism activities as a reason for using the existing existing opportunities. Positive national policies followed in recent years, government

supports and incentives have played an important role in the development of this sector, which has a fairly high added value. It is supported by many incentives and grants in order to develop the sector, increase its quality and increase its brand value. Health Tourism activities, which are subject to the permission and supervision of relevant public institutions and organizations, are encouraged by the state almost every step it takes. As a matter of fact, positive national policies followed in recent years, government supports and incentives have played an important role in the development of this sector, which has a fairly high added value. Therefore, significant increases have been observed in the types of incentives and incentive rates given by the government.

T.C. The Ministry of Commerce provides support in excess of TL 100 million to health institutions and health tourism companies operating in the field of international health tourism and accepting/bringing patients for therapeutic purposes. All health institutions operating in the fields of medical tourism, thermal tourism, SPA-Wellness or advanced age and disability care, as well as all health tourism companies providing guidance, counseling, transportation, accommodation and organizational services to patients from abroad can benefit from these non-refundable supports. These supports given in 13 different areas, ranging from advertising and promotional expenses to personnel employment expenses, are listed below.

1. Government Supports and Incentives for Health Tourism

Incentives, in a general sense, are the facilities provided by the state to enterprises/organizations. The incentives provided by the state are given in accordance with certain purposes or justifications and are offered for the benefit of the beneficiary institutions or organizations (Erdoğan and Ataklı, 2012). In Turkey, incentives are applied for education, research and development, improving the quality of the workforce, technology and innovation-oriented policies (Güven, 2007). At the same time, government incentives refer to the net transfer of resources to sectors determined in accordance with the investment targets set by the state (Sönmez, 2005).

The Ministry of Commerce has been in the technical consultancy sector since 2010; Since 2011, our country has been effectively implementing state aid programs that include comprehensive support elements for other service sectors, which are considered to be competitive and have significant potential. Within the scope of the aforementioned programs, health tourism, informatics, film-TV series, education, management consultancy, real estate, publishing, transportation, logistics, gastronomy, retail, accommodation sectors are supported with different support elements suitable for the relevant sector. (T.R. Ministry of Commerce, 2019).

Effective in 2022, the supports provided in accordance with the Decision

on Supporting Foreign Exchange Earning Service Trade for the development of health tourism have been reorganized. Health institutions operating in the health sector, licensed by the Ministry of Health, and a health tourism intermediary organization are covered by the support as part of the expenses related to the activities performed by beneficiaries resident in Turkey, operating in health tourism, in order to increase foreign exchange earning service revenues in Turkey and improve the international competitiveness of the health tourism sector. These supports are provided as non-refundable grants. The health tourism intermediary organization specified within the scope of these supports refers to a company operating in the field of health tourism within the framework of contracts concluded with health institutions in Turkey, having at least one foreign language website and an International Health Tourism Authorization Certificate issued in accordance with the Regulation on International Health Tourism and Tourist's Health, which entered into force by publishing in the Official Gazette dated July 13, 2017 and numbered 30123. A health institution refers to a private or public sector organization or a university health/care organization that operates under the permission and supervision of the relevant public institution, has a website in at least one foreign language and has an international service delivery infrastructure (T.C. Ticaret Bakanlığı, 2022).

1.1. Registration and Protection Support

Within the scope of this support; trademark / patent office services for trademark registration abroad, consulting, research on whether the trademark has been registered on behalf of another company /organization in that country, examination expenses, legal consulting and other legal expenses related to the protection of the trademark abroad are supported (T.C. Ticaret Bakanlığı, 2022). Expenditures of beneficiaries operating in the sector related to product and service registration and expenses related to registration and protection of domestically registered trademarks abroad are supported by 60% up to a maximum of TL 600,000 TL (TURSAB, 2022).

Applications for support of expenses related to trademark registration and protection are made to the examining institution together with the "registration and protection support application documents" by health institutions and health tourism intermediary organizations no later than 6 (six) months after the completion of the registration and protection process. Registration renewal expenses, on the other hand, are not included in the scope of support. The upper limits of support are set for the year 2022 and these limits are updated at the beginning of each calendar year (average CPI + average YI-PPI)/ 2 (T.C. Ticaret Bakanlığı, 2022).

1.2. Certification Support

Within the scope of this type of support; application and document review expenses, certification audit expenses, document usage fees, mandatory registration fees and supervision expenses are supported (T.C. Ticaret Bakanlığı, 2022). The expenses of the beneficiaries operating in the health tourism sector related to the documents, certificates or accreditations determined by the Ministry are supported at a rate of 60% and a maximum of 600,000 TL per document. JCI approved by the Ministry (Joint Commission International) Accreditation, TEMOS Certificates (International Patient Rights Certification), QHA Trent Akreditasyonu, Accreditation Canada, Australian Council on Healthcare Standards Onternational (ACHSI), TÜV Certifications, Ministry of Health Accreditation Standards in Health (SAS Certificate) application and document review expenses, certification audit expenses, document usage fees, registration fees for the first year, consulting expenses, training expenses are covered for quality documents (TURSAB, 2022).

The companies operating in the health tourism sector are concerned with the environment, quality and human health to comply with the technical legislation and to establish a pre-diagnosis center, office opening andcarried out to ensure its operation; quality, hygiene, environmental documents, relevant license andrequired or advantageous in entering a country market with permits Non-refundable grant support is given by the ministry for 60% of all kinds of certification expenses, including training and consultancy, regarding the documents/certificates that provide (T.C. Ticaret Bakanlığı, 2022).

1.3. Agency Commission Support

Agency commission expenses of health institutions are supported at a rate of 60% for a maximum of 5 years and at a maximum of 1,200,000 TL per year. Offices and polyclinics benefit from this support up to 240.000 TL per year, other health institutions and accommodation facilities benefit from a maximum of 1.200.000 TL per year, and the support period is 5 years (TURSAB, 2022).

Applications for supporting commission expenses paid to agencies for bringing health tourists to our country are made to the examining institution by the health institution or accommodation facility no later than 6 (six) months after the payment is made, together with the "application documents for agency commission support" by the medical institution or accommodation facility (T.R. Ministry of Commerce, 2022).

In the invoices received regarding the agency service, the nature of the activity in question must be determinable. In case there is no Decoupling between the activity requested for support and the invoice, the expenditure item in question is not supported. Commission expenses amounting to a

maximum of 30% of the main service amount are covered by the support, and no support payment is paid for the part exceeding the specified rate. The cost of treatment is taken as the basis in determining the main service amount for health institutions (T.C. Ticaret Bakanlığı, 2022).

1.4. Complication and Travel Health Insurance Support

With this support, it has been ensured that the entire insurance coverage of a person visiting our country for treatment is covered from the loss of his/her suitcase to the emergency medical service he/she may need during his/her visit and the complications that may arise after the treatment. Moreover, taking into account the importance of the insurance element, the support rate has been increased from 60 percent to 70 percent so that it will be specific only to this support item (Türkiye Odalar ve Borsalar Birliği, 2022). The complication and travel health insurance expenses that beneficiaries operating in the health tourism sector will have from insurance companies located in Turkey for patients who come to Turkey for treatment are supported by 70% and a maximum of TL 2,400,000 per beneficiary per year (TURSAB, 2022).

The application for support for complications and travel health insurance expenses that health institutions and health tourism intermediary organizations will pay to patients arriving in Turkey from insurance companies located in our country is made to the examining institution together with the "complication and travel health insurance support application documents" by the relevant beneficiary who insures the health tourist no later than 6 (six) months after the payment is made. In order to support insurance expenses; the insurance policy should be arranged to cover the treatment period in Turkey, only for treatments to be performed in Turkey and only for trips to Turkey. In any case, the support payment is paid after the patient's treatment at the health institution (T.C. Ticaret Bakanlığı, 2022).

1.5. Employment Support

The gross wages of the translator, call center staff, guide, social media specialist and marketing specialist employed by the beneficiaries operating in the health tourism sector and the care worker and social worker employed by the beneficiaries of health tourism operating in the field of care are supported by 60%, up to TL 18,000 per month per staff; up to TL 600,000 per practice and outpatient clinic per year, and up to TL 2,400,000 per year for other beneficiaries of the health tourism sector. Beneficiaries benefit from this support for a maximum of 5 years (TURSAB, 2022).

With this support; it has been stated that the employment of qualified personnel needed in the health service export value chain in a wide range from guide to translator, call center staff to social media and marketing specialist,

elderly and disabled care worker to social worker has been included within the scope of the incentive (The Union of Chambers and Commodity Exchanges of Turkey, 2022).

For the personnel employed by health institutions and health tourism intermediary organizations, a pre-approval application is made to the general directorate together with the "employment support pre-approval application documents". Expenses related to personnel who are not pre-approved are not supported. Paid paid employment support applications are submitted to the examining institution by the health institution or the health tourism intermediary institution at least for quarterly periods, no later than 6 (six) months from the realization of the payment, together with the "employment support payment application documents" (T.R. Ministry of Commerce, 2022).

1.6. Support for Foreign Language and Health Tourism Education

Training expenses for foreign language and health tourism, approved by the Ministry, are 60%; A maximum of 240,000 TL is taken per practice and polyclinic per year, and a maximum of 960,000 TL per year is supported per other health tourism sector beneficiaries. (TURSAB, 2022).

Applications for support of expenses for foreign language or health tourism education that employees of health institutions and health tourism intermediary organizations employ will receive online or in physical environment from higher education institutions located in Turkey, which have signed a protocol with the General Directorate and are included in the list of "supported higher education institutions, trainers and training programs" by the health institution or health tourism intermediary organization, it is made to the examining institution together with the "application documents for foreign language and health tourism education support" no later than 6 (six) months from the end date of the educational institution/program. In order to support the training expenses related to the relevant personnel, the personnel must have participated in at least 90% of the training program in question and have passed an exam or an average success score indicating the successful completion of the relevant training course/program (T.R. Ministry of Commerce, 2022).

1.7. Patient Pathway Support

The air ticket expenses of the patients brought to Turkey for treatment by health institutions and health tourism intermediary organizations that make protocols with the Ministry are supported at a rate of 60% per patient for a maximum of 5 years and a maximum of TL 12,000. Offices and polyclinics benefit from this support up to a maximum of 600,000 TL per year, other

health tourism beneficiaries benefit from a maximum of 6,000,000 TL per year (TURSAB, 2022).

Applications for supporting the airfare expenses of patients brought to Turkey and treated in Turkey are made to the examining institution by the health institution or the health tourism intermediary institution together with the "patient pathway support application documents " no later than 6 (six) months after the patient's registration with the health institution. Patients who do not register for admission to the health institution no later than 7 (seven) days from the date of entry into the country are not considered within the scope of support. The expenses of patients entering Turkey on the date of signature of the protocol by the General Directorate and after this date are supported (T.C. Ticaret Bakanlığı, 2022).

1.8. Advertising, Promotion and Marketing Support

The expenses of the beneficiaries and cooperation organizations related to advertising, promotion and marketing activities directed abroad are supported by 60% for beneficiaries and 70% for cooperation organizations for a maximum of 5 years. Offices and polyclinics benefit from this support up to 2.400.000 TL per year, other beneficiaries in the health tourism sector up to 6.000.000 TL per year, cooperation organizations up to 9.600.000 TL per year, beneficiaries and cooperation organizations operating in the sports tourism sector up to 1.200.000 TL per year. The support rate for beneficiaries who are members of the operating sectoral Internet portal is applied as 70% during the period of their participation in the portal. Sample of promotional material related to advertising, promotion and marketing activities spent on advertising, promotion and marketing activities/sample publication/visuals (catalog, brochure and promotional material sample/newspaper, magazine sample advertised/ photo, video recording, CD, etc.) are covered under this support (TURSAB, 2022).

Applications for supporting expenses for activities carried out abroad and included in the "List of Supported Advertising, Promotional and Marketing Activities" are made to the reviewing organization together with the "advertising, promotional and marketing support application documents" by beneficiaries and cooperation organizations operating in the health tourism and sports tourism sectors within 6 (six) months from the date of paying. It is essential to adequately reach the right target audience with the advertising, promotional and marketing activities carried out, to select an appropriate organization venue and to deliver the promotional message using effective communication methods, to benefit from the supports within the scope of this article (T.R. Ministry of Commerce, 2022).

1.9. Overseas Unit Support

Gross rental and commission expenses, including taxes, fees and fees, as well as certification expenses related to the opening or operation of up to 25 units of beneficiaries and cooperation organizations operating in the health tourism sector are supported for a maximum of 5 years for each country; 60% for beneficiaries, 70% for cooperation organizations and a maximum of TL 1,440,000 per unit per year. Offices and polyclinics benefit from this support for a maximum of 600,000 TL per unit per year (TURSAB, 2022).

Within the scope of gross rental expenses, expenses related to space rent, including taxes/pictures/fees related to the unit, are supported. If the rental service is purchased from a company that offers shared office services, the expenses related to the use of the office are evaluated within the scope of gross rental expenses. In order for units opened by the beneficiary / cooperation organization directly or by its company operating abroad to benefit from this support element, there must be an organic Decoupling between the beneficiary / cooperation organization in Turkey and the company abroad (T.C. Ticaret Bakanlığı, 2022).

1.10. International Event Participation Support

Expenses related to foreign events determined by the Ministry in which beneficiaries and cooperation organizations participate at the individual or national level are supported by 60% for beneficiaries, 70% for cooperation organizations and a maximum of 300,000 TL per event, at the same rates for up to 3 prestigious events held within a calendar year if the event is one of the prestigious events determined by the Ministry, and a maximum of 600,000 TL per event. The promotional expenses of cooperation organizations and national participation organizers related to the national participation event are supported at a rate of 70% and a maximum of 1,440,000 TL per event (TURSAB, 2022).

Transportation, registration fees and booth expenses as well as economy class transportation expenses of up to 2 (two) representatives of beneficiaries/ cooperation organizations are supported for physically organized events; participation and registration expenses are supported for events organized in virtual environment. Stand expenses include stand area, stand design, construction, installation and stand related expenses such as shelves, lighting, internet, electricity, cleaning, carpet, table, chair and do not include expenses related to security, hostess and interpreting services. In order to support the expenses, it is necessary to participate in the event with a stand (T.R. Ministry of Commerce, 2022).

1.11. Domestic Event Participation Support

The expenses of beneficiaries and cooperation organizations related to their individual participation in domestic events determined by the Ministry are supported at a rate of 60% for beneficiaries, at a rate of 70% for cooperation organizations and at a maximum of TL 300,000 per event (TURSAB, 2022).

Within the scope of this article, transportation, registration fees and stand expenses are supported. Stand expenses include the design, construction, installation of the stand and stand-related expenses such as shelves, lighting, internet, electricity, cleaning, carpet, table, chair and do not include expenses related to security, hostess and interpreting services. In order for the expenses to be supported, it is necessary to participate in this event with a stand (T.R. Ministry of Commerce, 2022).

1.12. Domestic Promotion and Training Support

The expenses of the beneficiaries and cooperation organizations operating in the health tourism sector related to the training and promotion programs organized exclusively for the promotion of their facilities and services in the country with the participation of academicians, experts, press members, experiencers, sectoral institutions or organization representatives invited from abroad, which are approved by the Ministry and carried out no more than 5 within one calendar year, are supported by 60% for beneficiaries and 70% for cooperation organizations. Beneficiaries of the domestic promotion/training program are supported up to a maximum of 600,000 TL per program, cooperation organizations are supported up to a maximum of 1,200,000 TL per program (TURSAB, 2022).

Within the scope of this support; (I) economy class transportation and transfer expenses of no more than two representatives of the organizing cooperation pole and foreign institutions/organizations participating in the organization, (II) accommodation (bed and breakfast) expenses up to 1,800 TL per person per day of the organizing troops and no more than 2 (two) foreign institutions/organizations participating in the organization, (III) translation expenses, (IV) organization expenses (ground rent, rental expenses for related technical tools and equipment), (V) visual, written and auditory expenses of the organizing pole and no more than two representatives of foreign institutions/organizations participating in the organization, (III) translation expenses, (IV) organization expenses (ground rent, rental expenses for related technical tools and equipment), (V) visual, written and auditory expenses of the organizing pole and foreign institutions/organizations participating in the organization, (II) accommodation (bed and breakfast) expenses up to 1,800 TL per person per day, accommodation (bed and breakfast) expenses up to 1,800 TL per person per day, (III) accommodation (bed and breakfast) expenses up to 1,800

TL per person, (III) promotional expenses, (VI)public referrals, consultancy and matching services expenses, (VII) catalog, weighted and promotional materials expenses are supported (T.C. Ticaret Bakanlığı, 2022).

1.13. Product Placement Support

Product placement expenses in cinema films, documentaries, series, animated films and program formats and digital games shown abroad are supported for a maximum of 5 years; 60% for beneficiaries and a maximum of 1,200,000 TL per year, 70% for cooperation organizations and a maximum of 2,400,000 TL per year. The expenses of cooperation organizations operating in the health tourism sector related to the sectoral trade delegation and sectoral procurement delegation program approved by the Ministry and carried out no more than 5 within one calendar year are supported by 70% and no more than 1,800,000 TL per program (TURSAB, 2022).

Applications to support product placement expenses in films and digital games screened abroad, due to films being screened abroad; Within 6 (six) months after the digital games are offered for sale abroad, the beneficiary or the cooperation organization that has the product placement made to the examining institution together with the "product placement support payment application documents". It is essential to reach the right target audience adequately with the product placement activity, to choose a suitable movie/digital game, and to deliver the promotional/marketing message using effective communication methods in order to benefit from the supports within the scope of this item (T.R. Ministry of Commerce, 2022).

1.14. HİSER (Needs Analysis, Training, Consultancy, Promotion, Employment, Trade/Procurement Delegation) Project Support

The expenses of the cooperation organizations operating in the health tourism sector for needs analysis, training, consultancy and promotion related to HISER projects approved by the Ministry are supported at a rate of 75% and a maximum of 6,000,000 TL per project. Within the scope of the HISER project, expenses related to sectoral trade delegation and sectoral purchasing delegation programs up to 5 per year within a calendar year are supported at a rate of 75% and up to TL 1,800,000 per program. For the planning of HISER projects on the basis of clustering understanding and the organization of project activities, 75% of the gross salary of up to 2 expert personnel employed by cooperation organizations at the same time is supported by a maximum of TL 18,000 per month per employee. The duration of the HISER project is 3 years. The duration of the project can be extended up to 2 years by the Ministry according to the performance of the project (TURSAB, 2022).

1.15. Virtual Fair Organization Support

The organization expenses of the cooperation organizations operating in the health tourism sector related to the virtual fair approved by the Ministry and carried out no more than 5 times within one calendar year are supported by 70% and no more than 960.000 TL per activity. Support for the development and promotion of the health and sports tourism sectors is provided by the associations deemed appropriate by the Ministry; (I)expenses related to the construction, maintenance, updating, operation, promotion of the portal and employment of portal call center personnel of the sectoral Internet portal for the development of health tourism services sectors and the creation of a positive Turkish service brand and image, (II)Sunday intelligence and information subscription expenses for health tourism sectors, (III)employment expenses related to the examination, tracking and finalization of applications for facilitating the entry of people who will visit our country within the scope of health tourism, it is supported at a rate of 100% for a maximum of 5 years and a maximum of 9,600,000 TL per year (TURSAB, 2022).

In order for a virtual fair organization to be included in the scope of support, the number of participants should not exceed 25 and the number of online visitors who will log in to the virtual fair portal during the fair should not exceed 150; the number of foreign visitors should not be less than half of the total number of visitors. Within the framework of the virtual fair organization organized by cooperation organizations; (I) advertising expenses in visual, written, audio media and internet/other digital media for the promotion of the virtual fair, as well as service expenses for advertising campaigns, (II) service expenses for the planning and coordination of the virtual fair organization, (III) expenses related to the organization of matching and bilateral business negotiations, and (IV) expenses related to the platforms where the virtual fair organization is carried out are supported (T.C. Ticaret Bakanlığı, 2022).

1.16. Competition and Event Support

Expenses related to no more than 1 competition and event organized by cooperation organizations for the health services sector and deemed appropriate by the Ministry within a calendar year are supported by 70% and no more than 2,400,000 TL per event (TURSAB, 2022).

Within the scope of this support; (I) international or intercity economy class transportation and transfer expenses of up to 2 (two) representatives of invited guests participating in the organization, (II) accommodation (bed and breakfast) expenses up to 1,800 TL per person per day for up to 2 (two) representatives of invited guests participating in the organization, (III) translation expenses, (IV) competition/event organization expenses (ground rent, rental expenses of related technical tools and equipment, food and catering expenses, etc.), (V) visual, written and audio promotional expenses, (VI) public relations and consulting service expenses and (VII) catalog, brochure and promotional materials expenses are supported (T.C. Ticaret Bakanlığı, 2022).

Table 1. *Government Supports and Incentives in Health Tourism in Turkey (Health Institution/Health Tourism Intermediary Organization)*

Health Institution / Health Tourism Intermediary Organization					
The Support Element	**Rate**	**Support Upper Limit (TL)**	**Other Limits**	**Pre-Approval**	**Extra (+10)**
Advertising, Promotion and Marketing	60%	Practice and polyclinic 4 million 809 thousand/year Other beneficiaries 12 million 23 thousand/year Sports tourism 2 million 404 thousand / year	5 year	1 month (special promotion)	✓
Participation in Foreign Events		601 thousand/event Prestigious event 1 million 202 thousand/event			✓
Domestic Event Participation		601 thousand/event			
Product Placement		2 million 404 thousand/year	5 year	1 month	
Agency Commission		Practice and outpatient clinic 480 thousand/year Other health institutions and accommodation facilities 2 million 404 thousand /year	5 year		
Health Institution / Health Tourism Intermediary Organization					
The Support Element	**Rate**	**Support Upper Limi1 million 202 thousand/document t (TL)**	**Other Limits**	**Pre-Approval**	**Extra (+10)**
Registration and Protection	60%	1 million 202 thousand/ year			✓
Market Entry Documents		1 million 202 thousand/document			If it is related to the climate ✓
Employment		Practice and polyclinic 1 million 202 thousand/year Other beneficiaries 4 million 809 thousand/year	5 years 21.6 thousand/ month/staff	✓	
Foreign Language and Health Tourism Education		Muayenehane ve poliklinik 480 bin/ yıl Other beneficiaries 1 million 923 thousand/year			
The Sick Road		Practice and polyclinic 1 million 202 thousand/year Other beneficiaries 12 million 23 thousand/year	5 years 24 thousand TL/ patient		
Unit		Practice and outpatient clinic 1 million 202 thousand/unit/year Other Beneficiaries 2 million 885 thousand/unit/year	5 Years maximum 25 units per country		✓
Domestic Promotion		1 million 202 thousand/program	Max 5 pcs/year	1 month	
Complications and Travel Health Insurance	70%	4 million 809 thousand/ year			

Kaynak: *T.R. Ministry of Commerce (2023). Sağlık ve Spor Turizmi Hizmetleri Sektörü - 5448 sayılı hizmet ihracatının tanımlanması, sınıflandırılmasıdesteklenmesi hakkında karar. https://ticaret.gov.tr/data/629dab6813b876408c889af6/2023%20%C3%96ZET%20TABLO.pdf (Erişim Tarihi: 16.05.2023).*

Table 2. *State Supports and Incentives in Health Tourism in Turkey (Cooperation Organizations)*

Cooperation Organizations - Health Tourism				
The Support Element	Oran	Support Upper Limit (TL)	Other Limits	Project Duration
Advertising, Promotion and Marketing	70%	Medical tourism 19 million 237 thousand / year	5 year	1 month
Participation in Foreign Events		601 thousand/event Prestigious event 1 million 202 thousand/event		2 months (national participation)
Introducing the National Participation Organization		2 million 885 thousand / event		
Domestic Event Participation		601 thousand/event		
product placement		4 million 809 thousand/year	5 year	1 month
Sectoral Trade/ Procurement Committee		3 million 607 thousand/ program	5 pcs each/year	2 month
Virtual Fair Organization		1 million 923 thousand / activity	5 pcs/year	1 month
Competition and Event		4 million 809 thousand / event	1 pcs/year	1 month
Domestic Promotion/ Training		2 million 404 thousand/ program	5 pcs each/year	1 month
Unit		2 million 885 thousand/unit/ year	5 Years per country, maximum 25 units	
Development and Promotion of Health Tourism Sectors (for Associations)	100%	19 million 237 thousand/ year	5 yıl	Before the activity
HISER (Service Sector Competitiveness Enhancement Project)				
Support Element	Rate	Support Upper Limit (TL)	Other Limits	Project Duration
Needs Analysis	75%	12 million 23 thousand / HISER		3 years / project + 2 years / project with the approval of the Ministry
Education				
Consultancy				
Promotion				
Employment		36 thousand / month / staff	Maximum of 2 staff	
trade/ purchase		3 million 607 thousand/ program	Maximum of 5 pieces each	

Kaynak: T.C. Ticaret Bakanlığı (2023). Sağlık ve Spor Turizmi Hizmetleri Sektörü - 5448 sayılı hizmet ihracatının tanımlanması, sınıflandırılması ve desteklenmesi hakkında karar. https://ticaret.gov.tr/data/629dab6813b876408c889af6/2023%20%C3%96ZET%20TABLO.pdf (Erişim Tarihi: 16.05.2023).

As can be seen in Table 1 and Table 2, all organizations operating in the field of health tourism in our country; registration and protection support, Sunday entry documents support, agency commission support, complication and travel health insurance support, employment support, foreign language and health tourism training support, patient pathway support, advertising/ promotion and marketing support, unit support, international event participation support, domestic event participation support, domestic promotion and training support, product placement support, hiser project support, virtual fair organization support and competition and event support are provided.

Result

Health tourism is a type of alternative tourism that is growing at an average annual rate of more than 20% in the world and in our country. The annual commercial volume of this sector in the world is about 100 billion USD, and more than 20 million people travel for the purpose of health tourism. Especially in tourism countries, the expansion of tourism to 12 months, the development of health tourism, which is the most important alternative type of tourism for the sustainability and profitability of the tourism sector, has been a necessity and an opportunity at the same time. Paying paying around 700 USD in total, tourists who come to our country for tourism purposes stay for an average of one week, while a foreign patient (medical tourist) who comes to our country for treatment stays for 10 days on average and pays an average of 10 thousand USD, a foreign patient who comes for rehabilitation stays for a month on average and only pays 7 thousand USD to the health institution. In addition, these foreign patients necessarily come with at least one companion, accommodation and other non-health expenses exceed 2-3 thousand USD. In short, although the number of health tourists is small, the amount of foreign currency they leave to the country is 10-15 times higher than normal tourists.

The countries that have been successful in health tourism in the last 10 years in the world have defined their processes before and made health tourism a country policy by determining their strategies. The keys to the success of the world health tourism policy can be expressed as strategic planning, policy making, action plan development, coordination, branding/ promotion. Government support and incentives play an important role in the development, branding and sustainability of the success of the health tourism sector. Government support and incentives are applied in countries such as India, Thailand, Singapore, Malaysia, Costa Rica, Mexico, USA, Spain, Hungary, Germany, which are among the brand destinations that have achieved success in health tourism, including Turkey Dec. In order to attract more investment to the country, make investments more efficient and increase global competitiveness, Turkey has directed incentives from fiscal

policy instruments to this sector, including government incentives for regional development, support of priority sectors and foreign exchange generating service trade, in particular.

In addition, the requirement of having an accreditation / authorization document will be introduced to benefit from incentive applications. It will be provided that institutions providing quality services (medical and thermal facilities, brokerage institutions, etc.) are accredited to benefit from the incentive system in order to support and improve the quality of service. In order to ensure the determined standard level and to keep the service quality at an acceptable level, the accreditation obligation is seen as the exit door for all kinds of actors in the sector

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Chapter 6

HEAT SHOCK PROTEINS AND HSP90 IN SILICO ANALYSIS WITH DISEASE ASSOCIATION

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Heat shock proteins (HSP) are a group of proteins that are known to have a molecular weight smaller than 100 kDa and are produced simply as a result of the cells encountering high temperatures (42-46°C). Molecular chaperones stabilise polypeptides, thereby preventing misfolding or facilitating folding of other denatured proteins (1). In fact, folding errors often occur at high temperatures. HSP production can increase due to infection, intake of trace elements, exposure to ultraviolet rays (UV), hypoxia, long-term fasting, alcohol and toxins (2). HSPs are found in almost all eukaryotic and prokaryotic cells and are activated in case of stress, so these proteins are also called as 'stress proteins' (3). HSPs prevent the collapse of various proteins as clusters, and recognise the denatured proteins and enable the refolding of them (4). Owing to the robust molecular structure of HSPs, they are less commonly damaged compared to the proteins. Their more robustness lies in their intracellular lipophilic packaging and having strong hydrogen bonds (5). The mechanism of the association of HSP proteins with tumors, which has recently been associated with cancer, is explained as maintaining the malignant structures by preserving the neoplastic cell properties (6). They have a role in cytoprotection under stress and in physiological events in the cell, signal transmission, neurodegenerative disorders (Alzheimer's, Parkinson's, Huntington's, etc.) and immunological development in addition to their role in cancer (7).

Heat shock proteins can be classified in four main groups in accordance with their weight as HSP 90 family, HSP 70 family, HSP 60 family and small HSPs.

HSP 90 Family

This family can be grouped as alpha and beta isoforms. Post-translational modifications such as acetylation, prenylation, phosphorylation, acylation, methylation, ubiquitination, lipidation, and glycosylation regulate the function of the HSP90 protein (8). They form complexes with HSP90 co-chaperones for stability and maturation. The HSP90 protein exists as a homodimer in cells. This protein generates a C-terminal dimerisation domain containing a tetratricopeptide rebinding (TRP) motif. It creates an adenine binding domain and controls the ATPase activity of cellular proteins. In addition, it creates an intermediate binding site for co-chaperones, which has high affinity (9).

HSP 90α: is responsible for intercellular communication and maintenance of their morphology. It is also closely associated with tumorigenesis. It is generally in anti-apoptotic position. It plays a role in signaling pathways such as Akt signaling pathway and receptor signaling and apoptotic pathways (10). It plays a role in transcription. Functionality of the "vascular growth endothelial factor" (VGEF) and "epidermal growth factor" (EGF) receptors is shaped in accordance with the HSP 90. It takes part in the regulation of the stability of steroid hormone receptors, which have important functions in the body (Androgenic hormone) (11).

HSP90β: This protein is suggested to have a key role in glucose metabolism. It has similar effects with the HSP100 molecule. Recent studies suggested that these two molecules act together (12). The most widely known effect of it is Calmodulin bindings. They are in interaction with the actin protein, however, they need Ca^{+2} for the activation of the effect mechanism (13).

HSP 70 family

HSP 70 has been the most investigated molecule to date. Along with HSP60, it can also be found outside the cell in cases such as cell necrosis. It has 2 forms as HSP 72, and HSP 73.

HSP 72 tends to increase in stress situations, while HSP 73 tends to be continuously produced. HSP 70 plays a role in conditions such as refolding by enabling the collapsed proteins to become soluble, preventing their collapse, controlling the protein activation, and transferring secretory proteins to the cell membrane (14). Therefore, they are like the guard of the cell and use ATP while performing these tasks. In addition, the presence of glutamine in the environment is very important. If the glutamine level is low, the expression of HSP 70 will also be lower (15). The control of the HSP 70 chaperone depends on its relationships with other chaperones and on HSP 70 gene control. J-domain protein (JDP) is a type of co-chaperone and works with HSP 70 in situations such as the collapse of the newly produced proteins. In addition to all these, the HSP 70 family encapsulates the hydrophobic ends of the newly produced protein and protects it from intracellular events (16). Studies have shown that HSP 70 prolongs the life span of malignant cells with the functional mechanisms of p53 and p21 proteins in cell culture tumor cells (17).

HSP 60 family

This family is often named as 'chaperonin'. Generally, it is located in the mitochondrial matrix and takes part in the folding-transport actions of the proteins. It is suggested to play a role in amino acid transport since it is also located in the cell membrane. In case of mutation due to its localisation, mitochondrial diseases are detected and the regional response of mitochondria under stress is again with HSP 60 (18). It is formed by the inclusion of HSP 10 in the assembling of multiple molecules. Its one of the most important tasks is to regulate the folding of polypeptide chains, and requires ATP for this process. Although it has similar characteristics with the HSP 70 family in terms of its protective effect, they use different mechanisms (19). On the other hand, it interacts with HSP 70 and is highly expressed in multi cancer types such as glioblastoma. Therefore, HSP 60 expression can be used as a biomarker for diagnosis and prognosis in many diseases (20). In addition, HSP 60 (GroEL) is required for the development of Escherichia coli (21).

Small HSP family

Multiple chaperone proteins with alpha crystal in its structure and weighing in the range of 12-43 kDA are in this group. The common point of these molecules is that they contain the α-crystallin domain, which is a 100 amino acid structure. This group is known to resemble the α-crystallin domain in the human eye lens and even prevents the formation of cataract (22). They can be localised in different sites in the cell. Together with HSP 60, 70 and 110, they regulate the newly produced proteins. By interacting with α-crystallin, they provide protection against factors such as chemical agents, oxidative stress, together with microtubules, actins and microfilaments, which are the skeletal structural elements. The refolding phase is under the control of the HSP 70 chaperone alone (23). They preserve their functionality owing to the presence of free carboxyl end in their structure, and they attach to other small HSPs. Oxygen radicals are located in the cell membrane, and this group protect themselves from myobacterium macrophages owing to the blocking properties of the chaperons. They protect the organs such as kidney, heart, and brain, and intestinal system against ischemic problems. In addition, small HSPs are known to have roles in the pathophysiology of the degenerative diseases such as Alzheimer's and Parkinson's when they are mutated(25).

Large HSP Family

This group can be gathered in 2 subgroups as HSP 110, and the glucose regulatory protein 170 (GRP 170). This group is also called as HSP170. They have a molecular weight of 110 kDa and are continuously synthesised at the basal level, even if there is no stress in the environment (26). It is a molecule that has positive results in cancer treatment due to its ability to stimulate the acquired and innate immune system. GRP 170 is the least studied chaperone in cancer diagnosis, however, HSP 110 is expressed at higher levels in many types of cancer (27). Large HSPs are functionally involved in the reorganisation of proteins. The HSP 100 functions in fragmentation of the protein into clusters. HSP 104, on the other hand, can enable the reuse of the newly clustered proteins. The chaperones in this group are suggested to cause the cell to become heat tolerant (28).

UPR Pathways and ER Stress

Eukaryotic cells contain many organelles, the most important of which is the endoplasmic reticulum (ER). The endoplasmic reticulum performs vital functions for the cell, such as maintaining Ca^{+2} balance, glycolysis, modification of protein amino acids, transport, and lipid synthesis (29). In some cases, the proteins synthesized in the ER may not function properly, and in this case, dysfunctional proteins undergo preteolysis after controlling. In addition, ER facilitates the normal functioning of the cell by denaturation of misfolded

proteins and precipitated proteins. The basis of this mechanism used for all this procedures is the stimulation of the unfolded protein response (UPR). Among the factors that cause the accumulation of misfolded proteins in the ER are the mutations in proteins, excessive alcohol consumption, accumulation of reactive oxygen species (ROS), lack of ATP, which is the energy source of the cell, and nutrient deprivation (30, 31). The UPR involves 3 signaling pathways that require the presence of protein kinase R (PKR)-like endoplasmic reticulum (PERK), activating transcription factor 6 (ATF6), enzyme 1 (IRE1) and which become active after activation of ER stress-sensing receptors. All pathways are located in the ER membrane and function against the stress factors that cause all these protein defects, if they cannot function well(in the inactive state), the stress receptors work with the binding immunoglobulin protein (BIP). BIP prefers to bind hydrophobic regions of the yet unfolded proteins, with the increased ER lumen load, and makes the receptors free. Thus, all the 3 pathways are activated. In addition, the UPR can facilitate the repair of the misfolded proteins by regulating the expression of chaperones. If the mechanism does not work properly, which may be due to mutation or advanced age, protein accumulation increases and the cell's system that controls proteins rises above its normal capacity. The cell terminates translation through the UPR to return to normal function. Thus, the expression of chaperones in the medium, and unfolded protein aggregates increase. This situation results with the inducing of the signals which lead the cell to apoptosis by UPR (Figure 1) (32).

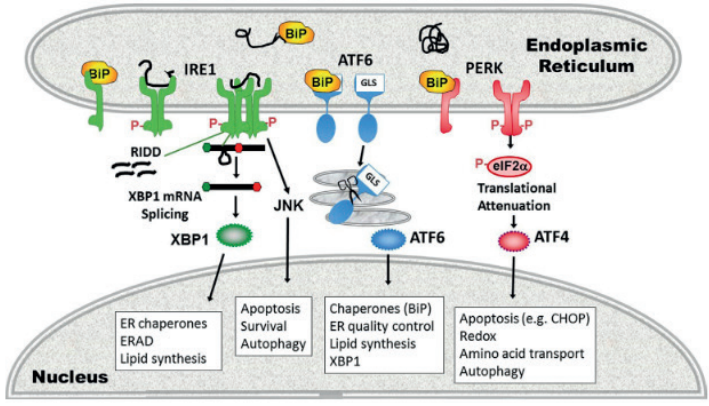


Figure 1: *Three pathways of the unfolded protein response (UPR) (32)*

Chemical, Molecular and Pharmacological Chaperones

Chaperones can be described as chemical, molecular and pharmacological chaperones (33). They are low molecular weight chaperones that support the stability of the protein in its possible conformation so that it is not denaturated.

One of the most well-known properties of chaperones is to ensure the correct folding of folded proteins. Molecular chaperones are the group which are most effective in this process. Current studies showed that the molecular chaperones have been shown to be actively involved in UPR pathways. The misfolded proteins especially in neurodegenerative diseases form aggregates in the brain, leading to clinical and pathophysiological findings. Targeting of the molecular chaperones in this respect may support the therapeutic approaches (34). Pharmacological chaperones, are the class of chaperones responsible for the correct folding of the protein in abnormal situations such as mutations. Pharmacological chaperones regulate the translation of the mutated protein, inducing the formation of more mature proteins. They can specifically bind to the protein. They control the protein level of the cell during the post-translational modifications. Unlike chemical chaperones, they are effective at lower levels (35).

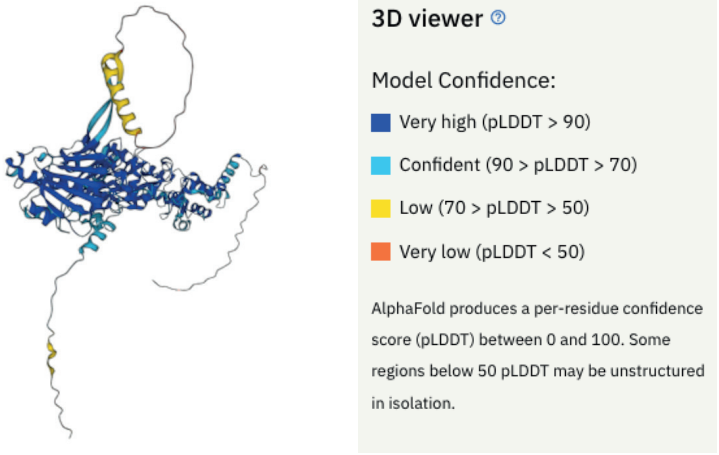
Omics Approach to HSP 90 Family

AlphaFold Protein Structure Database (GeneCards and UniProt)

Protein: Heat shock protein HSP 90-alpha

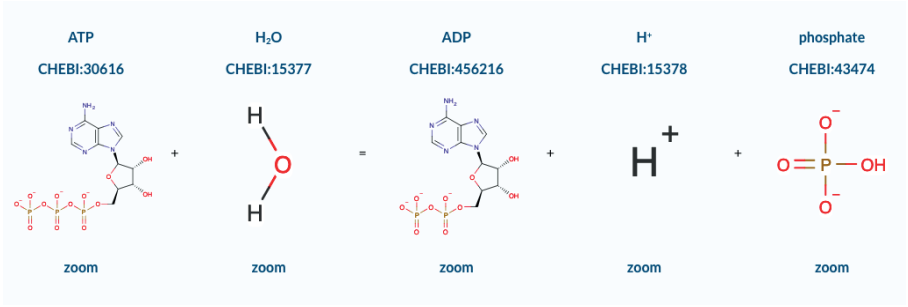
Gene: HSP90AA1

Source organism: Homo sapiens (Human)



AlphaFold produces a per-residue confidence score (pLDDT) between 0 and 100. Some regions below 50 pLDDT may be unstructured in isolation.

Catalytic activity: ATP + H2O = ADP + H+ + phosphate



Hsp90, consists of four structural domains:

-A highly preserved N-terminal domain of 25 kDa (NTD)

- A "loaded binding" domain which binds N-terminal to the medium region

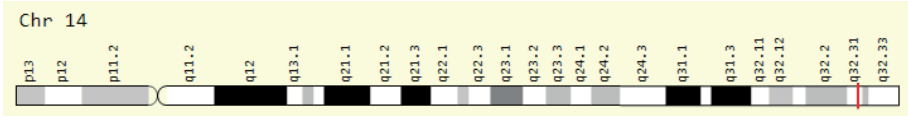
-A medium domain of 40 kDa (MD)

- A C-terminal domain of 12 kDa(CTD).

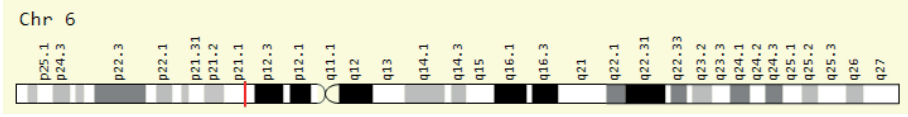
Chromosomal Location (GeneCards)

The gene encoding the cytoplasmic HSP90α is located on the chromosome 14. Hsp90 is a highly protected protein and is expressed in most organisms, and shows approximately 40% sequence identical, and 55% similarity in most human protein molecules including the prokaryotic analog HtpG (high temperature protein G).

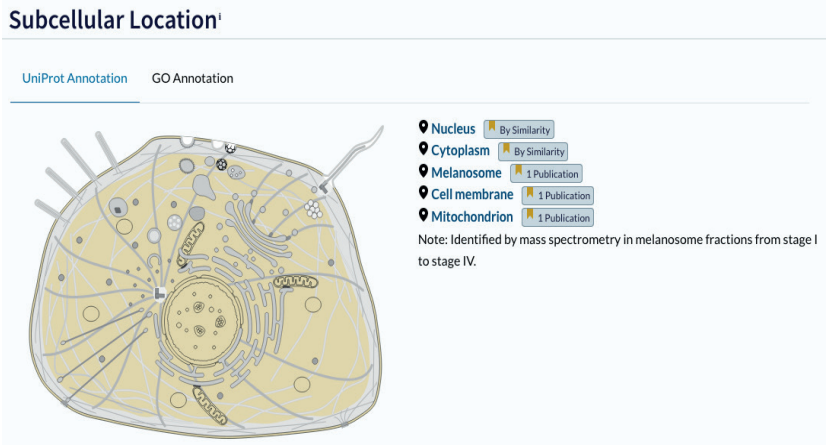
HSP90β is mainly located in the endoplasmic reticulum, and is encoded



on the 6th chromosome.



Cellular Location (GeneCards)

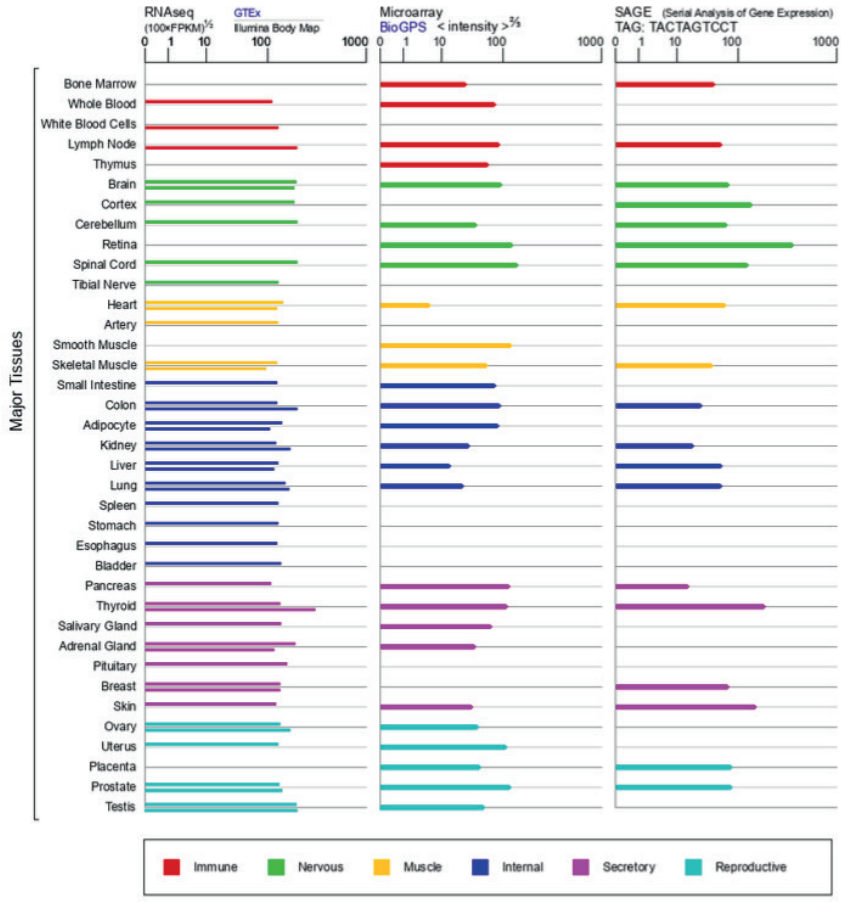


Shows the intracellular location of the UniProt HSP90α.

Compartment	Confidence
Lysosome	5
Cytosol	5
Nucleus	5
Mitochondrion	5
Extracellular	5
Plasma membrane	4
Golgi apparatus	3
Endosome	3
Endoplasmic reticulum	3
Cytoskeleton	3
Peroxisome	2

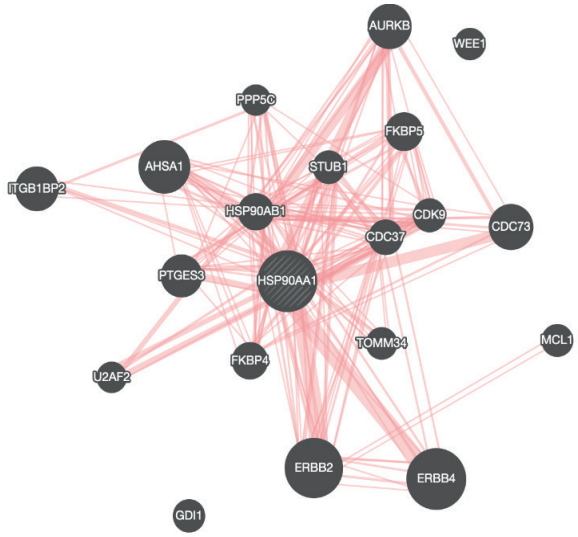
Tissue Expression (STRING/GeneCards)

Has been expressed in the tissues of abdomen (FDR=6.93e-07), atrium (FDR=0.0299), Gastrointestinal tract (FDR=0.0066), kidney (FDR=0.0453), liver (FDR=0.00018), lung(FDR=5.36e-07), ovary (FDR=0.0060), placenta (FDR=0.0114), skin (FDR=0.0012), testicle(FDR=0.0012), urinary system (FDR=0.0110) , and other various tissues.



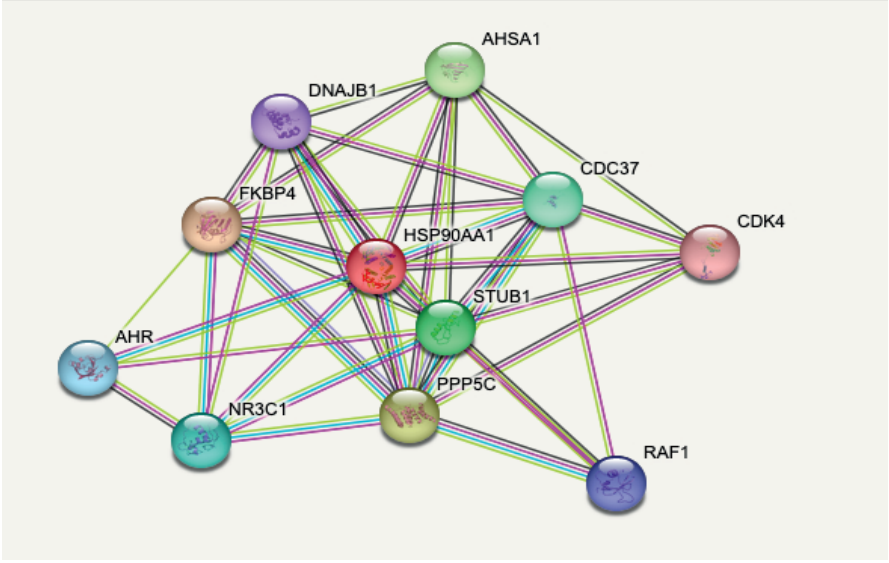
GeneMANIA

Using the GeneMania, the other molecules HSP90 protein physically interacts were shown.



STRING

Using the STRING, other molecules HSP90 protein physically interacts were shown.



Pathways

Using the KEGG Database and STRING Database, HSPs were shown to have significant association with the estrogen signaling pathway (FDR= 0.00024), NOD-like receptor signaling pathway (FDR=0.0076), PI3K-Akt signaling pathway (FDR=0.0413), Aryl hydrocarbon receptor pathway (FDR=5.52e-06),

NRF2 pathway (FDR=0.0109), nucleotide-binding oligomerization domain (NOD) pathway (FDR=0.0289), estrogen signaling pathways (FDR=0.00024).

Metabolic processes

The STRING Database showed that HSPs participated in metabolic processes such as protein processing in endoplasmic reticulum (FDR=0.00028), antigen processing and presentation (FDR=0.00066), cadherin binding (FDR=0.00019), and autophagy (FDR=0.00022) in statistically significant level.

Diseases

The STRING and GeneCards Database showed that HSPs have significant association with the fluid shear stress and atherosclerosis (FDR=0.0041), legionellosis (FDR=0.0252), infectious disease (FDR=0.0029), and Leishmania infection (FDR=0.0115). In addition, the GeneCards study showed that HSPs have association with liver cancer, Herpes simplex infections, flu, and systemic candida infections.

Conclusion

Studies have shown that the HSPs also have a key role in cancer including neurodegeneric diseases, infertilisation and infectious conditions after comprehending their mechanisms and roles in pathophysiological processes. The inhibition or activation of proteins in the UPR signal cascade are suggested to possibly affect the prognosis of diseases. In this respect, this protein family is seen as an important step for treatment and prognosis. Thus, it will be possible to get a faster response to treatment or to detect the disease in the early period. In addition to all these, their modulatory roles in many pathways and biological processes and its role in diseases have been clearly revealed after the recent studies.

Performing of the experimental studies with large cohorts will provide a better understanding of these molecules. In addition, the bioinformatics tools are suggested to guide new studies in terms of showing their interactions with other molecules.

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Chapter 7

IN VIVO DOSIMETRY IN EXTERNAL BEAM RADIOTHERAPY

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1. Introduction

Radiotherapy aims to give the ideal dose to the target tumor and to protect the surrounding critical organs in the best way (De Felice et al., 2018). In the application of radiotherapy, it is necessary to apply the radiation correctly and to take the necessary radiation protection measures. Undesirable events arising from human errors or systematic parameter errors in radiotherapy applications may adversely affect patient treatments. In radiotherapy, it is very important to accurately transfer the desired dose to the target volume (Burnet et al., 2004). Depending on the developing technology, there has been a great improvement in radiotherapy applications. Increasing vigilance is required for newly developed radiotherapy techniques and their clinical application to verify that the prescribed dose is given safely and accurately. Quality assurance (QA) programs have been initiated to ensure accurate dosing and minimise errors (Weber et al., 2011). These programs should verify that all components in the radiotherapy chain are functioning correctly, including the treatment planning and treatment delivery system. Verification controls in external beam radiotherapy (EBRT) help minimize errors, but are insufficient. For dose escalation and compatible high-dose-high-precision techniques in radiotherapy applications, very high accuracy, both at the true dose level and at geometric accuracy, should be the target (Mijnheer et al., 2013).

In addition, QA procedures are useful for measuring the uncertainty in actual dose delivery to patients treated according to advanced treatment techniques applied in radiotherapy. One way to assess uncertainty in dose delivery is to analyze the sequence of dosimetry procedures, including dose calculation, therapy machine calibration, and patient setup to deliver the prescribed dose to the patient (DeWerd et al., 2011; Paganetti et al., 2008). In addition, for individual patients, the actual accuracy in dosing may be less than desirable: for such an individual patient, systematic errors in dosing may occur due to the influence of patient contours, patient mobility, inhomogeneity, and visceral movements. In addition, errors can occur with the transfer of therapy data from the therapy planning system to the accelerator, errors in therapy machine settings, or positioning of the patient and beam modifiers (Benedict et al., 2010; Mans et al 2010). Final control of the actual dose delivered to an individual patient can only be performed at the patient level by means of in vivo dosimetry (IVD). For this reason, in vivo dose measurements are recommended by many international organizations (American Association of Physicists in Medicine (AAPM) and the International Commission on Radiation Units and Measurements (ICRU)) (Mijnheer et al., 2015; Olaciregui-Ruiz et al., 2020). The term "in vivo", which is a Latin word meaning "in vivo", means "measuring the dose given during treatment on the patient" in radiotherapy. In radiotherapy, IVD means measuring the radiation dose received by the patient before or during treatment using a phantom to represent the patient (Cilla et

al., 2016). In EBRT, IVD is performed by placing a type of detector on the skin at or near the target volume. It can be correlated to the dose inside the patient using a well-known relationship between the detector's characteristics and the doses at different locations. Many treatment errors can be identified with IVD that cannot be detected by treatment planning systems or other QA controls using pretreatment measures. In addition, IVD measurements are important in minimizing the side effects that may occur in the patient (Eaton et al., 2012)

Treatment planning algorithms cannot calculate skin doses with the desired accuracy, and in vivo, dosimetry can be used to decide when to protect the lungs, for example, during TBI. IVD checks the accuracy of the radiation dose administered. Treatment planning algorithms may not be able to accurately estimate out-of-field doses, in which case the actual dose can be measured with the use of IVD (Gul et al., 2023; Gul et al., 2023).

A useful dosimeter must have high accuracy and sensitivity, a wide dose range, high signal linearity and small directional dependence. Many types of dosimeters can be used in IVD applications. Different measurement devices such as film dosimetry, thermoluminescence dosimetry (TLD), ion chambers, MOSFET, semiconductor diode detectors and chemical dosimetry have been developed for in-vivo dosimetry measurements. The most commonly used in-vivo dosimetry systems are ion chambers, TLD and semiconductor diode detectors. This study will evaluate the properties of in vivo dosimeters commonly used in external radiotherapy.

2. In-Vivo dosimetry

As a result of the developments in modern technology, there have been rapid developments in radiotherapy treatment devices and planning systems. Depending on the progress in radiotherapy applications, the importance of QA has increased even more. The radiation sent to the patient during radiotherapy is measured with IVD systems. Thus, the dose defined before radiotherapy can be compared with the doses measured during irradiation. Thanks to a well-designed IVD system, the accuracy of the treatment can be controlled without prolonging the treatment period (Graves et al., 2015). Most radiation measuring devices are designed according to the ionization determination principle. Film dosimetry, thermoluminescence dosimetry (TLD), optically excited luminescence dosimetry (OSL), ion chambers, MOSFET, semiconductor diode detectors and chemical dosimeters are widely used in IVD measurements (Donmez., 2017; Damulira et al., 2019; Ashraf et al., 2020).

When choosing in-vivo dosimetry, care should be taken to ensure that the measurement system used is safe, reproducible and easy to use. In addition, it is desired that the dose responses of the detectors be independent of variables

such as energy, dose, dose rate and temperature(Ruiz et al., 2019; Tanderup et al., 2013). In-vivo dosimetry systems allow the elimination of errors encountered during radiotherapy application. With the in-vivo dosimetry system placed on the patient's skin, dose calculations can be made according to the dose value received by the skin surface. Thus, in-vivo input dose measurement, erroneous daily dose, erroneous beam energy treatment and set-up errors can be controlled by dosimetry systems. In addition, the efficiency of the treatment device and data problems in the planning system can be checked with IVD measurements (Nachbar et al., 2020; Bruza et al., 2018).

2.1. Film dosimetry

Radiochromic films have been used for many years in radiotherapy applications. The main advantages of radio chromic films are that they are tissue equivalent, have a high spatial resolution, have low energy dependence, have a wide energy range, are cheap, are thin, robust and flexible, are water resistant, and provide dose information in 2 dimensions (Casolaro et al., 2019). Radiochromic films change color after irradiation without the need for any chemical treatment. The optical density change that occurs in proportion to the dose absorbed by the film can be easily measured with any photometric device (Devic et al., 2016). It is the first radiochromic film model suitable for use. Gafchromic EBT2 film and Gafchromic EBT3 film started to be used in 2009 and 2011, respectively (Borca et al., 2013).

The active layer differs in radiochromic film models. The films became more sensitive to radiation with the use of lithium pentacosa-10, 12-diynoate (LIPCDA) in EBT models. EBT films can measure doses in the 0.2 Gy – 100 Gy range. 2% of the dose measurement uncertainty was attributed to the irregularity in the sensitive layer of the film. The most important difference between the EBT3 film and the EBT2 film is that the 28 μm thick active layer in its structure is the same (125 μm) in the thickness of the polyester layers below and above it. The structure of the EBT3 film is shown in Figure 1.

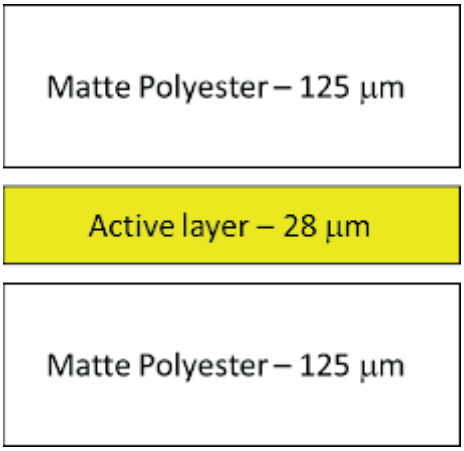

Figure 1. *EBT3 Gafchromic film structure*

The radiochromic film dosimetry system consists of 2 stages. The first stage is the calibration process. A calibration curve is required to convert the response of the radiochromic film to the absorbed dose. At this stage, dose values are obtained with the help of a film scanner, and an optical density-dose graph can be drawn. Care should be taken to draw the calibration curve separately for each film. Then, in the second step, using this curve function, the optical densities of the irradiated films are converted to unknown absorbed dose values. The net optical density change in the main film due to irradiation alone is obtained by subtracting the optical density change of the control film from the optical density change of the original film. The response of EBT3 radiochromic film is characterized by net optical density (netOD).

$$netOD = -log_{10}\frac{I}{I_0}$$

Where I0 and I are the reading for the unexposed and exposed film pieces, respectively. The following formula is used for standard deviation net optical density (SD(netOD)).

$$SD(netOD) = \frac{1}{ln\ 10}\sqrt{\left(\frac{SD(I_0)}{I_0}\right)^2 + \left(\frac{SD(I)}{I}\right)^2}$$

SD(I0) and SD(I) are the associated standard deviations I0 and I, respectively (Marroquin et al., 2016). Since the polymerisation continues for a long time in gafchromic films, 24 hours should wait for the ideal darkening of the film after irradiation (Mendez et al., 2021). Movie scanner; It scans

with three light colors: red, green and blue. It is recommended to use the red channel at low doses. In high doses, using the blue channel is recommended (van Hoof et al., 2012). Gafchromic EBT3 film is widely used in external RT, IMRT and brachytherapy treatments. The most significant advantage of EBT 3 film is that it is tissue equivalent and waterproof. For this reason, it is used in many dosimetric measurements in radiotherapy. Film dosimeters are used in phantom and on-patient for in vivo dose measurements.

2.2. Thermoluminescence dosimetry

Safe, reproducible and easy to use are among the essential features sought in ideal IVD systems. To be preferred, dose responses must be independent of variables such as energy, dose, dose rate and temperature. Thermoluminescence dosimetry (TLD), which can measure independently of many factors, is accepted as the most suitable IVD system (Moradi et al., 2021). TLDs are solid crystal materials that are semiconductors. Semiconductor materials conduct electricity depending on the temperature, and the conductivity of TLDs increases as the temperature increases. When TLDs are exposed to radiation, electrons in the valence band move towards the conduction band. Electrons that pass into the conduction band leave holes behind. This creates what is called an electron-hole pair (Figure 2).

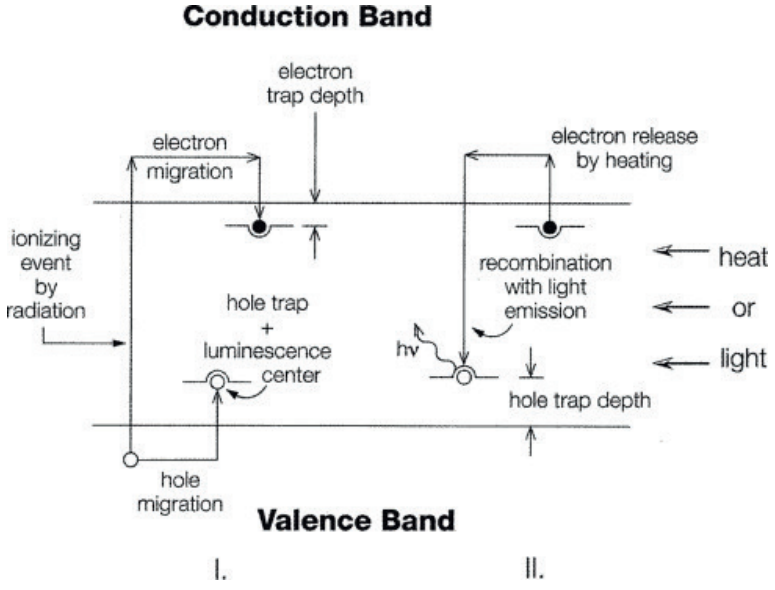

Figure 2. *Basic concepts of thermoluminescence.*

The amount of light emitted from the crystal is proportional to the number of electrons and holes in the traps. This is called thermoluminescence (TL). The photomultiplier detects the light emitted from the crystals and correlates with the absorbed dose (Bose, 2017). TL detectors are preferred for in vivo measurements in radiotherapy because of their small volume and high sensitivity. It also has the advantage of not being wired to an electrometer. TL materials are available in the form of powder or solid dosimeters. These dosimeters can be made of single crystal or polycrystalline extrusions (rod, chip). The most commonly used TLDs are doped phosphors such as lithium fluoride (LiF), lithium borate ($Li_2B_4O_7$), calcium sulfate ($CaSO_4$) and calcium fluoride (CaF_2) (Aramrun et al., 2018; Harvey et al., 2010). LiF(Mg, Ti), LiF(Mg, Ti, Na), $Li_2B_4O_7$:Mn, and $Li_2B_4O_7$:Cu are suitable TL substances that are equivalent to soft tissue and lung and used in radiotherapy. LiF is the most commonly used TLD for dose measurement in radiotherapy, with an effective atomic number of 8.14. The effective atomic number of tissue is 7.42 (Kim et al., 2008).

LiF is widely used in radiation measurement because it has a good energy response. The TLD system consists of crystal dosimetry, TLD furnace and a TLD reader. The schematic representation of the TLD reader is shown in figure 3.

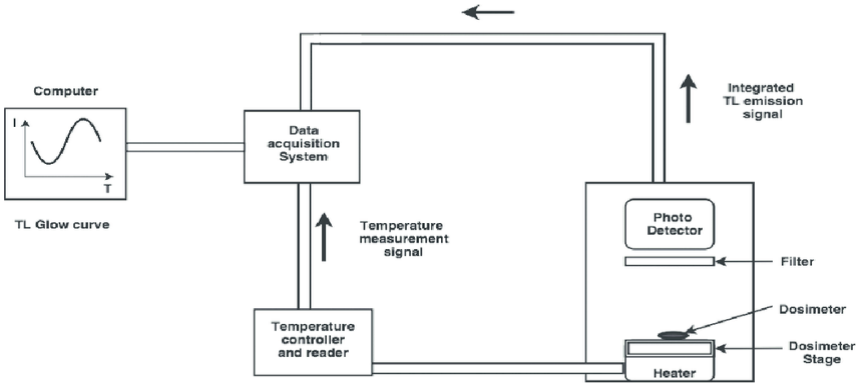

Figure 3. *Schematic representation of TLD reader*

The thermoluminescence glow curve changes with the heating method and heating temperature. Low unstable temperature curves after radiation exposure can be eliminated by preheating. High stable temperature peaks are used for dosimetry with readers reaching temperatures up to 400°C. Due to differences in production and structural properties, TLD crystals sensitivities are different. Therefore, the element correction coefficient (ECC) is defined to eliminate sensitivity differences. ECC is a characteristic coefficient approximating the load value measured from the TLD to the average load

value. The ratio of the corrected readings to the amount of radiation used during calibration is defined as the reader calibration factor (RCF) (HK et al., 2019). The most important part of TLD readings is the calibration process.

2.3. Optically stimulated luminescence dosimetry dosimetry

The phenomenon that occurs when the crystal is excited with light instead of thermal energy is called optically excited luminescence (OSL). The emitted beam intensity is proportional to the number of electrons captured in the traps and the total radiation dose absorbed by the crystal (Yuan et al., 2020). Modelling in OSL is done at trap levels that are most sensitive to light. In addition, the excitation process is regulated at room temperature. Three different methods can stimulate OSL dosimeters. These methods are divided into continuous wave OSL (CW-OSL), linear modulation OSL (LM-OSL), and pulsed OSL (POSL). In CW-OSL, the excitation light intensity is constant, and the OSL signal is observed throughout the excitation period. In the LM-OSL technique, the excitation intensity increases linearly while measuring OSL. In the POSL method, the excitation source is pulsed, and the OSL is observed only between pulses (Kitis et al., 2011; Bulur et al., 2000). The basic elements of an OSL reader are a light source for stimulation and a photomultiplier tube (PMT) for light detection (Figure 4.).

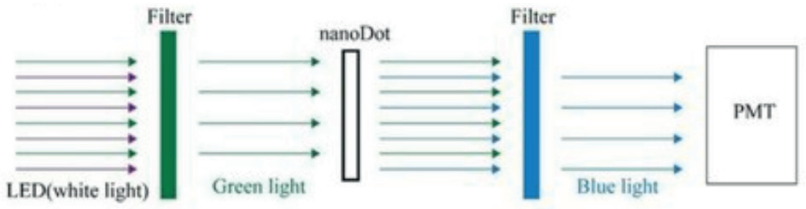

Figure 4. *Basic elements of OSL reader.*

For dose reading, the OSL is held up to the light source. With the stimulation filter, only green light is allowed to pass. OSL emits blue light. The blue light passes through the detection filter and is amplified by PMT. As a result of these steps, the dose value is obtained. The OSL intensity and the total light emitted are proportional to the absorbed dose (Pradhan et al., 2008). OSL dosimeters are widely preferred in radiotherapy because of their many advantages. OSL dosimeters do not require heating like TLD dosimeters. Therefore, it prevents luminescence efficiency problems that may occur with temperature. In addition, OSL dosimeters are dose rate independent.

2.4. Diode dosimetry

Semiconductor diode detectors have been used in radiotherapy for a long time (Koper et al., 2017). The most important reasons why diode detectors are preferred in radiotherapy are their small volume, mechanical robustness and real-time reading features. However, the disadvantages of these detectors include direction, energy, field size, temperature, dose rate and distance dependency. Like other in vivo dosimeters, diode detectors need calibration processes. The dose measurement of these detectors in radiotherapy is practical. The input dose can be measured by placing the detector on the skin. An n-type diode is formed by doping with an n-type silicon boundary acceptor. A p-type diode is formed by doping with a p-type silicon boundary donor. The main carriers of both types switch to the opposite type of silicon. Charge changes cause an electric field between the two regions. This region where the load changes occur is called the p-n junction (Figure 5.).

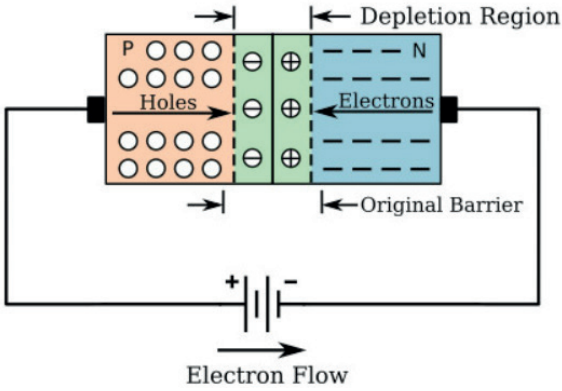

Figure 5. *p-n junction operation*

2.5. MOSFET (Metal-Oxide Semiconductor Field Effect Transistor) Dosimeter

The MOSFET consists of an insulating oxide layer separating a (p-type) positive silicon semiconductor substrate and an (n-type) negative silicon semiconductor layer. MOSFET detectors work according to the semiconductor ionizing radiation detector principle and are used as IVD in radiotherapy (Olaciregui-Ruiz et al., 2020). The absorbed ionizing radiation creates electron-hole pairs in the oxide structure. An electric pulse is formed due to the electrons accumulating in the traps. The absorbed radiation dose is proportional to the amplitude of this electrical pulse. The working mechanism of MOSFET is shown in Figure 6.

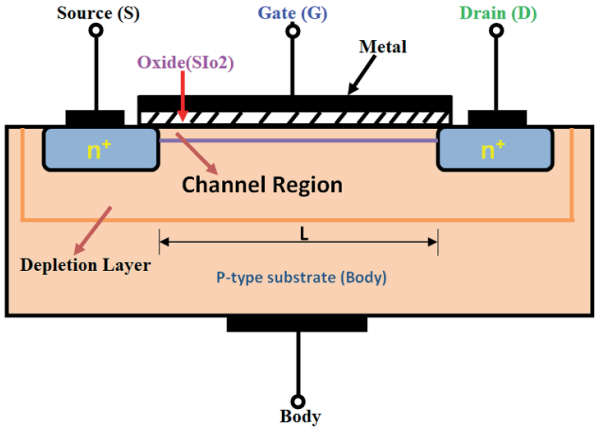

Figure 6. *The working mechanism of MOSFET*

Since they are active dosimeters, they are preferred as IVD in radiotherapy. The easy carrying of MOSFET dosimeters increases the application areas in radiotherapy. The advantages of MOSFET detectors include their small size and sufficient linearity protection. Their disadvantage is that they are sensitive to changes in bias voltage during irradiation.

3. Conclusion

In vivo, dosimeters (IVD) work with the principle of measuring the dose to achieve the goal of treatment in radiotherapy. With the use of IVD, the doses taken by the target volume and the surrounding critical organs can be measured. It is also accepted as an important quality control tool in IVD radiotherapy. The World Health Organization (WHO), International Commission on Radiological Protection (ICRP), and International Atomic Energy Agency (IAEA) recommended the use of IVD. The use of IVD helps to minimize the uncertainties that may occur in radiotherapy. The treatment planning system in radiotherapy cannot predict the skin doses exactly. By placing IVDs on the skin, the inlet doses can be accurately measured and help to apply the treatment more accurately. Many types of dosimeters can be used in IVD applications. Choosing the appropriate dosimeter according to the desired dose measurement is important. In addition to being used for dose measurements on the patient, IVDs can also be used for phantom measurements. In particular, by transferring the patient plans onto the phantom, the dose calculated by the treatment planning system can be compared with the dose measured. Before starting the treatment, the validation process should be effective, quick and error-free for the routine usability of the treatment. Therefore, the IVD method to be chosen is very important clinically.

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Chapter 8

VENOUS ULCERS

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DEFINITION

The most used definition for a leg ulcer is; a wound lesion on the skin of the leg (mostly the lower leg) due to the high pressure in the leg veins (1). While some definitions do not include ulcers that just affect the foot, others do include ulcers that affect the entire lower leg.

INTRODUCTION

Venous leg sores are defined as late signs of chronic venous insufficiency (2-3). Gastrocnemius and soleus muscle contraction and valves of the veins aid normal flow to the heart while avoiding reflux in the normal manner (4). However, the core component that leads to the development of this pathology is venous hypertension which results from reflux and valve dysfunction (5).

Venous leg ulcers are a costly medical issue that places a heavy burden on healthcare systems all around the world (6,7). With a predicted increase in the prevalence of chronic venous disease, the issue might get worse as the world's aging, obese, and sedentary population grows (8).

PATHOPHYSIOLOGY& ETIOLOGY

Although chronic venous insufficiency is a well-known factor in the development of VLU, ulceration only happens occasionally (5.1%) (9). Blood reflux, blockage, or a combination of both processes may cause CVI to develop, leading to circulatory dysfunction (4). Protein extravasation and fibrin cuff development are brought on by the elevated intraluminal pressure, which also triggers the inflammatory response and therefore obstructs the passage of oxygen and growth factors (10).

Degranulation of mast cells, some leukocytes, increased levels of prostacyclin and matrix metalloproteinase inhibitors, maturing of secretory smooth muscle cells, and fibroblast differentiation into myofibroblasts are the processes that contribute to vein wall remodeling and formation of varicose veins. Primarily M1 macrophages are the cause of the proinflammatory milieu. In the end, persistent inflammation and poor blood flow encourage advancing fibrosis and valvular damage (11). Together, these inflammatory reactions interfere with the healing process, which leads to the development of sores after trauma.

RISK FACTORS

Patients frequently exhibit many risk factors for the development of venous ulcers. These include musculoskeletal diseases, female sex, prior thrombosis or pulmonary thromboembolism, multiparity, and positive family history for thromboembolic events (2,4). Venous illness is also linked to modifiable risk factors like obesity and sedentarism (12).

Genetic characteristics, which appear to be an autosomal dominant feature with variable penetrance, maybe another predisposing factor (10). But no particular gene or gene site has been identified. Individuals with this pathology have been shown to have Forkhead box C2, which is a potential marker on chromosome 16q24 (13).

EPIDEMIOLOGY

The most prevalent kind of chronic wounds in the lower extremities are venous leg ulcers (14). In the United States and Europe, between 1 and 3 percent of the senior population is thought to be impacted. When visiting their physician for a variety of reasons, 2.21% of chronic venous insufficiency patients had an active or healed venous leg ulcer, according to an epidemiological survey that includes the regions; Asia, Eastern Europe, Latin America, and Western Europe (15).

Males experience a lower overall incidence than females do, however, it might vary depending on the research location, making it difficult to pinpoint the precise figure (16). A 25-year-old population research found that it typically takes 5 years from the diagnosis of chronic venous insufficiency to the development of a sore. In fact, according to another study, the risk for the first ulceration in chronic venous insufficiency patients rose from 4.49% after three years to 4.93% at five years (9)

HISTORY

When venous ulcers or varicose veins first appeared in homo sapiens is unknown. Despite certain animals having exceptionally long legs with high hydrostatic pressures, it appears that other mammals do not suffer from these disorders. Perhaps the groundwork was laid when our distant ancestors adopted an upright bipedal lifestyle by leaving the trees. Homo sapiens, who first appeared 36,000 years ago and are still alive today, is a more plausible contender. However, factors such as short lifespans, lots of squatting, and intense physical activity may have prevented the aforementioned issues in prehistoric society. The first ever proof of an ulcer-like lesion was discovered in an investigation of a mummy discovered in the Valley of the Kings under gross and microscopic conditions. The mummy, which is thought to date from the New Kingdom (1580–1085 BC), has a 3-cm sore on the lateral side of the lower leg. The ulcer was determined to be most consistent with a venous origin based on histologic tests that revealed hemosiderin accumulation (17). Before the advent of Christ, Hippocrates, known as the "Father of Medicine," was the most well-known and significant physician. He was born during the period of Socrates, and Plato, also known as the "Golden Age" of Greece, about 460 BC, on the little island of Cos, and he passed on around 377 BC (18). He said "... wetting all sorts of ulcers except with wine... for, the dry is nearer to the sound, and the wet to the unsound, since an ulcer is wet, but a sound part is

dry. And it is better to leave the part without a bandage unless a [poultice] be applied. … it is not expedient to stand; more especially if the ulcer be situated in the leg; but neither, also, is it proper to sit or walk. But quiet and rest are particularly expedient." Additionally advised were a limited diet, plenty of water, and gentle bowel cleansing. He recommended debridement of circular ulcers, periodic cleansing of the ulcer, and debridement (19). In the year 130, Claudius Galen was born in Pergamon a Greek city. His works dominated medical thought and practice.3 -26-In order to remove varicose veins, he recommended using a blunt hook. He also recommended fasting, purging, and bleeding to cure ulcers and rid the body of harmful humors (20).

Galen uses the example of a guy who had a persistent leg ulcer that was originally treated by removing varicose veins that were close to the ulcer. Although the surgical wound was open for a year, the ulcer recovered quickly. The 4 days of venesection enabled the wound to heal.5 Thus, making the lesion bleed became the preferred way to remove blood from wounds before it stagnated and turned bad. Two thousand years ago, venous medicine was in such a primitive stage. At this point, ulcers were distinguished from traumatic wounds that did not heal, and some of them appeared to be linked to varicose veins. They received treatment by being washed in vinegar or wine, having different poultices applied, and being bandaged, as well as the tried-and-true methods of purging and bleeding. Many surgeons in the 19th century accepted the theory that leg ulcers were caused by varicose veins and thought that cutting off the saphenous vein would hasten the healing of leg ulcers. Several quick and easy procedures were made popular by Sir Everard Home, Sir Benjamin Brodie, and the French surgeon Velpeau. The high incidence of infections (many of which were deadly), discomfort during surgery, and average outcomes, however, tempered the enthusiasm for surgery. John Gay argued that the term "varicose ulcer" was inaccurate and should be changed to "venous ulcer." In a book that was published in 1866, he observed that "varicose" ulcers might occur without varicose veins, and that, conversely, varicose veins could be present for many years without any kind of sore or color change of the skin. Instead, he discovered that (post-thrombotic) disease of the deep veins, of which varicose veins may also be a complication, was invariably associated with ulcers and skin changes. Gay observed that while ulcers may recover following varicose vein ligation, both the sores and the varices frequently recur on follow-up evaluation. He proposed the theory that healing might not be related to venous ligation but rather result from the postoperative period of bed rest (21).

DIFFERENTIAL DIAGNOSIS

Leg ulcer formation is a clinical symptom that many illnesses share. Leg ulcers often develop in the foot or lower leg, with venous ulcers more frequently seen in the gaiter region, close to the skin area affected by lipodermatosclerosis

however non-venous ulcers are more frequently found in the foot region. Numerous local and systemic causative elements might cause chronic sores of the lower limbs, giving rise to a general comparison between ulcers. According to estimates, venous origin contributes to about 65% of chronic leg ulcers, and this figure sharply rises if distal foot ulcers are taken into account. These figures are directly related to the observation of chronic venous disease symptoms such as edema, varicose veins, and skin changes in at least 25% of the population, which increases the likelihood of diagnosing chronic venous disease in patients with other types of ulcers. In addition to venous origin, additional frequent etiologies include arterial, mixed, neuropathic, diabetic, and pressure ulcers, whose frequency is correlated with population aging (22).

The differential diagnosis may be aided by knowing where the wound is. In actuality, arterial ulcers mostly occur in the distal areas of the extremities, while venous leg ulcers are typically situated in the lower leg region and show symptoms of chronic venous insufficiency (such as lipodermatosclerosis, edema, hyperpigmentation, white atrophy). Ulcers with arterial origin are typically accompanied by pain, a feeling of coolness, and changes in the color of the skin after leg elevation. Neuropathic sores in diabetes individuals develop in the plantar region, while diabetic ulcers are commonly seen in more distant portions of the extremities (such as the lateral or pretibial aspects of the thigh, the distal aspects of the forefeet and toes the dorsum of the foot) Numerous wounds can mimic ordinary venous leg ulcer, and due to coexisting risk factors, odd wounds are frequently misdiagnosed. Other factors, such as skin malignancies, metabolic abnormalities, inflammatory processes, infections, and other diseases, account for 10% of leg ulcers. During the diagnostic workup, several illnesses with hematological, immunological, connective tissue, and metabolic origins should be taken into account. Furthermore, persistent sores may develop malignant transformation (Marjolin's ulcer) and neoplastic illnesses may also cause leg ulcers (23). To make an accurate diagnosis of atypical wounds, histological analysis is required. It is advised to do skin edge biopsies on venous leg ulcers that have been present for 4 to 6 weeks or longer to check for other potential diseases, particularly if the lesion does not get better with specific wound treatment and compression therapy. Due to prolonged healing of the lesion, a leg ulcer misdiagnosis has a significant negative influence on both patient suffering and financial expenditures. The underlying condition may worsen as a result of incorrect therapies, which can also disguise symptoms, delay an accurate diagnosis, and increase morbidity or death (24).

DIAGNOSIS

The general rule is that to establish the diagnosis and choose the best course of action, the whole circulatory system must be evaluated. The lymphatics will strive to reabsorb the accumulated fluid if persistent edema is

present, but over time, it may suffer damage from the chronic inflammation that comes with chronic venous disease (25). Therefore, in such circumstances, a lymphatic system patency examination may also be necessary. Edema that extends proximally and history (such as surgery, malignancy, radiation, or trauma) are typical indications and symptoms of lymphatic involvement. Lymphoscintigraphy is presently the gold standard technique for the lymphatic system and can be beneficial for understanding the degree of involvement. Doppler ultrasound, color flow duplex ultrasound, air plethysmography, or venography can all be used to investigate the venous system. An examination of the micro- and macrocirculation is part of the inquiry of the arterial system. The macrocirculation is evaluated using the Ankle-Brachial pressure index, Doppler waveforms of arterial flow, duplex ultrasonography, angiography, and even magnetic resonance imaging. The microcirculation is evaluated using the measurements of pressure of transcutaneous oxygen (TcPO2), pressure of transcutaneous carbon dioxide (TcPCO2) also capillaroscopy, and laser Doppler flowmetry(24).

TREATMENT

Compression therapy for venous leg ulcers

Several conservative treatments can help with venous hypertension, including extracorporeal shockwave therapy, manual lymphatic drainage, intermittent pneumatic compression, and medical compression. The most easy, efficient, and economical technique for the treatment of venous leg ulcers is compression therapy (5). This calls for the use of different kinds of bandages, which may be single- or multi-layered, and differences in elasticity (26). Unlike inelastic wraps, elastic fabrics offer moldable compression during both rest and exercise (27). A Cochrane study found that multi-component, stretchable systems offer faster healing rates for venous ulcers than single-layered systems (28). Light (whose compression pressures are about 15 mmHg), moderate (whose compression pressures are about 21 mmHg), high (whose compression pressures are about 24 mmHg), or extra-high (whose compression pressures can be up to 60 mmHg) compression strengths are all possible, but the ideal strength is high compression, which should be at least knee-high. In situations of mild cases of peripheral arterial disease, as determined by the ABPI, light compression may be utilized (29,30).

Pentoxifylline or micronized, pure flavonoids is an efficient supplementary approach to compression therapy (31). In several systematic reviews and meta-analyses, both medications have demonstrated better healing especially combined with compression therapy (32). Other systemic medications such as aspirin, zinc, calcium dobesilate, stanozolol, and cilostazol are not supported by enough research to be advised (31). IPC may be used in individuals who cannot tolerate compression treatment or in addition to it. (32). A comprehensive study

found that dressings and delayed IPC delivery were inferior to regular IPC and quick IPC, respectively (33). Very little research has been done on lymphatic drainage and extracorporeal shockwave therapy for the treatment of VLU (34).

The cornerstone of the management of venous leg ulcers is compression therapy. Most of the venous sores will heal with proper wound therapy and compression treatment. The objectives of compression treatment include lesion healing, edema and pain reduction, and recurrence avoidance. Compression is used to treat venous leg ulcers because it narrows the veins, improves valve function, and lowers ambulatory venous pressure, all of which help to lessen venous reflux. Additionally, it quickens the capillary flow and therefore causes a decrease in capillary fluid leakage, this leads to a decrease of inflammatory cytokines and treat limb edema. Additionally, it mitigates lipodermatosclerosis, boosts lymphatic flow, promotes fibrinolysis, and boosts functionality (35).

Contraindications

The following conditions are not appropriate for compression therapy (36).

Advanced peripheral artery disease (defined as ankle brachial pressure index (ABPI) 0.8.)

Dolens' Phlegmasia cerulea

Decompensated congestive heart failure

Abscesses

Septic phlebitis

Peripheral neuropathy that has advanced.

According to some criteria, moderate compression is preferable to none (evidence level of A). A Cochrane review from 2009 (36) (evidence level A) compared compression with primary dressing, non-compressive bandages, and usual care, which was always devoid of compression, and came to the conclusion that compression accelerated the healing of venous ulcers and that any form of compression is preferable to none (37).

Antibiotics: Systemic antibiotics should only be used for individuals who have acute cellulitis symptoms or an ulcer that is clinically infected. There is no proof to back up the regular administration of systemic antibiotics to speed up the healing of venous leg ulcers (38).

Regular use of antibiotics for simple sores might lead to the establishment of resistant organisms. In one trial, 94%of patients treated with ciprofloxacin, 12% treated with trimethoprim, and 4% given a placebo developed resistance microbes to commonly used systemic antibiotics (39).

Systemic antibiotics are only prescribed to individuals who exhibit one or more of the following symptoms or indications that indicate a serious infection:

Heat and soreness in the area

An increase in the erythema of the nearby skin

Lymphangitis, which causes crimson streaks to go up a limb

Rapid expansion of the ulcer's size

Fever

Infection may prevent a wound from healing. A tissue sample should be submitted for gram stain, and culture if infection is clinically suspected. This can help confirm infection and direct the choice of antimicrobial medication. After irrigating the ulcer to remove any surface debris, a tissue sample is taken via biopsy from the ulcer's base and sent off for culture. Some medical professionals promptly remove the fluid by injecting 1 to 2 mL of sterile saline into the dermis around the ulcer. The fluid is then submitted for culturing. Patients with infections that are advancing quickly should be hospitalized and given broad-spectrum intravenous antibiotics, especially if they are also exhibiting fever and other symptoms of systemic toxicity. Diabetes increases the risk of infections that spread quickly (38,39).

Debridement of the ulcer — Debridement of the wound is a crucial part of managing venous ulcers. Devitalized tissue decreases the efficacy of topical treatments and systemic medicines, raises the risk of local bacterial infection and sepsis, and slows the rate at which wounds heal. The creation of healthy granulation tissue and improved re-epithelialization are aided by the surgical removal of necrotic tissue and fibrinous debris from venous ulcers (38,39).

Topical agents — Although a moist environment is necessary for wound healing, the majority of topical medications, such as topical antibiotics, topical antiseptics, and growth factors, do not increase ulcer healing rates (38). Silver sulfadiazine is an antiseptic solution used topically that has a long history of use in the treatment of wounds, such as burns, and donor sites for skin grafts. Most pathogenic microorganisms, including some species that are resistant to sulfonamides, are inhibited from growing in vitro by the release of silver ions in toxic amounts. Silver sulfadiazine has been used for infected lesions, but it is messy, develops a pseudoeschar that must be removed before the wound is dressed again, and it may color the skin. Due to their absorbent properties and convenience of use, silver-containing dressings have gained more popularity than silver cream formulations to treat some of these concerns. Silver sulfadiazine is frequently used to prevent infection or speed up the healing of venous ulcers, however, one comprehensive review found that there was

inadequate evidence to either support or contradict this practice (40) Another discovered no advantage (38).

The use of growth factors and cell-based therapies The process of healing a wound involves several growth factors, such as platelet-derived growth factor PDGF, epidermal growth factor EGF, fibroblast growth factor FGF, transforming growth factor TGF, and insulin-like growth factor IGF. A few well-controlled randomized trials that assessed the use of growth factors in the healing of different diabetic and pressure ulcers revealed conflicting results (41,42).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) injections into the skin appear to hasten the healing of chronic leg ulcers, particularly venous ulcers (43,44) Four weekly injections of either GM-CSF resulted in considerably greater healing rates than placebo at 13 weeks (57, 61, and 19 percent, respectively) according to a randomized trial. The discomfort of the injections limits the usage of intradermal GM-CSF (44).

Dressings for ulcers – Dressings are a crucial part of ulcer management. Dressings assist manage pain, keep the proper level of moisture in the air, and reduce exudate. Additionally, dressings preserve an environment that promotes epithelialization and hastens ulcer healing Venous ulcers can be dressed using semipermeable adhesive films, hydrogels, straightforward nonadherent dressings, hydrocolloids, paraffin gauze, alginates, and dressings or foams infused with silver (45).

The proportional benefits or drawbacks of the various dressing types depend on the features of the ulcer, how frequently the dressing is changed, and the cost. In real life, treating a venous ulcer involves using a variety of bandages (46).

Dressing types that could be used and their primary functions:

Alginates/CMC (carboxymethylcellulose)(effectively absorbs fluid promotes autolytic debridement) Foams(absorbs fluid and controls moisture) Honey (rehydrates also has antimicrobial action) Hydrocolloids (absorbs fluid promotes autolytic debridement) Hydrogels(rehydrates and cools the wound) Iodine (antimicrobial action) Low-adherent wound contact layer (silicone) (protects new tissue) PHMB (polyhexamethylene biguanide) (antimicrobial action) Protease modulating (controls protease levels) Silver(antimicrobial). Wound covering — Uncontrolled surgical studies and medical professionals' opinions support the use of skin grafting for extremely big venous ulcers or ulcers that have been present for more than a year (45).

Comparing compression and a basic dressing to bilayer artificial skin and compression bandages, a comprehensive review of clinical studies found that the latter improved ulcer healing (47). Although they shouldn't be considered

as a replacement for initial compression therapy for venous ulcers, human skin analogs could be an option for patients who don't improve with the treatment. ineffective or minimally effective supplements to ulcer treatment Hyperbaric oxygen Electromagnetic therapy Therapeutic ultrasound (48,49).

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Chapter 9

ENDOMETRIAL CANCERS

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1 Epidemiology

Although the incidence of endometrial cancer (EC) varies worldwide, it ranks fourth in the number of new cases after breast, lung and colorectal cancers according to cancer statistics by 2022 (1). In the world distribution, developed countries have the highest incidence rates and undeveloped countries have the lowest incidence rates (2). EC ranks fifth in the number of new cases in Turkey after breast, thyroid, colorectal and lung cancers (3). EC is the second most common gynecological malignancy after cervical cancer. In developed countries, it ranks first. The incidence is gradually increasing (4). It is thought that the reason for the increase in the incidence is the increasing obesity epidemic, the resulting hyperinsulinemia, population aging, and the decrease in the use of combined menopausal hormone therapy (5, 6). According to GLOBOCAN data, 417,367 new cases have been detected annually worldwide. 97,370 women died from this disease (1). In Turkey, the annual number of newly diagnosed patients was reported as 5918, and the number of those who lost their lives due to the disease was reported as 1589 (7).

2 Etiology and Risk Factors

2. 1 Estrogen

Endogenous and exogenous estrogen is the most important risk factor (8). Early menarche, late menopause, hormone replacement therapy (HRT), nulliparity, infertility, chronic anovulation (polycystic ovary syndrome (PCOS)) and tamoxifen are the causes of long-term estrogen exposure (9). Early menarche increases the risk of developing EC 9 times (10). The risk of EC increases 10-20 times in women taking HRT. However, it was found that combined HRT did not increase the risk (11). Nulliparity; It is a risk factor for endometrial, ovarian, breast and pancreatic cancer. The risk of endometrial cancer is 2-3 times higher in women who have never given birth (12). The risk of EC increases 3-4 times in women with PCOS (13). Tamoxifen is an antiestrogenic drug used in women with breast cancer risk and positive receptor breast cancer (14). However, it has a weak estrogenic effect in the endometrium. It has been found that the risk of EC increases 2 times in women using tamoxifen for 2-5 years, and 6.9 times in those using it for more than 5 years (15).

2. 2 Age

EC mostly affects postmenopausal women. The average age of onset is 60. About a quarter of EC is diagnosed between the ages of 65 and 74. Approximately one third of EC-related deaths occur in this age range (16).

2. 3 Family history

An increased risk of EC was found in the first-degree relatives of patients with a history of EC. The presence of EC more than doubles the risk. The risk of EC was found to be higher in women under 55 years of age with a family history (17).

2. 4 Having a history of cancer

Presence of breast and ovarian cancer in the patient's history increases the risk of EC. It is associated with increased estrogen. This risk is 3 times higher compared to individuals without a history of cancer (18).

2. 5 Diet

In general, natural fruits, vegetables and fibrous foods also reduce the risk of EC. In contrast, a high-fat and high-energy diet consisting of concentrated animal foods increases this risk (19).

2. 6 Obesity

While a decrease is observed in the incidence of many other cancers, an increase is observed in EC. This increase has been associated with obesity (20). Obesity is associated with high estrogen levels. This high estrogen level causes a decrease in the level of sex hormone binding globulin (SHBG) (21). At the same time, low progesterone levels are observed in obese women due to anovulation (22).

2. 7 Hypertension

The risk of developing EC was found to be three times higher in hypertensive patients (23).

2. 8 Diabetes

Presence of diabetes is a cause of increased risk of EC (24). Insulin, a stress hormone, increases estrogen levels. It stimulates the release of testosterone from the ovary. It also lowers circulating SHBG levels. It prevents the production of SHBG from the liver (25). The causes of increased risk in diabetic patients are thought to be increased insulin, insulin-like growth factor-1, irregularity of steroid synthesis in the ovaries, inflammatory mediators and adipokines (26).

2. 9 Radiotherapy

Radiotherapy given to the abdominopelvic region may rarely cause the development of EC. Radiation-related EC is associated with worse differentiation (27).

2. 10 Lynch syndrome

Lynch syndrome (LS) constitutes the majority of hereditary endometrial cancers. It constitutes 2-3% of all ECs (28). LS is an autosomal dominant disease. LS is associated with many types of cancer (renal pelvis, ovary, stomach, small intestine, etc.), especially colon and endometrial cancer. LS is caused by a heterozygous mutation of any of the MLH1, MSH2, MSH6 and PMS2 genes involved in the mismatch repair (MMR) pathway, which is one of the DNA repair mechanisms. The risk of developing LS, EK is 60% (29). EC, which occurs in LS, occurs at early ages. ECs associated with Lynch syndrome are generally poorly differentiated (30).

2. 11 Cowden syndrome

Cowden Syndrome (CS) is an autosomal dominant syndrome. CS is associated with PTEN mutation. There is an increased risk for breast, thyroid, kidney, endometrial, colon cancers and melanoma in this syndrome. The incidence of EC in women with Cowden syndrome is 25-30%. It can develop at a very early age (30).

2. 12 BRCA1 and BRCA2 genes

Patients with BRCA1 and BRCA2 gene mutations have a high incidence of breast and ovarian cancer. At the same time, BRCA2 gene mutation is associated with pancreatic cancer and malignant melanoma, and BRCA1 mutation is associated with the risk of EC (29). BRCA1 mutation also increased the risk of EC. ECs associated with BRCA 1 mutation are associated with poor prognosis (30).

2. 13 Other genetic causes

POLE, MUTYH, and NTHL1 gene mutations are other genetic causes of EK (31). POLE constitutes 8-10% of all EK. They have a very good prognosis The diagnosis of these patients is usually at an early stage. It is seen in young patients (28).

3 Protective Factors in Endometrial Cancer

3. 1 Combined oral contraceptives

Combined oral contraceptives (COCs) Estrogen has a stimulating effect on endometrial proliferation, and progesterone has an inhibitory effect (32). Therefore, a protective effect is observed with COCs. The risk of EC use is lower in those who use COCs compared to those who never use it, and COCs reduce the risk of EC by 30-50% (33).

3. 2 Gravida

The risk of developing EC with pregnancy is inversely proportional. Each year spent with pregnancy reduces the risk of cancer by 22% (34). The

advanced age of birth also reduces the risk of developing EC. Breastfeeding has a protective effect against EK. The protective effect against EC is the suppression of pituitary and ovarian hormones during breastfeeding and the reduction of stimulation of the endometrium with estrogen (35).

3. 3 Smoking

The relationship between smoking and the risk of EC is inverse. Endometrial thickness of smokers in the postmenopausal period was found to be more atrophic than non-smokers. These patients have a lower risk of endometrial hyperplasia. It has been shown that the risk of EC is reduced in postmenopausal women who smoke (36).

3. 4 Physical activity

With physical activity, insulin resistance, sex hormones, adipokines, inflammation and body fat are reduced. It has been shown that physical activity reduces the risk of EC recurrence and death by 46% (37).

3. 5 Coffee and Tea

Coffee and tea are protective factors against EK. A decrease in the risk of EC has been shown in women who drink one or more cups of coffee a day. It has been shown that consumption of 1 cup or more of tea per day protects against 5% EK (38).

3. 6 Aspirin

It has been reported that the consumption of aspirin 600 mg/day causes a decrease in the incidence of EC. It provides a secondary protection in women who are obese and at risk for cardiovascular disease, especially in EC patients (39).

4 Traditional Histopathological Classification of Endometrial Cancers

Bokhman in 1983; He divided the ECs into two. These are Type I (endometrioid type) and Type II (serous type) (40). The World Health Organization (WHO) has classified 2020 Endometrial epithelial tumors (Table 1) (41).

Type I EC constitutes the vast majority (80-90%), mostly endometrioid adenocarcinomas. Type I EK occurs in obese women with hyperlipidemia and hyperestrogenism. It is sensitive to progestins and has a good prognosis (42). It is usually low grade and has an early stage at the time of diagnosis. They develop on the background of a premalignant lesion with endometrial intraepithelial neoplasia (EIN) (43).

Type II ECs are predominantly serous carcinomas. It usually develops on the atrophic endometrium floor, independent of estrogen. The patient population is typically older, postmenopausal and frail women. It has

decreased sensitivity to progestins and has a poor prognosis. Although they constitute only 10% to 20% of cases, they constitute 40% of deaths (42). In these patients, p53 mutation is more common (44).

Table 1. 2020 World Health Organization (WHO) classification of endometrial epithelial tumors.

-Endometrioid Adenocarcinoma

-Serous Carcinoma

-Clear Cell Adenocarcinoma

-Undifferentiated Carcinoma

-Mixed Cell Adenocarcinoma

-Mesonephric Adenocarcinoma

-Squamous Cell Carcinoma

-Mucinous Carcinoma, Intestinal Type

-Mesonephric-Like Adenocarcinoma

-Carcinosarcoma

5. Molecular Classification of Endometrial Cancers

Bokhman's model remains valid. Four different endometrial cancers with different clinical, molecular and pathological features were defined by the Cancer Genome Atlas (TCGA) in 2013 in order to develop diagnostic test methods and targeted personalized therapies, different from the current histological classification (42,45). These are POLE Mutated Endometrioid Carcinoma, Microsatellite Unstable Endometrioid Carcinoma, P53 Mutant Endometrioid Carcinoma, and Endometrioid Carcinoma Without a Specific Molecular Profile (40,46).

POLE constitutes 8-10% of all EKs (35). POLE involved in nuclear DNA replication and repair is associated with somatic mutations. They have a very good prognosis even if they include high grade histology, severe nuclear atypia or p53 mutation. The majority are seen in young patients (46).

The MSI group constitutes 25-30% of EKs (46). There is loss of nuclear expression of MMR proteins (MLH1, MSH2, MSH6 and PMS2). Contains hypermutation. A large part is due to the epigenetic change in MLH1. 10% is associated with Lynch Syndrome. It is characterized by high mutation rates, KRAS and PTEN mutations. ECs associated with Lynch syndrome form a subset of MSI tumors (47).

The third group is Low Copy Number/Microsatellite Stable (CNS) tumors (49). These tumors are grade 1-2 and are of medium risk. Mutations

are frequently found in PTEN, PIK3CA, ARID1A and CTNNB1, SOX 17 and KRAS genes, which are involved in the Wnt signaling pathway (45, 48).

The fourth group consists of tumors with a high copy number (serous and serous-like). TP53 mutation is common. It constitutes approximately 26% of all endometrial cancers (46). The majority of almost all serous carcinomas (97.7%) and mixed type carcinomas (75%) belong to this group. It is the group with the worst prognosis (45, 48).

6. Histopathological Classification of Endometrial Cancers

6. 1 Endometrioid Endometrial Carcinoma (EEC)

EEC constitutes 70-80% of all ECs (52). EEC often develops from the background of atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN), and sometimes it can occur in the background of endometrial polyps. Endometrioid adenocarcinomas are tumors that form back-to-back complex glandular structures histomorphologically in a desmoblastic stroma (50). The glands are lined with stratified or pseudostratified columnar cells with round nuclei (51). Nuclear pleomorphism is low grade (49). There are numerous histological patterns of endometrioid adenocarcinoma characterized by squamous differentiation, villoglandular pattern, secretory pattern, microglandular pattern, small non-villous papilla, spindle cell pattern, serteliform pattern, mucinous pattern, sex cord-like structure and hyalinized pattern. These patterns are not associated with prognosis (53).

6. 2 Serous Carcinoma

Serous carcinoma (SC) constitutes 3-10% of ECs (49). SCs typically arise from the background of a polyp or atrophic endometrium (50) All SCs are considered high-grade tumors (49, 53). Histomorphologically, a desmoblastic stroma usually has a distinct papillary pattern with fibrovascular cores. It also contains less solid and glandular patterns. Nuclear pleomorphism exhibits high-grade features. Compared with EEC, SCs exhibit a high frequency of c-myc, C-erbB-2/HER2-neu overexpression and p53 mutation (54). Compared with EEC, SK has a lower frequency of PTEN and K-RAS mutations, as well as lower immunoreactivity for bcl-2, E-cadherin, beta-catenin, estrogen and progesterone receptors (55).

6. 3 Mucinous Adenocarcinoma (MC)

It constitutes 1-9% of all ECs (49). Intracytoplasmic mucin is found in more than 50% of tumor cells. If there is intracytoplasmic mucin in less than 50% of tumor cells, it is diagnosed as "endometrioid carcinoma with mucinous differentiation" (59). MC is low grade. Mucin-containing columnar cells with mild to moderate atypia form glandular or villoglandular structures.

Neutrophil infiltration is common in the tumor. Invasion usually does not exceed half of the myometrium (57). Unlike EK, the most common KRAS mutation is observed. Mitotic activity is not evident. The prognosis is very good (55).

6. 4 Clear Cell Adenocarcinoma

Clear cell adenocarcinoma (CHC) constitutes 3-6% of ECs (49). CAC develops on the background of atrophic endometrium and endometrial polyp. CHC is a high-grade, rare subtype. CACs have major structural skates: solid, papillary, and tubulocystic. Tumors have polygonal or hobnail cells with prominent nucleoli and clear cytoplasm, exhibiting prominent nuclear pleomorphism (58). Like SCs, but unlike ECC, CACs are estrogen and progesterone receptor negative and have a high Ki-67 index. Unlike SCs, it has low immunoreactivity for p53. They also exhibit positive expression with HNF-1β, napsin A and AMACR (59).

6. 5 Squamous Cell Carcinoma

It constitutes 0.1% of all ECs (60). This tumor has a poor prognosis. Squamous cell carcinoma has a 36% survival rate (55).

6. 6 Undifferentiated and Dedifferentiated Carcinoma

The frequency of undifferentiated carcinomas is 1-2%. Dedicated and undifferentiated carcinoma are usually very aggressive tumors, with recurrence and death rates in 55-95% of cases (52, 61).

6. 7 Transitional Cell Carcinoma

It is seen in elderly patients and exhibits aggressive behavior. Often with another type of carcinoma; it is usually associated with squamous, sometimes endometrioid or serous papillary carcinoma (55).

6. 8 Small Cell Carcinoma

It is quite rare, about 1%. Histologically, it resembles small cell carcinomas in other organs. It has a poor prognosis (55).

6. 9 Mixed Endometrial Carcinoma

Mixed endometrial carcinomas (MEC) constitute 3-10% of all ECs. It is the coexistence of type 1 and type 2 EK (62). Regardless of the percentage of the type II component, its presence establishes the diagnosis of MEC and determines the prognosis (52).

6. 10 Mesonephric Adenocarcinoma and Mesonephric-like Adenocarcinoma

Mesonephric adenocarcinoma (MA) is usually observed in the cervix and vagina. MA is very rare. In the uterus, it is observed in the endocervical region

where mesonephric remnants/hyperplasia attract attention. Histologically, cystic tubule structures, papillary structures, ductal and solid patterns are seen. It consists of uniform small and round cystic tubule structures lined with cuboidal cells, containing eosinophilic colloid-like material in their lumen. Immunohistochemically, although one or more of the CD10, TTF-1, calretinin and androgen receptors are characteristically positive, although not specific, staining with the estrogen receptor (ER) or progesterone receptor (PR) is not observed in MA (52). Mesonephric-like adenocarcinomas (MLA) have been described recently. These tumors are adenocarcinomas with mesonephric differentiation. They show morphological, immunohistochemical, and molecular similarity to MA arising from true mesonephric remnants. MLA can often be confused with other endometrial neoplasms, but it has an aggressive clinical behavior and tends to metastasize early to the lungs (63).

6. 11 Carcinosarcoma

Carcinosarcomas account for 5-9% of all uterine neoplasms. It is a rare gynecological malignancy that usually has a poor prognosis (64). Carcinosarcomas have both epithelial and mesenchymal components. The malignant epithelial component is typically endometrioid adenocarcinoma. However, serous, mucinous, clear cell, and squamous cell carcinomas can also form the epithelial component. The sarcomatous component may be endometrial stromal sarcoma, leiomyosarcoma, rhabdomyosarcoma, osteosarcoma, chondrosarcoma, and liposarcoma (52). In both cases, lymphovascular invasion is common. Carcinosarcomas are very aggressive tumors. 60% of cases present with extrauterine disease (64). Despite surgical and adjuvant therapy, recurrence occurs in more than 50% of cases. Compared with high-grade endometrial carcinomas, multiple studies have shown that carcinosarcomas are a much more aggressive tumor. Estimated 5-year survival is 33-39%. Even in the case of disease limited to the uterine corpus, the recurrence rate is high (65).

7. Prognostic Factors

7.1 Age

In general, older women have a worse prognosis and lower survival than younger women. Cases diagnosed at an advanced age have a higher risk of recurrence (66).

7. 2 Histological type

Type I EC is usually confined to the uterus and has a higher survival. Type II EC has advanced disease and high mortality rates (67).

7. 3 Histological grade

Histological grade is related to prognosis, stage, lymph node metastasis and myometrial invasion. In a study, five-year survival rates were reported as 94% in grade 1, 84% in grade 2, and 72% in grade 3 (67).

7. 4 Stage of the tumor

Tumor stage is still the strongest prognostic marker. (68).

7. 5 Lymphovascular invasion

Presence of lymphovascular invasion is the strongest independent prognostic factor for regional recurrence, distant recurrence, and overall survival (68). The presence of lymphovascular invasion is associated with reduced survival. It is associated with poor prognosis even in patients without lymph node metastases (69).

7. 6 Myometrial invasion

Myometrial invasion depth is an independent predictor of overall prognosis. As the depth of myometrial invasion increases, the survival rate decreases. If there is no invasion of the myometrium, survival is 94% if the tumor is limited to the endometrium, 91% if the tumor has not exceeded half of the myometrium, and 59% if it has invaded more than half of the myometrium. The increase in myometrium involvement also increases the recurrence rate (68).

7. 7 Cervical involvement

Presence of cervical stromal invasion in EC makes it stage II. Cervical stromal invasion is associated with worse prognosis and recurrence. (68).

7. 8 Parametrial spread

Parametrial spread is related to the stage of the disease. As the stage increases, the rate of parametrial spread also increases. While stage II tumors show 6% parametrial spread, this rate is 17% in stage III tumors. At the same time, the presence of parametrial spread is associated with high recurrence rates and poor survival (70).

7. 9 Peritoneal fluid

Peritoneal fluid performed as part of the surgical procedure in EC patients is evaluated cytologically. However, it is not currently used for EC staging. In addition, if there is no extrauterine spread, its prognostic significance is controversial. However, the presence of positive peritoneal cytology may be considered as a negative factor for the adjuvant treatment protocol (68).

7. 10 Adnexal involvement

EC patients may have adnexal involvement. Adnexal involvement adversely affects the survival of patients. In addition to involvement, lymphovascular invasion, lymph node metastasis, cervical stromal invasion, uterine serosa invasion, and distant organ metastasis incidence are increased in these patients (71).

7. 11 Lymph node metastasis

Presence of lymph node metastasis is the main prognostic marker for the prognosis and survival of the patient (72). In patients with pelvic or paraaortic lymph node metastases, 5-year disease-free survival is 36%. If pelvic lymph node metastasis is present, the risk of involvement of paraaortic lymph nodes is 50%, if not, this rate is less than 5%. In early stage EC, the depth of myometrial invasion is associated with lymph node metastasis. If there is an invasion that does not exceed half of the myometrium, the risk of lymph node metastasis is 5%, and if there is an invasion that exceeds half of the myometrium, the risk is 33% (70).

7. 12 DNA ploidy

DNA ploidy is an important prognostic marker for EC. 20-35% of EC cases are aneuploid. Less than half of myometrial invasion is observed in diploid EC cases (73). These patients have longer survival. The disease-free survival rate for early-stage diploid EC was 94%, and 64% for aneuploid carcinomas (74).

7. 13 Hormone receptors

20% of the cases with EC have advanced disease, poor prognosis and limited treatment options at the time of diagnosis. On the other hand, cases with grade 1-2 EC that express more than 50% of estrogen and progesterone have higher disease-free survival with hormone therapy (75).

7. 14 Bcl-2

Bcl-2 is a proto-oncogene. Inhibits programmed cell death. Bcl 2 is increased in endometrial hyperplasia, but decreased in adenocarcinoma. Loss of Bcl-2 expression is associated with increased depth of myometrial invasion, increased lymph node metastasis rate, poor prognosis, and decreased survival (76).

7. 15 CerbB2

C-erbB-2 (HER-2) is a transmembrane receptor protein with tyrosine kinase activity. 10-40% C-erbB-2 overexpression occurs in EK. C-erbB2 is a poor prognostic factor (77).

7. 16 Proliferation markers

The Ki67 proliferation index is the most common biomarker of cell proliferation. Ki 67 proliferation index is associated with prognosis in late stage EC. Early stage EK Ki 67 proliferation index low is a better prognostic indicator, while a high Ki-67 proliferation index is a poor prognostic indicator for Type II EC (59).

7. 17 Microsatellite instability (MSI)

MLH1, MLH6, MSH2, MSH3, and PMS2 are genes responsible for microsatellite instability (MSI) involved in DNA repair. MSI, especially endometrioid EC, is associated with a good prognosis. Cases with MSI in EC have a 20% better survival rate (78).

7. 18 K-ras

K-ras mutations are seen in 10-30% of ECs. K-ras mutations are associated with the depth of myometrial invasion, tumor stage and survival in EC cases (79).

7. 19 PI3K/AKT/mTOR pathway

In EC, changes in the PI3K/AKT/mTOR pathway are observed in 92% of type I ECs and 60% of type II ECs. In Type 1 EC, this change mechanism is loss of PTEN and/or PI3K mutation. In Type II EC, however, it is associated with higher mTOR expression and lower rates of PTEN loss (80).

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Chapter 10

PEDIATRIC DENTAL CROWN RESTORATIONS AND APPLICATION TECHNIQUES

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Tooth decay is defined as the destruction of dental tissues caused by acids that occur as a result of the fermentation of carbohydrates taken into the body by bacteria (Ustun and Koruyucu, 2021). Dental caries is an infectious disease and should still be considered a serious public health problem today. According to the WHO report in 2003, 60-90% of school-age children are affected by dental caries (Garg et al., 2016).Tasks of primary teeth include providing aesthetics, phonation and function until permanent teeth erupt, guiding permanent teeth and maintaining space (Ustun and Koruyucu, 2021). The presence of primary teeth in the mouth until exfoliation time is important for the jaw development of children. While decayed primary teeth negatively affect the growth and development of the child with pain and infection, it causes a decrease in the child's self-confidence with its negative effect on aesthetics and also affects the psychological and social development of the child (Sztyler et al., 2022). There are some factors to consider when treating severely destructed teeth in pediatric patients. These are primarily cooperation of the child, the maintenance of the tooth structure and parental satisfaction. (Garg et al., 2016; Rezvi et al., 2021)

The primary tooth restoration should be durable, aesthetic, retentive and strong. Because it is expected to present in the mouth and serve the function until the primary tooth is exfoliated (Yang and Mani, 2016). For many years, restorations in teeth with excessive tissue loss have been performed with glass ionomer cements (GIC), amalgam, compomer and resin composite materials. However, the resin restoration technique shows failures such as postoperative sensitivity, secondary caries formation, tooth or resin fracture, and long-term discoloration (Pei and Chen, 2023). Therefore, in recent years, AAPD recommends that teeth with excessive tissue loss, developmental defects, pulpotomy or pulpectomy should be restored with crowns (AAPD, 2022). Thus, it can be ensured that the mesio-distal dimension of the primary teeth is preserved, the structural integrity of the tooth is preserved and the life span of the tooth is increased (Mulder et al., 2018). Although each restorative material used has its own advantages and disadvantages, studies comparing these materials in terms of survival rate, secondary caries formation and durability report that the most successful results are seen in restorations performed with prefabricated crowns. (Roberts et al., 2005; Sztyler et al., 2022).

Indications for full coronal restoration of primary teeth are multisurface caries, incisal edge involvement, severe cervical decalcification, poor oral hygiene, following pulpotomy or pulpectomy, the loss of the majority of the tooth structure, developmental defects, and discolored teeth that are aesthetically unpleasing (Waggoner et al., 2002; Sathyaprasad et al., 2020; Shrestha et al., 2020).Pediatric crowns used in the clinic should be easy to apply, resistant to chewing forces, not harming the opposing tooth, biocompatible with the surrounding tissues, and not preventing oral hygiene

(Sztyler et al., 2022). Full coronal restoration of anterior teeth with excessive tissue loss is a treatment method preferred by both pediatric patients and their parents, especially in terms of aesthetics and phonation. However, aesthetic restoration of anterior teeth is difficult due to the close proximity of primary tooth pulp to the surface, small size of the teeth, relatively thin enamel and surface area for bonding, concerns with child behavior and finally the expense of the treatment. (Shah et al., 2004; Sathyaprasad et al., 2020).

Today, stainless steel crowns (SSC), open-faced SSC, preveneered SSC, polycarbonate crowns, strip crowns, crowns produced with a CAD/CAM system, and zirconium crowns are the most commonly used crown restorations in the treatment of primary teeth. (Ustun and Koruyucu, 2021). Open-faced SSC, preveneered SSC, strip crown, zirconia crown and fibreglass crowns (Figaro crowns) are among the most preferred crown materials in anterior restorations. Pediatric dental crowns are classified according to their cementation methods and material composition in the literature (Table 1) (Shrestha et al., 2020).

Table 1. Classification of pediatric dental crowns

Pediatric Dental Crowns		
According to Cementation Method		**According to Material Composition**
Cemented/Luted	***Bonded***	Polymer crowns
Stainless steel crowns (SSC)	Polycarbonate crowns	Polycarbonate crowns
Open-faced SSC	Strip crowns	Strip crowns
Preveneered SSC	Pedo jacket crowns	Pedo jacket crowns
Aluminium veneered tooth colored crowns	Composite resin based crowns	Acrylic crowns
Zirconia crowns		Composite based crowns
Composite based crowns		Artglass crowns
		Fibreglass crowns
		Lab enhanced customized crowns
		Preveneered SSC
		Aluminium veneered tooth colored crowns
		Zirconia crowns

Each material and technique has benefits and drawbacks of its own. The quantity of the remaining tooth tissue, the patient's financial situation, the ability to provide adequate moisture control, the cooperation of the child, and the aesthetic expectations all play a role in the material choice. The dentist should choose a practical, proper and durable solution that is fulfilled by thorough familiarity with various crown forms. Pediatric dentists have challenges in providing these teeth with pleasing restorations, improving esthetics, and also managing space and function (Sathyaprasad et al., 2020).

STAINLESS STEEL CROWNS

Crown restorations were first introduced in 1947 by the Rocky Mountain Company. In 1950, Engel described stainless steel crowns (SSC), but they became popular with Dr. William Humphrey and began to be used as a space maintainer and crown restoration (Ustun and Koruyucu, 2021; Amlani and Brizuela, 2023). Since then, changes in design have made the fitting process easier and the crown's morphology better, more closely mimicking the anatomy of primary molar teeth. Stainless steel crowns can be defined as semi-temporary crowns used on primary and young permanent teeth. They are prefabricated metal crown forms that are placed on the individual teeth and cemented by using a biocompatible luting agent (AAPD, 2022).

They went by the abbreviation SSC and were made of stainless steel. However, the initial metal was shortly replaced with nickel-chromium, and today it is most commonly referred to as a preformed metal crown (PMC) (Garg et al., 2016). PMC typically comprises 67% iron, 17-19% chromium, 10-13% nickel, and 4% of minor elements, though the exact composition vary significantly depending on the brand. According to reports in the literature, PMCs last longer than amalgams (Einwag and Dunninger, 1996; Sztyler et al., 2022).

Typically, their initial designs had excessively wide, straight, and lengthy sides. Numerous processes, including trimming, contouring, crimping, and finishing, were necessary for proper adaptation to teeth. Many drawbacks associated with the first SSCs were eliminated with the introduction of nickel-chromium crowns. First off, Cr-Ni crowns are perfectly formed and flaw-resistant. Second, they hardly ever need trimming due to improved anatomical precision. Untrimmed, pretrimmed, and precountoured crowns are the three primary types of preformed metal crowns (PMCs) available on the market. Untrimmed crowns need significant modifying because there was no trimming or contouring performed during manufacture. Pre-trimmed crowns, on the other hand, have straight sides that are adorned to match the gingival line. They still need to be trimmed sometimes and contoured. Lastly, pre-contoured crowns have pre-contoured and festooned sides. Even though they provide the finest replica of the tooth anatomy, the adaption process sometimes necessitates slight trimming and recontouring (Sztyler et al., 2022).

There are some indications and contraindications for using PMC in primary and permanent teeth (Subramaniam et al., 2010; Dhar et al, 2015; Garg et al., 2016; AAPD, 2022; Amlani and Brizuela, 2023).

Indications for application on primary teeth

1. Multisurface caries of primary teeth
2. Following pulpotomy or pulpectomy

3. When other restorative materials fail to provide satisfactory results

4. Cervical decalcification or developmental defects

5. As an abutment for a space maintainer or denture

6. As a prevention restoration in high caries risk patients

7. When children need general anesthesia for dental treatment, stainless steel crowns should be seriously considered

8. Severe bruxism

9. Patients who will not regularly come to follow-up appointments

Indications for application on permanent molar teeth

1. As a temporary restoration for a fractured or damaged tooth while waiting for a permanent restoration to be completed or the determination of the future orthodontic status

2. In clinically suitable cases, permanent PMCs are helpful as a semi-permanent, affordable restoration when financial concerns are an issue

3. The crowns help young patients with developmental defects by correcting the occlusion and minimizing any sensitivity.

4. If a full coronal restoration is necessary for the restoration of the permanent molar

Contraindications for use of PMCs

1. Primary teeth that show more than 50% root resorption or are almost ready to exfoliate

2. In case of excessive mobility of the tooth

3. If the patient has a nickel allergy

4. Lack of cooperation prevents the crown from being properly placed

Advantages

1. They have long life span and they are extremely durable

2. They protect the remaining tooth structure which have been weakened after caries removal

3. During the placement and cementation process, they are barely sensitive to oral conditions, so there is little chance of mistakes

4. They are reasonably cost-effective over the long term.

5. They have a low failure rate

Drawbacks

1. They have poor aesthetic appearance
2. When the tooth has just partially erupted, it cannot be used

The unaesthetic appearance of stainless steel crowns emerge as a significant disadvantage for both, anterior and posterior primary tooth. To address this drawback, preveneered stainless steel crowns and open-faced stainless steel crowns have been created over time. (Kindelan et al., 2008). Long-term controlled studies in the literature report that SSC shows high stability and success rate. In contrast, restorations performed with prevenered SSC and resin crowns showed more embrittlement. There is still a need for more options with both more aesthetic appearance and high stability, especially for anterior restoration (Pei and Chen, 2023). In studies comparing stainless steel crowns with other restorative materials, there is evidence that SSCs provide more successful clinical results than amalgam restorations in the long term (Dhar et al, 2015). A review of five studies found that the average five-year failure rate for Class II amalgam was 26% while the average five-year failure rate for prefabricated metal crowns was 7% (Randall, 2002). In studies with pulpotomized teeth, SSC did not have a significant advantage over other restorative materials. However, the pulpotomized teeth with multisurface composite restorations showed more marginal leakage and impaired marginal integrity. So they required more maintenance than those with SSC (AAPD, 2022).

In the literature, conflicting results have been reported in studies comparing gingival health after PMC and resin restoration. Some studies reported gingival health with PMC as good as resin restorations (Hutcheson et al., 2012), while others reported more gingival bleeding in PMC restorations (Atieh, 2008). Numerous studies demonstrate that moderate or good crown fit does not significantly increase plaque accumulation or gingival problems. It is suggested that an inadequately contoured crown and cement residues that have already set but are still in contact with the gingival sulcus are the main causes of gingivitis in people who have PMCs. A preventive regimen that includes oral hygiene training is advised to be include into the treatment strategy. (Dhar et al, 2015).

Preparation Technique

1. The dentist should consult with the child and his parents, and get their consent before PMC treatment;

2. The crown size should be determined before the preparation. In order to determine the appropriate crown size, it is advised to measure the mesial-distal width between the contact points of the adjacent teeth with calipers. If the distance cannot be measured due to loss of mesio-distal dimension, it

is recommended to measure the mesio-distal dimension of the contralateral tooth. Or the dentist can determine the crown size by trial/error method after preparation. A correctly fitting crown should snap into place at try-in.

3. There are some factors to be considered before preparation. These are whether there is extrusion in the opposing tooth, whether there is loss of mesio-distal distance due to caries in the related tooth, evaluation of crowding or the presence of physiological diastema in the occlusion, and recording of occlusion and midline relations.

4. Before starting the procedure, local anesthesia and dental isolation should be provided first.

5. If necessary, caries should be removed, pulp treatments should be completed, and the tooth should be restored with GIC, compomer or composite resin.

* According to studies, it is possible to access the proximal regions of the tooth more easily if the occlusal surface is first prepared. The best course of action is to initially reduce the occlusal surface before reducing the proximal surfaces.

6. The occlusal surface should be reduced by 1-1.5 mm. The original anatomy of the tooth must be preserved. If there is tissue loss on the occlusal surface due to caries, the marginal ridges of the adjacent tooth can be referenced.

7. Proximal reduction (mesial and distal surfaces) should be maximum 1 mm, the probe should be able to pass easily through. Without harming adjacent teeth, proximal surfaces must create space for the crown and achieve a feather finish gingivally. Again, the normal anatomy of the tooth should be preserved, a 2-5 degree taper should be obtained and the clinician should ensure that all line angles are rounded.

8. No buccal/lingual reduction or a minimal reduction of 0.5 mm is sufficient. The cervical third of a primary molar tooth is where it becomes most bulbous, and it is this undercut region at the gingival margin, especially buccally and lingually, that provides retention to the crown. To place a crown easier, several authors advised preparing the buccal and lingual walls to form a gingivally inclined long bevel. Others, on the other hand, argued that the vestibular and lingual aspects of the crown should not be prepared at all or very little (0.5-1 mm), unless there is noticeable enamel convexity that should only be slightly reduced. All line and point angles should be rounded. The angles between the buccal and occlusal teeth are beveled at a 45-degree angle.

9. For retention and gingival protection, the crown must adapt properly. A crown that is not properly fitted serves as a source of plaque accumulation,

leading to gingivitis and recurrent cervical caries. When placing the crown, it should be placed from the lingual to the buccal. First of all, it should be positioned lingually and by applying pressure in the buccal direction, it should be slipped from the buccal surface and placed in the gingival sulcus. The crown will slip over the buccal bulge with some resistance.

Ask the patient to bite over the prepared tooth after the chosen crown has been placed. A preliminary occlusal and marginal ridge relationship should be checked. After the placement; the crown should only extend 1 mm below the gingiva, if it goes deeper, it requires adaptation with trimming and crimping. By taking a radiograph, the dentist may confirm the gingival contour and extension and ensure that the entire tooth is covered in the final adaption of the crown.

10. Glass ionomer, resin modified glass ionomer, zinc polycarboxylate, and zinc phosphate cements are often used for PMC cementation. However, it is generally preferred to use a fluoride-releasing cement. As the cement sets, the crown should be kept firmly in place on the prepared tooth. The child may be asked to bite hard for 2-3 minutes, or alternatively, strong finger pressure may be applied. This process is crucial to avoid the crown dislocation and impairing the isolation. Excess cement should be removed and dental floss should be used to remove residual cement at the contact points. The whiteness of the gums disappears in a few minutes. If there is an occlusal inadaptability, it often resolves within a few weeks (Guelmann et al., 2003; Ramazani and Ranjbar, 2015; Virupaxi et al., 2020; Sztyler et al., 2022; Amlani and Brizuela, 2023).

Hall Technique

The Hall technique was first introduced in Scotland in 2006 by a general dentist, Dr. Hall and has been used and developed for over 15 years. It is a method in which teeth are restored with stainless steel crowns without the need for caries removal, tooth preparation, and local anesthesia. It can be easily accepted by the patient due to its non-invasive design, and it is thought that it can reduce the rate of untreated primary teeth due to its long-lasting restoration (Tatlı and Ozer, 2017; Altoukhi and El-Housseiny, 2020).

In the Hall technique, isolation of the tooth from its surroundings causes the carbohydrates needed for the formation of caries by microorganisms to be cut off, and thus the plaque content changes. If the caries is effectively isolated from the oral environment, the profile of the bacteria in caries changes significantly, it becomes less cariogenic and the progression of the carious lesion is stopped. This technique is based on isolating primary molar teeth from the oral environment with SSCs and thus stopping caries (Tatlı and Ozer, 2017; Altoukhi and El-Housseiny, 2020).Hall technique may be considered as a treatment option, when traditional SSC technique is impractical for treating

carious primary molars due to restrictions like poor cooperation or barriers to care (AAPD, 2022). The indications and contraindications of this technique can be listed as follows (Skucha-Nowak et al., 2015; Tatlı and Ozer, 2017):

Indications

- If the tooth with moderate occlusal caries cannot be restored with adhesive restorative materials under good isolation conditions,
- In Class 2 caries with or without cavitation, if the patient does not accept partial caries removal or conventional restoration and there are no clinical signs of pulpal pathology,
- If the patient does not accept conventional methods or fissure sealant application in Class 1 caries lesions without cavitation,
- If the patient does not accept conventional methods or partial caries removal in Class 1 caries lesions with cavitation,
- If there is sufficient sound tissue to retain the crown
- In cases where the child has poor cooperation for restorative treatment with local anesthesia

Contraindications

- Teeth with exposed pulp
- Presence of clinical or radiographic findings indicating pulpal pathology (irreversible pulpitis, pulp polyp, etc.),
- If the tooth cannot be restored with SSC,
- If there is a tissue loss in the tooth that cannot be treated with conventional methods,
- In teeth that can be cleaned by themselves, where restoration is unnecessary, and only follow-up is required,
- In decayed primary molar teeth, which do not require restoration, and there is short time left for the permanent tooth to erupt,
- If the patient's cooperation is not sufficient to protect the airway, the use of the technique is contraindicated,
- If the patient is at risk for bacterial endocarditis, conventional methods in which all caries tissue is removed should be preferred

Procedure

1. This technique's first step is to separate the tooth from the contact points with dental floss, separating plier or an orthodontic separator, thus making room for the crown. Separators are left in for around four to five days.

2. Afterwards, the separators are removed and the smallest possible crown is selected. It is desirable that the crown covers all the cusps, is compatible with the contact points and gives a 'spring back' effect.

3. If necessary, the crown should be adjusted using band-forming pliers.

4. Afterwards, the crown is cemented to the tooth by using glass ionomer cement. The dentist presses down with his or her fingers or the child bites down to apply pressure to the tooth for placing the crown. Until the cement sets, the tooth must be kept in occlusion. Finally, excess cement should be removed (Altoukhi and El-Housseiny, 2020; Sztyler et al., 2022).

It has been reported that restorations of decayed primary molar teeth using this technique show similar success rates to traditional restorative methods (Ludwig et al., 2014).

OPEN-FACED STAINLESS STEEL CROWNS

Although the clinical success of stainless steel crowns is high, the most important disadvantage is their poor aesthetic appearance. For this reason, there was a need to develop crowns with a more acceptable aesthetic appearance. Open-faced stainless steel crowns were first introduced in 1983 by Hartmann (Wiedenfeld et al., 1994). While these crowns still have the strength and durability of SSCs, they also allow for the improvement of their aesthetic appearance (Gilchrist et al., 2013). During the application of open-faced SSCs, after the SSC is placed on the tooth, a cavity is opened on the labial surface and glass ionomer cement or resin composite is applied in this prepared cavity (Shrestha et al., 2020).

Among the advantages of these crowns, being economical and robust, esthetically pleasing, well adapted to tooth and easy to use can be counted (Champagne et al., 2007). Although open-faced stainless steel crowns are produced as an aesthetic alternative to SSCs, they have some drawbacks. The procedure takes time, additional preparation and the usage of numerous materials is needed. Therefore, the chairside time has increased. It is difficult to manipulate. In order to apply composite facings, clinicians must control bleeding and isolate saliva. They may have poor color stability under oral conditions. Fractures may appear due to occlusal forces. Finally, since metal margins are seen, it is reported that they are still not aesthetically sufficient (Waggoner and Kupietzky, 2001; Shrestha et al., 2020, Ustun and Koruyucu, 2021).

PREVENEERED STAINLESS STEEL CROWNS

Preveneered stainless steel crowns (PVSSCs) have been created as an alternative to open-faced stainless steel crowns in order to overcome their drawbacks. These crowns can be supplied with a variety of facing materials,

such as composite resin or thermoplastic resin, bonded to the stainless steel crown. Various mechanical and chemical bonding techniques are used to keep esthetic veneers bonded to stainless steel crowns. PVSSCs are aesthetic-looking crowns that can be cemented in a single session without the need for a secondary preparation in the patient's mouth. They offer the mechanical properties of SSC with esthetics of resin facing. They were produced primarily for primary anterior teeth, but today there are varieties that can be used on both anterior and posterior teeth (Roberts et al., 2001; Garg et al., 2016). The various available preveneered crown brands are Cheng crowns, Nu-Smile crowns, Kinder Crowns and Dura crown (Guelmann et al., 2003). As with any material, PVSSCs have some advantages and disadvantages (Roberts et al., 2001; Garg et al, 2016; Ustun and Koruyucu, 2021):

Advantages

- Aesthetically pleasing result is obtained
- Because the manufacturer prepares the aesthetic components, the clinician spends less time with the patient
- It is reported that they are less affected by the saliva and moisture than open-faced stainless steel crowns
- Color stable
- Does not cause wear on the opposing tooth

Disadvantages

- Only the labial side has facing. Crimping should only be performed on the palatinal/lingual surface, as fractures may occur on the labial surfaces during crimping.
- Since there are few color options, achieving a natural appearance is challenging.
- Due to the huge volume and the restricted size alternatives, it is challenging to apply to children who have crowding or space loss.
- In comparison to a conventional SSC, the addition of resin results in a thicker SSC. It requires more tooth preparation due to passive fit to tooth structure.
- When exposed to strong force, resin face material which is generally rigid and brittle, has a tendency to break.
- Since it is impossible to fix the chipped area, the complete crown must be replaced if parents or children are concerned about some metal showing.
- Poor gingival health. It doesn't fit as precisely as a steel crown without veneer since the labial area of the margin cannot be crimped.

- PVSSCs cannot be autoclaved because heat will damage the veneer material. It has been observed, that heat sterilization causes to weaken the bond strength and color stability for these crowns. Using the steam sterilization is recommended.
- High cost compared to SSC

PEDO PEARLS

Pedo Pearls are aluminum veneered and tooth colored crowns. They were first introduced in 1980. They serve as permanent crowns for primary teeth and are made of metal with an epoxy resin coating. The distinction was that aluminum was utilized rather than stainless steel. Compared to stainless steel, aluminum surfaces have higher adhesion to the epoxy resin coating. They manufactured in a standard size and can be used on either side. It is simple to modify them by cutting and crimping (Sztyler et al., 2022).

However, because aluminum crowns are relatively soft, long-term permanency issues could arise. The white coating typically starts to fade off in places of heavy occlusion. Despite the issues they present, these crowns are considered among the aesthetically acceptable crowns. It is advised to use dual-cure of self-cure composite rather than normal luting cement when using these crowns. More composite can be used to patch up the area where the epoxy resin coating has worn away where it meets the opposing tooth. In bruxism cases it should be avoided from placing Pedo Pearls crowns. Among the drawbacks are their soft structure and possible reduced durability (Sathyaprasad et al., 2020; Sztyler et al., 2022).

POLYCARBONATE CROWNS

Polycarbonate crowns were introduced by Mink JW in the 1970s and began to be used by clinicians. These crowns are heat-molded and produced as prefabricated. They have great impact strength and rigidity. Due to their ability to be heated and molded into the desired shape as solids, polycarbonates are known as thermoplastic resins. Although they are aesthetically better than stainless steel crowns, their clinical use has declined because of their fragility and poor abrasion resistance. With the production of strip crowns, which are easy to apply, they have lost their popularity even more (Venkataraghavan et al., 2014; Shrestha et al., 2020). In 1990, as a result of the use of new production techniques, it started to be produced as thinner and stronger than self-cure acrylic resin. Thus, their use in the clinic has increased again (Garg et al., 2016). Pedo Natural Crowns, Kudos Polycarbonate Crowns and 3M ESPE Polycarbonate Crowns are some of the commercially available polycarbonate crowns.

For the application of polycarbonate crowns, the periodontal tissues of the teeth must be healthy and the clinical crown length must be sufficient

for retention. A prefabricated polycarbonate crown that is appropriate for the prepared tooth is chosen during application, and after being countured, it is attached to the tooth with acrylic resin. After margin correction and polishing, the restoration process is completed (Ustun and Koruyucu, 2021). It should be warned that polycarbonate crowns are brittle and susceptible to separate from the tooth entirely. Their clinical utilization is lower compared to other crowns because of these drawbacks.

Although polycarbonate crowns have comparable indications to stainless steel crowns, there are some circumstances in which they should not be used (Myers, 1976; Venkataraghavan et al., 2014). Their indications includes full coverage restorations of severely decayed maxillary anterior teeth (children exhibiting early childhood caries usually need full coverage restorations), fractured or malformed teeth, discolored teeth and following pulp treatments, while deep-bite cases, progressive resorption of the root, significant tissue loss of the teeth, unhealthy periodontal tissue, crowded anterior teeth cases and bruxism or excessive tooth wear (since these crowns cannot withstand the strong abrasive forces) are among their contraindications.

Advantages of polycarbonate crowns include that they are more aesthetic than SSC, easy to trim and adjust, extreme dimensional stability and need less chair side time. The flexibility of the crowns allows for crimping. Additionally, they are not affected by weak mineral and organic acids (Sathyaprasad et al., 2020). Polycarbonate crowns have a number of drawbacks that make them less practical for use, including poor abrasion resistance, discoloration, difficult placement, and frequent dislodgement (Shrestha et al., 2020).

STRIP CROWNS

In order to achieve natural-looking tooth form, function, and aesthetics in the treatment of severely damaged primary teeth, strip crowns have been developed in the late 1970s. Since then, they have been used frequently in the restoration of primary teeth. They are seamless, transparent prefabricated plastic/celluloid crown forms without long cervical collars. This is frequently the first option for clinicians, instead of the stainless steel crowns that have been used for years, especially in anterior primary teeth with excessive material loss or fracture, as it provides excellent aesthetics, has a wide usage area, and is easy to repair if any chipping off or fractures occur. Strip crowns for posterior teeth have been developed as an alternative to stainless steel crowns, although their usage has not been as widespread as SSCs. (Ustun and Koruyucu, 2021; Revzi et al., 2021). Studies in the literature show that although strip crowns are more aesthetic than SSC, SSCs are clinically more successful and long lasting than strip crowns (Tate et al., 2002).

Indications of strip crowns are similar to SSC. On the other hand, strip crowns are contraindicated if the remaining tooth surface after the removal of

the caries is insufficient for bonding, if there is extensive subgingival caries, if bleeding and moisture cannot be controlled, if there is deep overbite, if there is periodontal disease and if the patient has bruxism (Garg et al., 2016). The advantages and drawbacks of this material can be listed as follow (Kupietzky, 2002; Ram et al., 2003; Amrutha, 2019):

Advantages

- Good aesthetics
- Parent/patient satisfaction is high
- Obtaining a shiny and smooth surface
- Cost effective
- Very similar result to natural appearance can be obtained
- Easy to apply and easy to repair
- Less chairside time
- Sufficient strength

Drawbacks

- One of the biggest drawbacks is that they are technique sensitive.
- In order to give the material a good shape and color, it is crucial to avoid contamination with blood and saliva. Contamination affects resin bonding.
- In order for proper bonding of the bonding material and composite, the remained tissue after the tooth preparation must be in sufficient amount. The amount of sound tooth tissue that is available for crown placement determines the success rate of strip crowns.
- Prior to starting treatment, ideal oral hygiene is recommended but not always achievable. Many children who need treatment have inflamed gingiva, which could prevent the restorations from curing properly and cause excessive bleeding throughout the curing process, resulting in discolored crowns.
- It is difficult to apply in crowded teeth.
- Furthermore, because behavior management problems, applying strip crowns to very young children might be challenging.

Preparation Technique

1. Local anesthesia is provided and teeth are isolated.
2. Crown size is selected by measuring mesio-distal dimension of the space available.

3. Caries is removed by using a round bur.

4. To make room for the bulk of the resin, the teeth are prepared as though for a crown. Using a high speed tapered diamond or tungsten carbide bur, the length of the crown is initially shortened. At the gingival edges, proximal surfaces are prepared with a tapered knife edge.

5. The appropriate composite resin shade is selected.

6. Trimming of celluloid crowns using curved scissors.

7. Trying of crown fitting on tooth, length and cervical fit should be checked.

8. Vent holes at the incisal-edge of the crown allow air and excess material to escape, when it is filled with composite resin.

9. Acid etching of the tooth.

10. Composite filled strip crown placed on the tooth and excess material is removed.

11. After the composite resin has cured, the strip crown is stripped off with an excavator or probe.

12. Final adjustments include smoothening and polishing (Revzi et al., 2021)

PEDIATRIC JACKET CROWNS

Pediatric jacket crowns are an alternative type of crown used in cases of severe caries or trauma. The Pedo Jacket crown is used in a manner similar to how a celluloid strip crown form is, with the exception that the "jacket" is made of a tooth-colored copolyester material, is filled with resin material, and is then kept on the tooth following polymerization rather than being stripped off as is the case with the celluloid crown. They can be cut into desired shapes by using scissors. They are attached to the prepared tooth by using acid and bonding agent. If isolation can be ensured, the jacket crown is adapted and cemented to the tooth by using a composite resin; if not, resin-modified glass ionomer cement is used. (Garg et al., 2016; Ustun and Koruyucu, 2021).

The amount of time required for intraoral working has decreased because some preparations can be completed extraorally. This offers a considerable advantage when using it, particularly with children who are noncooperative. In addition, the fact that the crown can be placed in a single session, being cost effective and requiring minimal tooth reduction for multiple adjacent restorations are among its other advantages (Sadrapyasad et al.; 2020). Disadvantages of the pediatric jacket crown include poor wear resistance against occlusal forces and discoloration over time. Another issue is that these crowns are only available in one very white shade, making it challenging to

match them to adjacent, unrestored teeth. The copolyester used to make the crowns prevents them from being trimmed or reshaped with a high-speed finishing bur, because doing so would melt the copolyester (Dumne et al., 2021).

The detachment of the crown from the cement is usually the reason for failure. When the copolyester crown separates, the composite resin or resin-modified glass ionomer cement used as an adhesive remains on the tooth and resembles a crown without the need for further restoration. (Shrestha et al., 2020; Ustun and Koruyucu, 2021).

COMPOSITE BASED CROWNS/INDIRECT COMPOSITE RESIN CROWNS

Indirect composite resin shell crowns are offered as prefabricated crowns that are produced to order in a laboratory. Examples of prefabricated composite crowns enhanced with glass fibers include the Artglass, Pediatric Edelweiss, New Millennium and Figaro crowns. In comparison to direct composite strip crowns, customized lab enhanced composite resin crowns provide excellent quality with more complete polymerization, improved wear resistance, and less chairside time (Shrestha et al., 2020).

ARTGLASS CROWNS

These crowns are forms of full coronal restorations with high esthetic properties for the primary dentition. Artglass crown are made up of artglass. Artglass is a polymer glass that offers the aesthetics and durability of porcelain with the natural feel and bonding ability of composite (Dumne et al., 2021). It is a bifunctional and multifunctional methacrylate that creates a strongly cross-linked, three-dimensional molecular network. They are also referred to as "organic crowns" because of the structural nature of the crown. It consist of 20% silica filler and 55% microglass. There are six sizes and one shade available. The insufficient bonding to the teeth may be the cause of their failure (Sathrapyasad et al., 2020; Shrestha et al., 2020).

Among its advantages are that it is durable, aesthetic like a natural tooth, its wear is similar to enamel, it is color stable and plaque resistant thanks to the inorganic fillers it contains, it can be easily adjusted and repaired, and it requires minimum chairside work (Sathyaprasad et al., 2020).

NEW MILLENNIUM CROWNS

These crowns are composed of a lab-enhanced composite resin material, and extremely similar to the pedo jacket and strip crowns. They can be polished and trimmed with high-speed bur, unlike pedo jacket crowns, and they are quite aesthetically pleasing. Additionally, they are resin-filled and bonded to the tooth. They have some drawbacks, including the fact that they

are expensive, brittle, and require sufficient moisture control (Yang and Mani, 2016).

CROWNS PRODUCED WITH CAD/CAM

Primary teeth with severe caries have been restored with a variety of restorative materials, but no material that is both long-lasting, aesthetically pleasing and meets all expectations has yet been discovered. The drawbacks of current crowns have made it clear that more esthetic crowns that can be produced and applied more practically in combination with technology advancements are required.

The use of crowns created via computer-aided design and manufacturing has gained popularity in recent years. This system is called Computer Aided Design/ Computer Aided Manufacturing (CAD/CAM). In the 1980s, CAD/CAM technologies were introduced to the dental industry. The collection of data in a computer, the construction of a three-dimensional model, the realization of designs using these data, and the production using these designs form the basis of the system. As a result of the collaboration of a precision milling machine and computer software, various products can be produced from ceramic, composite, metal or hybrid blocks. Today, there are indications for CAD/CAM systems such as inlay, onlay, laminate veneer, partial crown, full crown and bridge restorations. Despite the fact that CAD/CAM restorations have started to be used in clinical practice of pediatric dentistry, they are not frequently used in the restoration of primary teeth, with the exception of research. However, its use has become widespread to restore the permanent teeth of pediatric patients (Lui, 2005; Miyazaki et al., 2009; Tolidis et al., 2019; Gulbahce et al., 2022).

Onlay and endocron restorations produced with CAD/CAM offer pediatric dentists an effective and perfectly compatible conservative treatment option for the restoration of endodontically treated teeth. Smaller composite blocks can also be formed for primary teeth, thus shortening the production time of the restoration. The ceramic blocks come in a wide range of shades and it is choosed according to the adjacent teeth. In pediatric patients, instead of ceramic crowns, it is preferred to use resin-containing or hybrid pediatric zirconia crowns to prevent wear on opposing teeth (Miyazaki et al., 2009; Gulbahce et al., 2022).

With the help of CAD/CAM technology, it is possible to obtain restorations that are more compatible and accurately mimic the anatomy of the tooth while avoiding the need to remove unnecessary dental hard tissue. These restorations can be performed at the chairside, in a single appointment, and are customized for the tooth that has to be restored. Treatments with CAD/CAM in primary and permanent teeth also have advantages such as being aesthetic, being very durable, easy archiving of data and providing

high quality control. However, like every material and system, CAD/CAM systems also have some disadvantages. These are the high production cost of restorations, the need for experienced personnel to use the equipment, and the difficulty of transferring teeth with deep subgingival margins to the computer system (Lui, 2005; Tolidis et al., 2019).

PEDIATRIC ZIRCONIA CROWNS

Stainless steel crowns are considered to be the most durable restorative materials that can be used in the restoration of primary molars and that protects dental tissue best. SSCs have several advantages, but because of their poor esthetics, researchers still aim to develop new materials. Pediatric zirconia crowns have been produced recently to fulfill the aesthetic requirements of children and parents as well as to restore severely damaged primary teeth. (Babaji, 2015).

Zirconia is a highly resistant material to wear, corrosion, and temperature. Zirconia is a reactive metal, so when it exposed to air or a solution, an oxide layer develops on its surface. It is protected from corrosion by the generated oxide layer. It has been claimed that zirconium has substantially better physical characteristics than other ceramics. It is known that the use of zirconia as a biomaterial started with the construction of hip prosthesis by Hellmer and Driskell in 1969 (Ustun and Koruyucu, 2021). The strongest dental ceramic now available is zirconia, which also aesthetically appealing. Despite being generally acknowledged as a restorative material for the permanent dentition, zirconia is a relatively new restorative material for the primary dentition (Garg et al., 2016).The first introduced prefabricated zirconium crowns were EZ-Pedo™ Crowns (Loomis, California, USA) in 2008. Today, there are forms prepared for use in both primary incisors and primary molars. Pediatric zirconia crowns are prefabricated, anatomically contoured, biocompatible and metal-free crowns for primary dentition. They are also an alternative for patients with Ni-Cr allergy. These crowns have a mechanical strength that is comparable to stainless steel crowns (Larsson, 2011; Sadhyaprasad et al., 2020; Revzi et al., 2021). Zirconia crowns that are prefabricated and available for children have been created to be far more durable and stronger than enamel structure. They also have high fracture resistance. The translucent feature of zirconium crowns, provides them very good aesthetics. (Tote et al., 2015).

Today, there are different brands and features of pediatric zirconia crowns on the market. To improve clinical success, grooves that provide mechanical retention within EZ-Pedo crowns were prepared using a patented technology called Zir-Lock retention. Later, preformed zirconia crowns with different properties were introduced by companies including Nusmile, Kinder Crowns, Signature Crowns, Cheng Crowns and many others. For example, try-in crowns in Nu smile crowns reduce the extra disinfection stage, while

Kinder crowns have an internal retention system (Babaji, 2015; Ninawe et al., 2022). In Turkey, there are pediatric zirconia crowns produced by the Prof Zr Crown brand in recent years and their use is becoming more widespread.

Pediatric zirconia crowns have some indications and contraindications (Ninawe et al., 2022). Its indications include multisurface caries, following pulpotomy or pulpectomy, early childhood caries, patients with high caries risk, caries involving incisal edge, fractured teeth and discolored anterior teeth, while its contraindications are gingival inflammation surrounding the tooth and excessive crowding of teeth.

Zirconia crowns have high esthetic appearance with good patient acceptance and they have high durability. They have also some drawbacks. Since crimping is not an option for these crowns, retention is mostly determined by cementation and internal surface patterns, such as crimp lock retentive design in Cheng crowns, zir-lock ultra grooves in EZ Pedo and internal retention bands in zirconia Kinder Krowns. Although these crowns are simple to manipulate, precise operational skills must be used because trimming with high speed burs can cause microcracks within the crown, forcing a seat can cause a fracture, and finishing subgingivally can result in bleeding from the gums, lengthening the operatory time for placement. The cement that will bond the zirconia crown to the tooth may not set if there is gingival bleeding. Because a passive fit for the crown is required, these crowns need more tooth reduction than what is necessary for an SSC. Adjustment of zirconia crown is difficult and lastly they are expensive compare to other crowns (Khatri, 2017; Shrestha et al., 2020; AAPD 2022). Dentists emphasized that while applying zirconia crowns, it takes time because it is a new material at the beginning, but the application becomes easier and faster as they gain experience. They also stated that if a dental preparation is to be performed in the clinic, the patient must be cooperative, otherwise the procedure can be performed under sedation or general anesthesia. (Lopez Cazaux et al., 2017).

One limitation of zirconia crowns is that, while there are wide variety crown options accessible to dentists for restoring primary anterior teeth, the majority of these crowns are exclusively made for maxillary primary incisors. In cases where complete coronal restoration of mandibular incisors is necessary, dentists typically adopt a maxillary lateral crown, which unfortunately leads to a very massive-looking restored incisor. However, crowns for lower anterior teeth are manufactured by only the recent additions like Pediatric Edelweiss crowns and Figaro crowns (Sathrapyasad et al., 2020). The Prof Zr Crown brand has also started to meet this need by introducing lower incisor crowns to the market in recent years. However, more studies are needed in the literature to evaluate their clinical success.

Preparation Technique

There are some points to be considered before preparation. Adequate space, correct angulations, and knife-edge finish lines that are clinically obvious help to maintain gingival health and prevent plaque accumulation. The aesthetics are greatly enhanced by proper tooth preparation, which also lowers the risk of veneer fracture and shortens chair time (Ninawe et al., 2022).

1. Explain the procedure to the child and the parents and get their consent;

2. Crown selection; Appropriate size of crown should be selected by measuring mesio-distal width using vernier caliper or simple divider.

3. Local anesthesia is applied and tooth isolation is provided;

4. Incisal reduction of around 1.5–2 mm or occlusal reduction around 2 mm. The marginal ridge of the adjacent teeth can be used as a reference point for posterior teeth.

5. Buco-lingual reduction around 1-1,5 mm. Bur should be kept parallel to the tooth. Complete removal of the cingulum area is important for proper seating.

6. Interproximal reduction around 1 mm is achieved using a .368 or .330 tapered flame-shaped diamond bur.

7. Adequate knife edge subgingival preparation about 1–2 mm; ending with a feathered margin.

8. Examine the occlusion to determine whether there is sufficient space between the opposing teeth and the prepared tooth;

9. Crown try-in and check fit. To do this, align the zirconia crown's incisal edge with the incisal edge of a neighboring tooth. It should be checked whether the selected crown is sitting passively. Adjusting occlusally and interproximally is not advised since it will lose the crown's glazing and create a weak area of thin ceramic. Since these crowns are rigid, it is crucial that they fit passively; otherwise, trying to force it in place will cause a fracture. If the crown encounters resistance while being placed, more tooth structure must be removed. In a subgingival position, the crown should passively and completely fit without altering the gingival tissue.

10. Bleeding should be controlled using pressure or hemostat. Before the cementation, the crown should be cleaned with alcohol and tap water to remove any saliva or blood.

11. Cementation: A resin cement or a dual-cure resin cement can be used as luting agents.

12. Removal of excess cement (Planells del Pozo and Fuks, 2014; Karaca et al., 2013; Ninawe et al., 2022).

There are many studies in the literature evaluating the success of zirconia crowns. In studies evaluating the parental satisfaction and clinical succes of anterior pediatric zirconium crowns, it has been reported that crowns show successful results in terms of retention, gingival health, opposing tooth wear, color and parental satisfaction (Holsinger et al., 2016; Lopez Cazaux wt al., 2017). Similarly, in a meta-analysis zirconia crowns for primary teeth were found to be associated with better gingival health, high fracture resistance, good retention and marginal adaptation, color stability, high parental satisfaction, smooth cosmetic resistance surface, and no recurrent caries (Alzanbaqi et al., 2022). However, zirconia is harder than tooth. In studies with 6 months to 1 year follow-up, abrasion was observed on the opposing natural teeth. When zirconia crowns and resin materials are compared in anterior tooth restorations, zirconia crowns are observed to be stronger, while it has been reported that zirconias are more prone to fracture or chips in primary molar tooth restorations than SSCs (Pei and Chen, 2023). According to a study comparing parents' satisfaction with various crowns, patients/parents had the highest satisfaction with zirconia crowns, preveneered crowns, and strip crowns respectively (Salami et al., 2015). In a meta-analysis comparing the clinical success and gingival compatibility of SSCs and zirconia crowns, it was claimed that the gingival compliance of zirconia crowns was more successful and the plaque accumulation was less than that of SSC. However, it was emphasized that the choice of material should be made by evaluating the clinical condition of the patient, since wear on the opposing tooth can be observed in zirconia crowns (Pei and Chen, 2023).

MOST RECENTLY INTRODUCED CROWN MATERIALS

GOLDEN STAINLLESS STEEL CROWN

The titanium-coated golden stainless steel crowns (Kids crown, Shinghung, Seoul, Korea) that Shinhung Co. Ltd. has introduced offers an added advantage above traditional stainless steel crowns. They are SS crowns with a titanium coating that gives them a natural golden luster, a high-quality aesthetic with simple adaptability and decreased chair side time (Vamshi et al., 2021).

EDELWEISS PEDIATRIC CROWNS

Edelweiss pediatric crowns, which were introduced in 2018, are the newest additions to the array of pediatric full coverage restorations. These exhibit excellent biocompatibility, natural abrasion behavior, as well as simple repairability and are constructed of densely filled composite with layer-sintered barium glass. Although there are different size options, it offers a sizing guide to facilitate crown selection. The inside of the crown needs to be somewhat roughened, then etched and bonded, and finally bonded to the tooth with composite (Shrestha et al., 2020). However, these crowns are expensive and more clinical studies are needed on this material.

FIGARO CROWNS (FIBREGLASS CROWNS)

Figaro crowns are introduced in 2017. They are advertised as being entirely white, metal-free, Bisphenol-free, and produced from the best, safest materials now utilized in dentistry and medicine. Figaro Crowns are produced in the United States and have all of the ISO Certifications needed by the FDA and Canada Health (Vamshi et al., 2021). They are consist of either fiberglass or quartz fibers/filaments embedded with an outer of cosmetic composite resin material, and manufactured in 5 sizes for each tooth as well as a universal style for lower incisors (Shrestha et al., 2020).

The properties of Figaro crowns are that they are biocompatible, strong, easy to place, cost effective and autoclavable. With these properties, they might be a viable option among many other crowns. The Figaro crowns mimics the accurate anatomy of a natural tooth. Zirconia and SSC are limited in their capacity to mimic the shape of the tooth and more closely resemble hills and valleys, but the Figaro crown respects the original tooth's anatomy and produces an aesthetically pleasing outcome with cusps and grooves (Ghosh and Zahir, 2021). Additionally, these crowns have a perfect fit thanks to their flex-fit technology and therefore the preparation technique is similar to technique of stainless steel crowns. Since less tooth preparation is performed, the chairside time is also shorter. They are less technique sensitive than strip crowns or zirconia crowns. These crowns have the disadvantages of being unable to be crimped and of being not clearly visible on radiographs (Shrestha et al., 2020). Manufacturer claimed that these crowns may be adjusted gently on occlusal/incisal surface only (just like a resin filling would) and because of their pre-beveled margins, adjusting the margins is not recommended. It is recommended to use any glass ionomer or any resin-modified glass ionomer cement for cementation. Figaro crowns may be modified for aesthetic, grinding, or eccentric occlusion reasons. No other preformed crowns have this property (Figaro Crowns FAQs).

Studies with Figaro crowns are limited in the literature. In a study comparing Figaro crowns (fibreglass crowns) and Nusmile zirconia crowns reported that Figaro crowns presented higher fracture resistance and could be used as alternatives to zirconia crowns as they require less tooth structure reduction (Çiftçi et al., 2022)

NU SMILE BIOFLX

Nu Smile BioFLX is the latest pediatric crown material introduced by the company Nu Smile (Houston, USA). The Bioflx crown is constructed from a biocompatible, high-impact hybrid resin polymer that is utilized in the medical sector when extreme strength, durability, and flexibility are required. They are metal-free, Bis-GMA-free and autoclavable. They are distinctively flexible, therefore it is easy to place on the tooth. With a modest resistance

known as a flex or active fit, BioFlx crowns should fit snugly on the tooth. It will flex over small convexities of the tooth. Monochromatic tooth-colored crowns are able to hide the discoloration of arrested caries. They are also stain resistant. According to manufacturer wear resistance of Bioflx crowns is similar to SSC. Laser marking on the inner occlusal surface provide optimal esthetics.

It is said to be that the occlusal reduction should be around 1-1.5 mm, similar to an SSC preparation. Therefore, the chairside time is also short. If the occlusion of the crown is slightly high, it will progressively self-adapt and may eventually develop a dimple in that location, rather than wearing. BioFlx crowns are not recommended for bruxism, the Hall technique, or manipulation by crimping, but they can be trimmed with crown scissors if needed. For cementation self-setting resin modified glass ionomer cements or glass ionomer cements recommended. A light-cured cement is not recommended, because the opacity of the crown will not let light pass through to set the cement. (NuSmile BioFlx FAQs). Since Bioflx is the most recent material, there is no study in the literature comparing it with other materials. Future studies on this material are needed.

CONCLUSION

Until now, aesthetics was prioritized in the anterior region when determining the treatment strategy to be used in the entire coronal restoration of teeth with significant tissue loss in pediatric patients, whereas function was gaining importance in the posterior region. Today, aesthetics has become the primary choice of families in both the anterior and posterior regions. However, due to the high cost of materials with high aesthetics, the use of stainless steel crowns and their more aesthetically pleasing modifications still often continues.

Each technique and material carries its own advantages and drawbacks. Although there isn't enough evidence to say one restoration is superior to another, dentists have been using many of these crowns with effectiveness. The final decision regarding the crown material is influenced by the preferences of the clinician, economical factors, age of the child, aesthetic and phonation needs, ease of application of the material, lifespan and clinical success of the material, duration of treatment, the behavior of the child, bleeding management, and moisture control.

Although the materials used in the past for full coronal restorations of pediatric patients are still being used, new materials that are more aesthetic, easy to apply and with high clinical success are primarily preferred. However, further clinical studies with longer follow-up periods are needed for these new materials and newer materials will continue to be developed until clinicians find the ideal crown material.

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Chapter 11

THE IMPORTANCE OF ITS1-ITS2 SEQUENCES IN THE PYHLOGENETICALLY EVALUATION OF *POTENTILLA* L. TAXA AND COMPARISON OF THESE SEQUENCES AS BARCODING REGIONS

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INTRODUCTION

The genus *Potentilla* L. belongs to the family Rosaceae and it is one of the biggest plant groups according to the distribution and number of the species within the family. The genus has over 400 species which have wide distribution area in the world (Soják, 2009; Bean, 2015; Persson et al., 2020a; Faghir et al., 2021). The northern hemisphere and especially its temperate and boreal regions are the richest region for *Potentilla* taxa based on species diversity (Bean, 2015). The most of the species belonging to the genus *Potentilla* are herbaceous perennials with yellow flowered (Persson et al., 2020a).

Taxonomy of the genus and taxon names within the genus are very controversial from Linnaeus to current (Eriksson et al., 2022). For example; it is stated by Bean (2015) that *Potentilla anserina* L. is recently reinstated as *Argentina anserina* (L.) Rydb. within the genus Argentina. Similarly, the relationships between the genus *Potentilla* and *Sibbaldia* L. based on the identification of some species are taxonomically problematic (Lundberg et al., 2009; Paule and Sojak, 2009; Dobes and Paule, 2010; Eriksson et al., 2015). Some species belonging to the genus *Potentilla* (*P. miyabei* Makino, *P. cuneifolia* Bertol., and *P. tridentata* Sol.) that is closely related with *Sibbaldia* are stated by Lundberg et al. (2009). Furthermore, these species were evaluated within the *Sibbaldia* by Paule and Sojak (2009). On the contrary of this sample, some species evaluated in the genus *Sibbaldia* are today described in the genus *Potentilla* such as *Sibbaldia tetrandra→Potentilla tetrandra*, *Sibbaldia sikkimensis→Potentilla sikkimensis*, *Sibbaldia purpurea→Potentilla purpurea*. Many similar samples related to the changes of taxon names in the genus *Potentilla* are described today (Eriksson et al., 2003; Lundberg et al., 2009; Paule and Sojak, 2009; Dobes and Paule, 2010; Eriksson et al., 2015; Feng et al., 2017).

Rosaceae is the family in which polyploids are common (Ilnicki and Kolodziejek, 2008). Polyploidy is another situation increasing taxonomic problems and commonly observed in the genus *Potentilla*. The genome duplications caused by within species and interspecific hybridization called as autopolyploidization and allopolyploidization are important process in the evolution of the genus. Töpel et al. (2011) mention about sterile offspring sample by hybridization, chromosomes duplication of offspring, finally fertile polyploid individual evolved in their study based on the molecular data. Similarly, it is mentioned about hybrid individuals with reproductive barriers and finally new species evolved by de Queiroz (2005) and Persson et al. (2020b), as a result of the polyploidization and hybridization.

Different chromosome numbers for some species belonging to the genus *Potentilla* were determined by many researchers because of different ploidy levels observed (Skalinska, 1950; Müntzing, 1958; Ilnicki and Kolodziejek,

2008; Töpel et al., 2011). It is reported the pentaploid chromosome numbers in previous studies on *Potentilla* taxa (Müntzing, 1931; Müntzing, 1958; Ilnicki and Kolodziejek, 2008). Furthermore, it is observed that hexaploid type of *P. argentea* L. predominates in Poland (Skalin´ska and Czapik, 1958). Similarly, Asker (1985) reported different chromosome numbers in *Potentilla* taxa. In other words, there are different ploidy levels changing of up to hexadecaploid (16x) in the genus (Kalkman, 2004; Persson et al., 2020a). The basic chromosome number of the genus based on the karyotype analysis revealed in previous studies is reported as n=7 (Goswami and Matfield, 1975; Ilnicki and Kolodziejek, 2008; Töpel et al., 2011).

Morphological characters are crucial to differentiating and identification of species. However, these are insufficient in plant groups that exhibit widely hybridization behaviours like the genus *Potentilla*. Individuals that have intermediate morphological characters or sometimes high morphological variations because of interspecific hybridization can be seen in the genus. Moreover, wide geographical distribution of the genus and morphological variations caused by geographical and ecological conditions are other frequently observed situations in the genus.

All of these make problematic the genus *Potentilla* in aspect of phylogenetic relationships of taxa and the taxonomy of the genus.

The molecular studies in taxonomically problematic plant groups like *Potentilla* are frequently used to understand phylogenetic and taxonomic relations more comprehensively, besides improving available taxonomic problems and identification in species level. Especially, DNA barcoding studies which contain the short DNA sequences information with sufficiently preserved and enough variation to reveal the relationship between species have accelerated with the development of sequence analysis techniques in recent years. DNA barcoding is frequently used for the purpose of the determining of species diversity and the evaluation of phylogenetic relationships, in addition to the solving of taxonomic problems. Furthermore, sequence information belonging to nuclear and plastid DNA are tried in order to find the most suitable regions for aims stated.

The sequences of cytochrome c oxidase-1 (CO1) gene are frequently preferred because of their enough nucleotide differentiation rates and highly efficient discrimination ability in many groups like vertebrate and invertebrate of animals, also this region in DNA barcoding studies is used as an universal barcode (Hebert et al., 2004; Ward et al., 2005; Hürkan, 2017; Rodrigues et al., 2017; Yılmaz, 2020). However, it is unsuitable in plant groups (Chase et al., 2005; Kress et al., 2005; Hollingsworth et al., 2009; Yılmaz, 2020). For this reason, alternative barcode regions giving the best results are still screened in plant groups. The regions belonging to the nuclear and chloroplast genome such as

internal transcribed spacer regions (ITS1-ITS2) between rDNA genes, rpoC1, matK, rbcL, trnK, trnL/trnF, psbA-trnH, trnK-matK, atpB-rbcL are frequently and effectively used to understand and resolve the taxonomy and phylogenetic relationships in taxonomically problematic plant groups (Eriksson et al., 2003; Töpel et al., 2011; Faghir et al., 2014; Feng et al., 2017; Yılmaz, 2023). However, the regions preferred for barcoding do not exhibit adequate contribution every time based on species identification and taxonomic discrimination. Moreover, the DNA sequences preferred for barcoding can show variability in aspect of species identification and taxonomic discrimination abilities from a plant group to other. This makes necessary the determination of the species identification and separation abilities of the regions preferred for each plant groups. In other words, the determination of the most successful barcoding regions in plant group studied would have a considerable effect, especially with using together of regions that exhibit the most beneficially the taxonomic and phylogenetic relationships.

ITS1 and ITS2 sequences between rDNA genes belonging to the nuclear DNA are the commonly using regions for plant phylogenetic studies (Alvarez and Wendel, 2003; Sramko, 2008; Persson et al., 2020a; Yılmaz and Yeltekin, 2022). The most important reasons the using frequently of ITS regions are that they have sequences showing high variations in the separation of species besides their species identification abilities. However, there is no universal barcoding region in plants, and for this reason, the regions with the best discrimination ability in terms of generally expressed characteristics are determined and used together. One of the most important of these regions is the ITS sequences.

In this study, the sequence data for *Potentilla* taxa based on ITS1 and ITS2 regions between rDNA genes were provided from National Center for Biotechnology Information (NCBI) and then examined both of ITS1 and ITS2 sequences to provide contributions to taxonomy of the genus. 117 *Potentilla* taxa for ITS1 sequences and 83 *Potentilla* taxa for ITS2 sequences were evaluated in aspect of taxonomic and phylogenetic relationships of the genus. The objective of this study is to compare the regions (ITS1 and ITS2) used frequently because of their species identification and discrimination abilities besides the evaluation taxonomically and phylogenetically of the genus *Potentilla*. Furthermore, it is aimed to provide important contributions to better understand the genus phylogenetically and taxonomically by using as many taxa as possible belonging to the related sequences, in addition to the solving the complexity of the genus. Another aim of this study is to make comparison of the data analysed with previous studies to provide more comprehensive and accurate results.

MATERIAL AND METHODS FOR PHYLOGENETIC ANALYSIS BASED ON ITS1 AND ITS2 SEQUENCES OF *POTENTILLA* TAXA

The all sequence data of ITS1 and ITS2 regions between rDNA genes belonging to the nuclear DNA were acquired from NCBI for the evaluation of *Potentilla* taxa. After the all data containing sequence information belonging to ITS1 and ITS2 for the genus *Potentilla* were separately provided from NCBI, the data for each regions stated were examined and preferred according to the compatibility of sequence information to make more accurate phylogenetically evaluation of the taxa studied. In other words, the compatible sequences for *Potentilla* taxa from both of ITS1 and ITS2 regions were determined and then used for the analyses. Moreover, the data belonging to the researchers sharing the sequence information at different times in NCBI for the purpose of using as many taxa as possible were examined and analysed for more contributions to the taxonomy of the genus and to better understand the relationships among *Potentilla* taxa. For this aim, some taxa in this study were represented by more than one samples and analysed the relationships with other samples in phylogenetic tree.

117 *Potentilla* taxa belonging to 158 samples for ITS1 region and 83 *Potentilla* taxa belonging to 123 samples for ITS2 region were investigated based on sequence information (Appendix). ITS1 and ITS2 sequences between rDNA genes were extracted from these regions because of more effective analysis.

Firstly, the multiple sequence alignments for taxa examined were performed using the Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura et al., 2021). The variable sites and parsim-info sites in addition to the alignment lengths of taxa examined were computed.

The probabilities of substitution from one base to another base (Table 1, 3) were determined and then transitional base substitutions (%), transversional base substitutions and transition/transversion ratios for purines and pyrimidines were computed for ITS1 and ITS2 regions separately. Nucleotide frequencies (A+T/U % and G+C %) of ITS1 and ITS2 regions were also computed and showed in Table 2 and 4.

Finally, the dendrogram to show phylogenetic relationships among taxa examined and to evaluate the taxonomy of the genus *Potentilla* was inferred using the Maximum Parsimony (MP) method. The MP dendrogram showing the evolutionary history with bootstrap values on branches were used with the option of hide values lower than 50% (Figure 1, 2). Furthermore, the bootstrap test was performed with 500 replicates and shown next to the branches with values %. The positions with gaps treated as missing data were eliminated with option of the program for more effective analyses.

ANALYSIS RESULTS PROVIDED FROM ITS1 SEQUENCES OF *POTENTILLA* TAXA

ITS1 sequences were extracted from the regions containing rDNA genes such as 18S rDNA- ITS1-5.8S rDNA and 18S rDNA-ITS1-5,8S rDNA-ITS2-28S rDNA. The sequence information for ITS1 were provided from NCBI database. 158 samples which contain 117 *Potentilla* taxa for ITS1 region were collected based on the compatibility of sequence lengths and examined for subsequent analysis (Appendix).

Firstly, ITS1 sequences for *Potentilla* taxa were aligned and alignment length was determined as 349 bp. Then, variable sites and parsimony informative sites which are important indicators in the taxonomical relationships between species studied were observed in 159 and 116 nucleotides, respectively. As a result of this, it can be stated that ITS1 region exhibits high variation for *Potentilla* taxa. In addition to these analysis, base substitutions were examined and the probabilities of all base substitutions were determined (Table 1). The highest base substitutions were observed between T and C bases. Base substitutions from T to C and then, from C to T were determined in the rate of 42.32% and 21.10%, respectively. The rates of transitional substitutions were observed as higher than transversional substitutions and shown in bold in Table 1.

Table 1.

The probability of base substitutions (r) from one base (row) to another base (column) for ITS1 sequences

	A	T	C	G
A	-	1.24	2.49	**12.51**
T	1.39	-	**42.32**	1.64
C	1.39	**21.1**	-	1.64
G	**10.56**	1.24	2.49	-

The total rate of transitional substitutions (the substitutions between the same base groups) was computed as 86.49%. Transversional substitutions was observed as 13.51%. Besides the rates of transitional and transversional substitutions, the transition/transversion rate for purines (k_1) and pyrimidines (k_2) were determined as 7.61 and 17.02, respectively. Moreover, the overall transition/transversion rate (R) was determined as 6.18. Nucleotide frequencies based on ITS1 sequences for *Potentilla* taxa were determined as 38.87% for A+T/U, 61.13% for C+G. It can be stated that *Potentilla* taxa for the

region examined consist of C+G sequence (%) in high level. All data for ITS1 sequences belonging to *Potentilla* taxa were shown in Table 2.

Table 2.

The information of taxa examined based on ITS1 sequences

Taxon	Alignment length (bp)	Variable site	Parsim-info site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion rate Purines (k_1)	Pyrimidines (k_2)	Overall (R)	Nucleotide freq. (%) A+T/U	G+C
117	349	159	116	86.49	13.51	7.61	17.02	6.18	38.87	61.13

The dendrogram provided from MP method was examined to show the phylogenetic relationships among taxa studied and to determine the species identification ability of ITS1 sequences for *Potentilla* taxa. Many researchers states in their studies the presence of the major clades: Alba, Anserina, Argentea, Fragarioides, Ivesioid and Reptans in the genus *Potentilla* (Töpel et al., 2011; Feng et al., 2017). In addition to these clades, Himalayan clade that contain old *Sibbaldia* species was stated by Eriksson et al. (2015). In this study, all clades stated were observed in the MP dendrogram provided from ITS1 sequences of *Potentilla* taxa (Figure 1).

Phylogenetic tree separated the species in eight groups. Group VIII is the largest group that contain the most species in the dendrogram. Taxa evaluated in Argentea clade were grouped together and they formed a distinct group from the rest of samples examined. Furthermore, it can be stated that group VIII consists of outmost species belonging to the Argentea clade in phylogenetic tree. Group VII is represented by three species: *P. gordonii*, *P. biennis* and *P. norvegica*. All of these species are evaluated in Ivesioid clade and grouped together in phylogenetic tree as close to Argentea clade. The taxa evaluated in the Anserina clade were clustered in group VI and showed similarity with the taxa belonging to group V. *P. miyabei*, *P. cuneifolia* and *P. tridentata* stated as closely related with the genus *Sibbaldia* by Lundberg et al. (2009) were determined as very close to taxa from Anserina clade (in Group V).

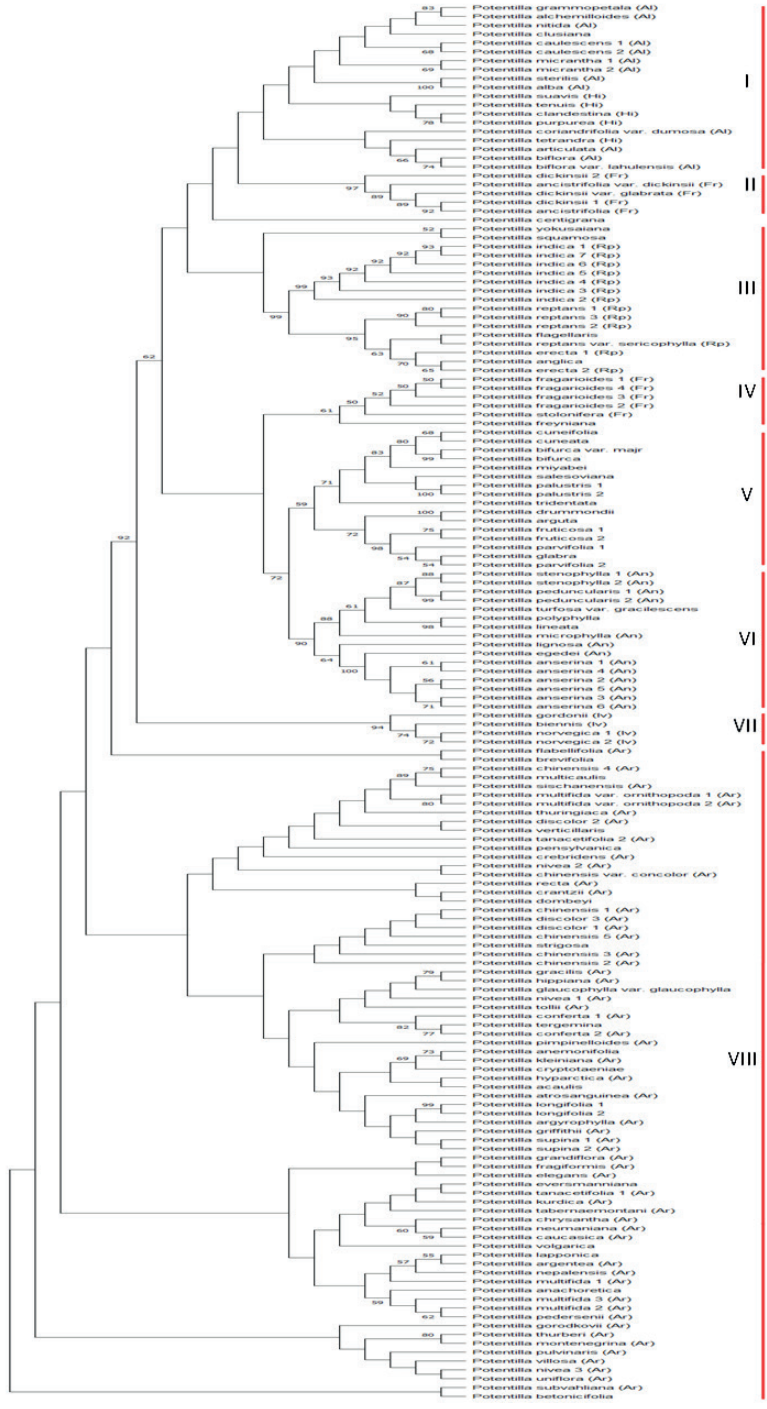

Figure 1. *Maximum Parsimony tree provided from ITS1 sequences of Potentilla taxa (Al: Alba, An: Anserina, Ar: Argentea, Fr: Fragarioides, Hi: Himalayan, Iv: Ivesioid, Rp: Reptans)*

The taxa belonging to Fragarioides clade such as *P. fragarioides* and *P. stolonifera* were clustered together in group IV as close to these two groups. *P. dickinsii* and *P. ancistrifolia* are other species belonging to the Fragarioides clade. These were clustered together in group II and formed separate group from other species represented in Fragarioides clade. The taxa belonging to the Reptans clade such as *P. indica*, *P. reptans* and *P. erecta* were clustered in group III with a few taxa unknown their clade. All taxa clustered in group I belong to the Alba and Himalayan clades. Himalayan species (*P. suavis*, *P. tenuis*, *P. clandestine*, *P. purpurea* and *P. terandra*) were resolved as close with each other in group I. Furthermore, it can be stated that taxa belonging to Himalayan clades showed proximity to taxa from Alba clade in MP dendrogram.

In the comparison of the clades with each other according to the dendrogram:

i. Argentea clade with the highest species number formed outmost group. Similar result was observed in the study based on trnL/trnF IGS region belonging to the cpDNA (Yılmaz, 2023).

ii. Ivesioid clade was resolved as close to Argentea clade. *P. norvegica* was evaluated in Ivesioid clade with *P. gordonii* and *P. biennis*. However, it is observed that *P. norvegica* is evaluated in the Argentea clade based on cpDNA studies on the contrary of nuclear DNA studies evaluated in the Ivesioid clade (Eriksson et al., 1998; Dobeš and Paule, 2010; Töpel et al., 2011; Yılmaz, 2023). This can be interpreted as a possible connection between Ivesioid and Argentea clades. This relationship between Argentae and Ivesioid clades was supported by Eriksson et al. (2022) in the study based on the phylogeny of Potentillinae.

iii. Himalayan clade was resolved as very close to Alba clade. All taxa evaluated in Himalayan clade were found to be nested in Alba clade. Similar results were observed in the studies based on nuclear and cpDNA (Feng et al., 2017; Yılmaz, 2023).

iv. Reptans clade showed proximity to Alba and Himalayan clades.

v. The taxa belonging to the Fragarioides clade were clustered in two distinct groups in phylogenetic tree. One of these was resolved as close to Alba clade and other to Anserina clade. *P. dickinsii* and *P. ancistrifolia* (Group II) evaluated in Fragarioides clade were clustered between Alba and Reptans clades in the study by Persson et al. (2020 a). The results provided from this study show similarity based on the relationships of these taxa with other clades.

vi. The all taxa belonging to Anserina clade were clustered together in group VI and showed similarity to group V.

ANALYSIS RESULTS PROVIDED FROM ITS2 SEQUENCES OF *POTENTILLA* TAXA

ITS2 sequences between rDNA genes were provided from NCBI database and examined taking into account of their sequence compatibility. Afterwards, the sequences that exhibit compatibility were extracted from these regions which contain the rDNA genes. 83 *Potentilla* taxa belonging to 123 samples were analysed to determine the ability of ITS2 sequences in aspect of better understand the genus taxonomy and phylogenetic relationships of the species (Appendix). For this aim, all sequence information belonging to ITS2 region of *Potentilla* taxa were aligned and alignment length was determined as 445 bp.

The variable sites and parsimony informative sites are very important in the characterization of species identification ability of the region preferred besides the importance in taxonomic and phylogenetic relations of species. Both of them were analysed for their importance. The variable sites and parsimony informative sites were observed in 169 and 101 nucleotides, respectively. It can be stated based on these two sites that ITS2 region exhibits high variation for *Potentilla* taxa like ITS1 region.

Base substitutions from one base to another base were determined for ITS2 region and showed in Table 3. The highest base substitutions were observed between A and G bases. Base substitutions from A to G and then, from T to C were determined in the rate of 32.53% and 19.69%, respectively.

The rates of transitional substitutions in comparison to the transversional substitutions were observed as higher and shown in bold in Table 3. The rate of transitional and transversional substitutions were computed as 73.44% and 26.56%, respectively.

Table 3.

The probability of substitution (r) from one base (row) to another base (column) for ITS2 sequences

	A	T	C	G
A	-	1.71	5.3	**32.53**
T	1.97	-	**19.69**	4.31
C	1.97	**6.37**	-	4.31
G	**14.85**	1.71	5.3	-

In addition the rates of transitional and transversional substitutions, the transition/transversion rates were determined for purines (k_1) and pyrimidines (k_2) as 7.55 and 3.17, respectively. The overall transition/transversion rate (R) was determined as 2.22. Nucleotide frequencies of *Potentilla* taxa based on ITS2 sequences were determined as 27.69% for A+T/U, 72.31% for C+G. In other words, it can be stated that *Potentilla* taxa for ITS2 region consist of C+G sequence (%) in high level like ITS1 region. All data for ITS2 sequences belonging to *Potentilla* taxa were shown in Table 4.

Table 4.

The information of taxa examined based on ITS2 sequences

Taxon	Alignment length (bp)	Variable site	Parsim-info site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion rate Purines (k_1)	Pyrimidines (k_2)	Overall (R)	Nucleotide freq. (%) A+T/U	G+C
83	445	169	101	73.44	26.56	7.55	3.17	2.22	27.69	72.31

The MP dendrogram provided from of ITS2 data of *Potentilla* taxa was created to determine the species identification ability of the region related and to show the phylogenetic relationships among taxa examined. All clades stated in ITS1 analysis were observed here (Figure 2).

Group VII that consist of taxa from Argentea clade is represented by the most samples in dendrogram. Furthermore, these taxa were clustered together and formed the outmost group in phylogenetic tree. It can be stated that there is similarity with the results provided from ITS1 data in aspect of the location of Argentea clade in dendrogram. Group VI was represented by five species from Alba clade (*P. micrantha*, *P. caulescens*, *P. alba*, *P. alchemilloides* and *P. grammopetala*) and *P. dickinsii* from Fragarioides. *P. norvegica* and *P. biennis* evaluated in Ivesioid clade were clustered in Group V as close to group VI. All Himalayan taxa (*P. tenuis*, *P. suavis*, P. tetrandra, *P. clandestina* and *P. purpurea*) were clustered together in group IV with a few taxa from Alba clade. Group VI, V and IV are very close with each other in phylogenetic tree. Himalayan and Alba clades were resolved as close in dendrogram such as ITS1 data. However, Ivesioid clade was clustered between Alba and Himalayan clades contrary to ITS1 results. The totally 13 samples belonging to *P. indica*, *P. reptans* and *P. erecta* evaluated in Reptans clade were clustered together in group III. All taxa evaluated in Anserina clade were clustered in group II and showed the most similarity with the taxa from group I.

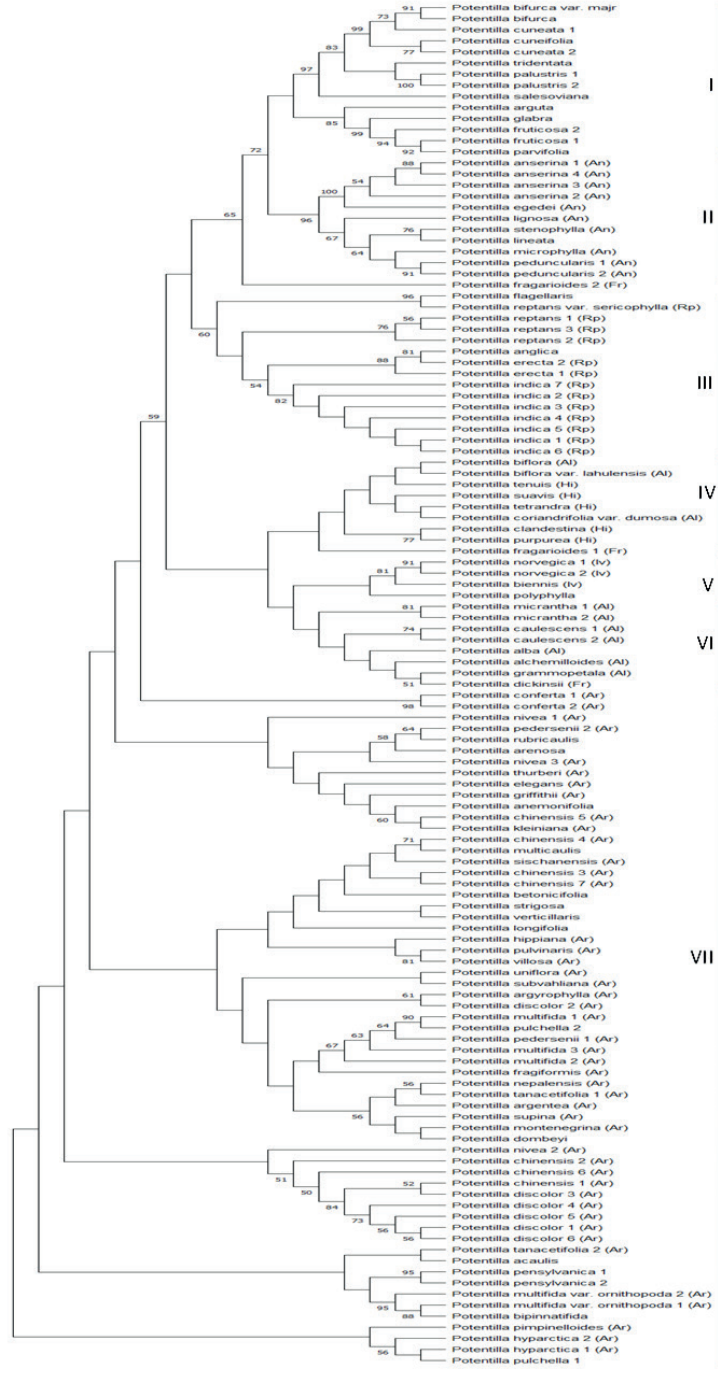

Figure 2. *Maximum Parsimony tree provided from ITS2 sequences of Potentilla taxa (Al: Alba, An: Anserina, Ar: Argentea, Fr: Fragarioides, Hi: Himalayan, Iv: Ivesioid, Rp: Reptans)*

P. cuneifolia and *P. tridentata* were determined as closely related with taxa from Anserina clade (in Group I) like ITS1 dendrogram. The taxa from Fragarioides clade (*P. fragarioides* and *P. dickinsii*) were clustered unexpected positions in dendrogram that show the phylogenetic relationships of *Potentilla* taxa. While *P. dickinsii* with one of the samples belonging to *P. fragarioides* were clustered as close in phylogenetic tree, other sample from *P. fragarioides* were separated from these taxa and placed as close to Anserina clade.

In the comparison of the clades with each other according to the dendrogram:

i. Argentea clade formed outmost group with the highest species number in dendrogram like ITS1 analysis.

ii. Ivesioid clade was resolved between Alba and Himalayan clades on the contrary of ITS1 dendrogram. According to ITS1 data, Ivesioid clade represented by *P. norvegica*, *P. gordonii* and *P. biennis* formed distinct group in phylogenetic tree. However, according to ITS2 data, although the taxa belonging to Ivesioid clade were clustered together in group V, it was not clearly separated from the taxa evaluated in Alba and then Himalayan clades. Furthermore, *P. norvegica* was similarly evaluated in Ivesioid clade like stated by previous studies based on nuclear DNA (Töpel et al., 2011; Yılmaz, 2023), but the relationship between Argentea and Ivesioid clades observed in dendrogram provided from ITS1 sequences was not determined here.

iii. All taxa from Himalayan clade were clustered together in group IV. Similarly, taxa evaluated in Alba clade formed distinct group in phylogenetic tree. Similar results were observed in the comparison of dendrograms provided from ITS1 and ITS2 sequences in aspect of the relationships between Alba and Himalayan clades. Furthermore, the taxa from Himalayan clade were observed to be nested within Alba clade in ITS2 dendrograms same as ITS1 dendrogram.

iv. Anserina clade (Group II) was resolved as close to group I and both of them (Group I and II) showed proximity Reptans clade.

v. Reptans and Anserina clades were resolved as very close in dendrogram unlike the results of ITS1.

The sequence information uploaded to NCBI by many researchers in different periods were used in this study to show the variations of same species that have different habitats, besides evaluation the accuracy of data from NCBI. Another important aim of this study is to make comparison of ITS1 and ITS2 data between rDNA genes used frequently in DNA barcoding studies in aspect of taxonomically and phylogenetically. As a result, both of them provide very valuable data in the taxonomical and phylogenetic evaluation of *Potentilla* taxa and they are strongly recommended. However, it

can be stated that ITS1 sequences have more comprehensive data than ITS2 in the taxonomical relationships of the genus *Potentilla*.

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Appendix

ITS1

MF964096, AY635025, AJ511775, FN430828, KP875300, KF912900, AY862574, U90792, AF163478, AM114842, MN872939, MN872964, MN872947, MN872946, FN430818, AJ511776, MN872940, MN872925, FN555612, FN555611, FN555610, KP994573, KF912908, AH006926, FN555607, AJ511780, MG456718, FN430824, KP994563, KF954772, U90788, AJ511773, AJ511778, FN430820, AJ511774, FN430825, KP994569, KP875298, KF912931, FN430775, U90785, FN666607, FN555609, FN555608, FN430829, FN430827, FN430826, FN430823, AH006919, FN430822, FN430821, FN430819, FJ356170, FN430817, U90790, FN430816, KF912940, AH006920, FN430815, MW357749, U90784, FN430814, FN430813, FN430812, FN430811, FN430810, FN430809, FN430808, FN430807, FN430806, FN430805, FN430804, FN430803, FN430802, FN430801, FN430800, FN430799, FN430797, KP994561, FN430796, FN430795, FN430794, KP875299, KF912938, FN430793, FN430792, FN430791, FN430790, FN430786, FN430785, FN430784, FN430783, FN430782, FN430781, FN430780, AH006918, FN430779, FN430778, FN430777, FN430776, FN430774, AJ511779, AJ511777, U90789 , KP875295, KP994568, KP875293, KF912946, KP994562, KF912925, KF912920, KF912893, KF912883, KP994572, KP994571, KF912944, KP994570, KP994567, KP875296, KP994566, KP994560, KP994559, KP994558, KP994557, KP994556, KP875313, KP875308, KP875307, KP875306, KP875305, KP875304, KP875302, KP875301, KP875297, KP875294, KP875291, KM353011, KF954762, KF912935, KF912932, KF912917, KF912914, KF912895, KF912890, KF912887, KF954768, HM453948, FJ356172, FJ356171, FJ356169, FJ356168, KP994565, MK802481, MK802480, MK802442, U90791, U90787, U90786

ITS2

MF964096, AY635025, AJ511775, FN430828, KP875300, AY862574, U90792, MF785707, AF163478, AM114842, AJ511780, AJ511778, FN430820, AJ511776, AJ511774, FN430825, KP994569, KP875298, KR611748, OM180102, MH349341, AJ511773, FN430824, KP994563, U90788, FN430829, FN430827, FN430826, FN430823, AH006919, FN430822, FN430821, FN430819, FJ356170, FN430818, AJ511776, FN430817, U90790, FN430816, AH006920, JN999413, FN430815, U90784, MW357749, FN430809, FN430808, FN430804,

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Chapter 12

CURRENT PIT AND FISSURE SEALENTS

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Dental caries is one of the most prevalent childhood disease that develops over time as a result of interactions between acid-producing bacteria, a substrate that the bacteria can metabolize, and a variety of host factors, such as saliva and teeth. Dental caries is caused by an ecological imbalance in the physiological balance between oral bacteria, biofilms and dental minerals (Young et al., 2015). Nonetheless, dental caries is a chronic disease, with significant medical, social, and financial repercussions for both the individual patient and the public as a whole. While the general public must pay for treatment and potential lost productivity of those affected, the individual patient has discomfort, oral system dysfunction, and a lower quality of life (Petersen, 2008). Data from the National Health and Nutrition Examination Survey (NHANES) 2011-2012 showed that dental caries in primary teeth was diagnosed in 37% of children between the ages of 2 and 8, 21% of children between the ages of 6 and 11, and 58% of children between the ages of 12 and 19 were found with dental caries in their permanent teeth (Dye et al., 2015). When comparing this data to the prior study from 1999-2004, there has been a general fall in the overall incidence of caries in primary teeth and a minor decline in the percent of permanent teeth with caries. Although the prevalence of caries has decreased, especially in children and adolescents, occlusal surface caries have not decreased at the same rate as smooth surface caries. Deep pits and fissures are linked to the increased prevalence of caries on occlusal surfaces. It has been generally accepted that the use of caries prevention approaches such as fluoridation of community waters, topical fluoride treatment, plaque control, and sugar control in the diet have a greater impact on the decrease of caries prevalence in general, especially smooth surface caries lesions (Naaman et al., 2017).

1. Pit and Fissure Morphology

Occlusal surfaces of teeth are prone to caries because of the anatomical structure of pits and fissures. Pits and fissures create a retentive area on the tooth surface and cause nutrient and bacterial accumulation. In addition, the thinner enamel in these regions causes demineralization to accelerate (Nowak et al., 2018). The risk of caries on the occlusal surfaces is also affected by the angle between the tubercle slopes of the fissures. It has been reported that the caries risk is low in shallow fissures where the angle between tubercle inclinations is approximately 70-90°, and caries susceptibility is high in fissures with this angle less than 70° (König, 1963).

Pits and fissures are divided into five groups according to their morphological features as V, U, I, IK, and Y types (Nagano, 1960) **(Fig. 1)**;

- **V type**: The fissure is wide at the top and narrows toward the base (34%),
- **IK type**: Hourglass-like (26%),

- **I type:** A narrow slit from the top of the fissure to its base (19%)
- **U type:** The fissure is the same width (14%) from top to bottom (14%),
- **Y type:** These are narrow fissures at the top and widening at the base (7%).

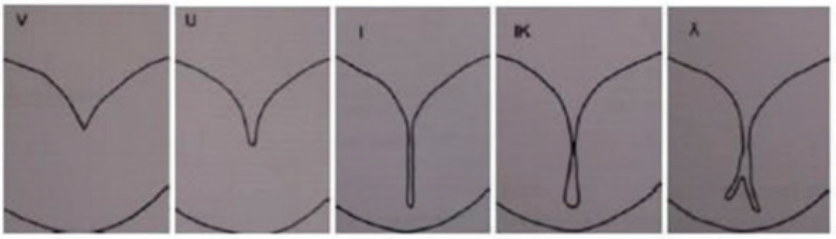

Figure 1. Fissure types according to morphological structures (Nagano, 1960)

According to the Nagano classification, the risk of caries in fissures is indicated as V<U<I<IK<Y from low to high. While the formation of caries is less in V and U-shaped fissures specified in this classification, caries formation is more common in deep and narrow fissures of I and IK type.

Classification of fissures according to their depth (Symons et al., 1996);

- **Fissure of shallow depth**; fissure where the base of the fissure is seen between the tubercule when examined with light, and the tubercule slopes merge with each other at a wide angle
- **Fissure of medium depth**; fissure where the base of the fissure is seen when examined with transillumination, where the tubercle slopes merge with each other at a narrower angle
- **Fissure of deep depth;** deep fissure where the base of the fissure is not visible with light and the tubercule slopes meet at an acute angle

According to the Symons classification, it is more common in deep and narrow-angle fissures due to the risk of caries in fissures, high microorganism and nutrient retention, and not being easily cleaned (König, 1963).

2. Pit and Fissure Caries

Pit and fissure caries constitute more than 80% of dental caries. The anatomical structure of the pits and fissures of the teeth is an element that should be considered in terms of caries formation. Pit and fissure caries are formed by the fusion of two independent lesions at the base that progress along the enamel prismas. The width of the enamel-dentin junction, which is prone to caries, and the depth of the fissure determine the rate of caries progression (Hicks & Flaitz, 2009; Özer et al., 2016). Depending on the anatomical structure

of fissures, the enamel thickness at their base differs. Enamel thickness is approximately 1.5-2 mm on the majority of tooth surfaces, whereas it is only 0.2 mm or less in deep fissures. Therefore, the spread of caries lesions that start in deep fissures to dentin is faster (Sungurtekin et al., 2010).

2.1. Pit and Fissure Caries Prevention

The basis of protective treatments are applications related to biofilm control. Some methods are being developed to prevent dental caries and oral diseases caused by biofilm. These methods are:

- Reducing the number of detrimental pathogens present in the biofilm
- Increasing the resistance of dental hard tissues and protecting gingival health
- Increasing the repair ability of the tooth and surrounding tissues.

Today, the methods applied to prevent pit and fissure caries can be classified as ***mechanical plaque control***, ***chemical plaque control*** and ***pit and fissure sealant*** applications (Ulusoy., 2010).

Permanent molars' occlusal surfaces have anatomical grooves, pits, and fissures that trap food particles and promote the growth of bacterial biofilm, which from the perspective of primary prevention raises the risk of developing carious lesions. A thorough caries prevention strategy includes properly infiltrating and sealing these surfaces with a dental material, like pit-and-fissure sealants, which can help prevent lesions. There is evidence that sealants can reduce the progression of noncavitated carious lesions from the perspective of secondary prevention. When deciding on the ideal intervention for noncavitated carious lesions, the clinician must consider the use of sealants to stop or slow the progression of the lesions (Wright et al., 2016).

3. Pit and Fissure Sealents

Pit and fissure sealants are materials that are applied to teeth with occlusal pits and fissures that are susceptible to caries. This forms a protective covering that is micromechanically linked and inhibits caries-causing bacteria from getting to their source of nutrients. The narrow widths and irregular depths of fissures are an ideal environment for acid-producing bacteria to accumulate. The cleaning function of saliva is insufficient for pits and fissures in molars. Also, the bristle diameters of the toothbrush are too large to clean most fissure surfaces. For this reason, fissure sealants act as physical barriers to acids produced in plaque (Simonsen., 2002).

3.1. Pit and Fissure Sealents: A Brief History

At the beginning of the 18th century, it was thought that covering the occlusal surfaces of molars with a suitable material could prevent possible

caries lesions. The first treatment approach was initiated by Wilson by covering the fissures with zinc phosphate cement. In the following period, a more aggressive method was developed by Hyatt in 1923, "prophylactic odontomy" technique, in which the deep pits and fissures with suspected caries are mechanically abraded and the created class 1 cavities are filled with amalgam (Hyatt., 1924). In 1929, Bodecker advocated cleaning the fissures with sond and dripping oxyphosphate cement into them, then proposed the "fissure eradication technique" in which occlusal fissures are flattened by grinding with a bur to create self-cleaning areas in the fissures (Naaman et al., 2017).

The emergence of pit and fissure sealants is based on a study by Buonocore that allows the resin material to infiltrate into the microvoids created by the etching of the enamel, increasing the retention of the material. Using 85% phosphoric acid for 30 seconds, he described the acid etching process as a method to enhance the adhesion of self-curing methyl methacrylate resin materials to dental enamel. In fact, this research marked the start of a new era in clinical dentistry (Buonocore., 1955). The first clinical applications of fissure sealants were started by Cueto and Buonocure in 1967, with the sealing of etched occlusal surfaces using cyanoacrylate. However, the use of cyanoacrylates has been abandoned due to the toxic effects on the skin and oral mucosa, difficulty in application, bacterial deterioration over time in a humid environment, dissolution in oral fluids and low bond strength (Cueto & Buonocore, 1967). Later that, Bowen developed a viscous resin known as bisphenol-A-glycidyl dimethacrylate, or BIS-GMA. It has been found that this material is resistant to deterioration and successfully adheres to etched enamel (Bowen., 1965).

In 1970, Buonocore published its first article on pit and fissure sealer, detailing the effective application of BIS-GMA resin using UV light (Buonocore., 1970). The use of fissure sealant materials containing bis-phenol A methacrylate (Bis-GMA) resin monomer was authorized by the American Dental Association (ADA) in the 1980s (Bowen., 1982) **(Fig. 2).**

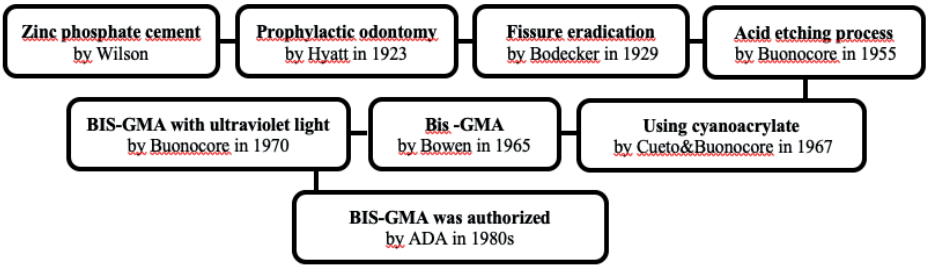

Figure 2. History of Pit and Fissure Sealents

3.2. Pit and Fissure Sealent Materials

3.2.1. Resin-Based Fissure Sealent Materials

Resin-based dental materials consist of the organic part consisting of the resin matrix, the inorganic part formed by the filler content and the intermediate phase that connects the matrix and fillers. Bisphenol A glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), glycol dimethacrylate (TEGDMA), hydroxyethyl methacrylate (HEMA) are the most preferred among the monomers that constitute the resin matrix (Roberson et al., 2006).

Bis-GMA is an epoxy resin-like hybrid material in which epoxy groups are replaced by methacrylate groups. Bis-GMA combines the rapid polymerization of methyl methacrylate with the minimal polymerization shrinkage of epoxy resin. UDMA, is a monomer with a lower molecular weight, more fluid, resistant to color change and better adhesion compared to Bis-GMA. However, since it has a lower molecular weight than Bis-GMA, it undergoes more polymerization shrinkage. In order to decrease the viscosity of the polymer matrix structure and improve the penetrating ability of the fissure sealant, monomers like TEGDMA or HEMA are included to it (Roberson et al. 2006; Hicks & Flaitz, 2009). It is not advised to use resin-based fissure sealants when technical perfection prevents isolation from being achieved (Garg et al., 2018).

Resin-based sealents can be classified into according to their polymerization mechanism, filler content, colour (Naaman et al., 2017).

3.2.1.1. Classification of Resin-Based Fissure Sealents According to Polymerization Mechanism

According to the mechanism of polymerization, resin-based fissure sealants can be divided into four generations.

- Ultraviolet light-cured resin-based sealants (1st generation fissure sealants),
- Chemically-cured resin-based sealants (2nd generation fissure sealants),
- Visible light-polymerizing resin-based sealants (3rd generation fissure sealants),
- Fluoride-releasing resin-based sealants (4th generation fissure sealants).

First Generation Fissure Sealents: Ultraviolet light's impact on the material's initiators, which cause polymerization, resulted in the formation of the first generation of resin-based fissure sealants. However, this method was

abandoned because the wavelength of ultraviolet light cannot be stabilized and prolonged exposure to ultraviolet light causes retinal damage. For this reason, this type of fissure sealents are no longer used. The first sealant to hit the market was Nuva-Seal® (LD. Caulk Co., Milford, USA), which is an example of a resin-based sealer polymerized by a UV light source (Pinkham et al., 2005).

Second Generation Fissure Sealents: The second generation of resin-based fissure sealants were chemically cured or auto-polymerizing resin-based sealants. This type of fissur sealent includes two parts; it contains a part containing benzoyl peroxide and Bis-GMA as an initiator and another part consisting of 5% organic amine as an accelerator, and by mixing these two parts, a chemically exothermic reaction occurs and the hardening reaction takes place within 1-2 minutes. The interaction of these two components generates free radicals, which start the polymerization of the resin sealant materials. It has been reported that the retention of second generation fissure sealants is more successful than first generation fissure sealants (Geiger et al., 2000).

Third Generation Fissure Sealents: The third generation, which consists of resin-based sealants that are polymerized by visible light, has mostly succeeded resin-based sealants that are autopolymerized. These are polymerized with visible (blue) light with a wavelength of approximately 480 nm. There are camphoroquinones and amines that initiate the polymerization reaction in the structure of the 3rd generation fissure sealants. The light sources used for the polymerization reaction are; halogen light sources are modified type visible blue light sources, laser light sources, light emitting diodes, quartz tungsten halogen light sources (QTH) and plasma arc light sources (Santini et al., 2013). The advantage of third generation fissure sealants; prolonged working time, polymerization control by the clinician, faster hardening, decrease in porosity due to no mixing process and homogeneous distribution of the material (Pinkham et al., 2005).

Fourth Generation Fissure Sealents: The fluoride releasing resin-based sealants are the fourth generation. The result of adding fluoride releasing particles to light polymerizing resin-based sealents in an effort to prevent caries is fluoride resin-based sealant. However, the research states that fluoride-releasing resin-based sealents cannot be regarded as a fluoride reservoir that releases fluoride over time, and as a result, this type of sealant does not assist light polymerizing resin-based sealents clinically in any way (Simonsen., 2002)

3.2.1.2. Classification of Resin-Based Fissure Sealents According to Filler Content

Resin-based fissure sealents can be classified according to filler content;

- Filled Resin-Based Fissure Sealents
- Semi-filled Resin-Based Fissure Sealents
- Unfilled Resin-Based Fissure Sealents

Clinical results appear to be only minimally affected by the use of filler particles in the fissure sealent material. Filled sealants are less able to enter fissures despite having a better wear resistance. In general, the filled sealants necessiate occlusal adjustments, which extend the process needlessly. On the other hand, unfilled resin sealants have a lower viscosity and offer better retention and more penetration into fissures (Reddy et al., 2015).

3.2.1.3. Classification of Resin-Based Fissure Sealents According to Colour

Resin-based fissure sealants can be opaque (tooth-colored or white) or transparent (transparent, amber, transparent pink). The fact that white opaque fissure sealants are easily visible on the teeth during application, control appointments and by the family and the child ensures that they can be easily recognized when there is any loss of material from the fissure sealant. It has been reported that transparent fissure sealants are useful in evaluating caries formation in the substrate. However, the choice of sealant material is typically a matter of preference (Simonsen., 2002).

The ability to change color has been incorporated thanks to advancements in resin sealant technology. Either during the curing step, as with Clinpro (3M ESPE, Saint Paul, MN, USA), or during the post-polymerization phase, as with Helioseal Clear (Ivoclar Vivadent, Schaan, Liechtenstein), this color property changes. Although its benefits have not yet been completely confirmed, this technique may in fact offer the benefit of improved sealing surface recognition (Dean., 2016). Therefore, it would seem that the ***opaque, unfilled, light polymerizing sealant*** would be the best option for a resin-based fissure sealant (Naaman et al., 2017).

3.2.2. Glass Ionomer Based Fissure Sealent Materials

Glass ionomer cements, which were first introduced to the market by Wilson and Kent in 1972. Glass ionomer cements have various uses in dentistry as base material, filling, adhesive cement and fissure sealant. Glass ionomer cements are formed as a result of the acid-base reaction between polycarboxylic acid and silicate glass powder and its adhesion to dental hard tissues occurs chemically. This chemical bonding occurs as a result of the interaction of phosphate ions on the dental surface and carboxylate groups in the cement (Wilson et al., 1983).

The advantages of glass ionomer cement are that it is biocompatible with dental tissues, is resistant to acids, contains no residual monomer, can release active fluoride, has anticariogenic effects, and can be chemically bonded to dental enamel and dentin (Croll., 1990). There are also disadvantages such as not being as good in color as composite resins, high sensitivity to moisture during curing, lower resistance than resin-based fissure sealent materials, high rate of microleakage and limited working time (Savaş et al., 2015).

In general, the continuous fluoride release and fluoride recharge capabilities of a glass ionomer cement-based sealant are its key benefits. Because some parts of sealant may still be present deep inside the fissures, its preventative function might even endure after the substance has been visibly lost. Unlike sealants made of hydrophobic resin-based sealents, it is not sensitive to moisture, is easy to install, and is moisture-friendly (Pinkham et al., 2005). When resin-based sealants cannot be used, such as on partially erupted permanent teeth, it can be used as a temporary sealant, especially if the operculum is covering the distal portion of the occlusal surface. Additionally, primary molars with deep fissures that are challenging to isolate because of a child's pre-cooperative behavior may benefit from glass ionomer-based sealant (Antonson et al., 2012). When better isolation is possible, it must be replaced with a resin-based sealant because it is only a temporary sealant (AAPD, 2016).

3.2.3. Resin Modified Glass Ionomer Based Fissure Sealent Materials

Resin modified glass ionomer cements, which are more resilient, was accomplished by adding resin to the cement structure in order to improve the insufficient physical attributes of glass ionomers, such as insufficient color compatibility, low abrasion resistance, and susceptibility to moisture. The material contains 23% flouride and releases more flouride than conventional glass ionomer cement. The material consists of 80% glass ionomer and 20% resin content and was first used in 1992. The curing mechanism first starts with the light activation and then continues with the acid-base reaction. Thus, the material is bonded to the tooth both micromechanically and chemically (Hes et al., 1999).

The powder part of resin modified glass ionomer cements contains fluoroaluminosilicate glass powders, the liquid part contains HEMA, methacrylate groups, tartaric acid, polyacrylic acid and 8% water (Kanık & Türkün., 2016). It has been stated that the biocompatibility of resin modified glass ionomer is lower than that of conventional glass ionomer due to the HEMA it contains (Nicholson et al., 2008).

In a study comparing resin modified glass ionomer based fissure sealents and conventional glass ionomer based fissure sealants, it was reported that the retention of resin modified glass ionomer fissure sealants was better than

conventional glass ionomer based fissure sealants, but lower than resin-based fissure sealants (Papacchini et al., 2005). As a result of the another study comparing the surface properties, retention and caries prevention properties of resin modified glass ionomer and a resin-based fissure sealant, it has been shown that the adhesion, abrasion resistance, retention and caries preventive properties of resin modified glass ionomer at 1-year follow-up were as good as a resin-based fissure sealant. Therefore, it has been stated that resin modified glass ionomer could be an alternative to resin-based fissure sealants (Oliveira et al., 2008).

3.2.4. Polyacid-Modified Resin-Based Fissure Sealent Materials

As a fissure sealant, polyacid modified resin-based composite material, also known as compomer, has been used. It contains 70-80% composite resin, 20-30% glass ionomer cement and 13% fluoride. The physical properties of polyacid-modified resin composites are similar to composites, but their wear is more and their fracture resistance is lower than composites (Ünlügenç & Bolgül., 2019).

A polyacid-modified resin-based sealents are materials that combine the aesthetic properties of composite resins with the fluoride release and chemical bonding properties of glass ionomer cements. It has superior adhesive characteristics to enamel and dentin in addition to being less water-soluble than glass ionomer-based sealant material and less technique-sensitive than resin based sealants (Puppin-Ronatni et al., 2006).

3.2.5. Ormoser-Based Fissure Sealent Materials

Organically modified ceramics (ormosers) were introduced in 1998 in order to improve the physical properties of composite resins and to minimize polymerization shrinkage. The increase in the molecular size of the material increases the polymerization shrinkage, reduces wear and monomer release. For this reason, it is preferred to be used as a matrix under composite restorations. In addition, the fact that the ormoser material contains cross-linked organic inorganic copolymers instead of conventional monomers (UDMA, Bis-GMA) ensures that polymerization shrinkage is lower than composite resins (Bottenberg et al., 2007; Tauböck et al., 2019).

According to a study assessing the clinical success of ormoser-based fissure sealants, ormoser had higher of clinical success than compomer-based sealants and was comparable to resin based and resin modified glass ionomer based sealants (Yılmaz et al., 2010). In a study, secondary caries, retention rates, and marginal adaption of glass ionomer- and ormoser-based fissure sealants were compared. Similar findings from the study were observed in terms of marginal adaption and retention. Fissure sealant containing glass ionomer was found to be more successful at preventing the development of

secondary caries (Guler et al., 2013)

3.2.6. Giomer-Based Fissure Sealent Materials

Giomers are hybrid materials formed by adding ***pre-reacted glass ionomer*** (S-PRG) fillers to the resin matrix. It includes basic components of composite resins and glass ionomer cements. Fluoride release and fluoride recharging features are similar to glass ionomer cements, while aesthetic, biocompatibility, easy polishing and wear-resistant properties are similar to composite resins (Durham et al., 2017; Mungara et al., 2013).

While applying giomer-based fissure sealant, self-etch primer is applied instead of etching. In this way, the negativities that occur during etching are eliminated and time savings are provided for the patient and the clinician. However, research has shown that the giomer-based fissure sealant made without etching has a lower bond strength and microleakage value than the resin-based fissure sealant and the giomer-based fissure sealant used with etching (Durham et al., 2017).

Fluoride release properties of giomer-based fissure sealants were found to be higher than compomer and lower than glass ionomer (Bansal & Bansal., 2015). It was observed that the ability of the material to release fluoride was low in the first days and reached the highest value at the end of the following 21 days (Okuyama et al., 2006). In a study evaluating the penetration depths of fissure sealants, it was reported that the penetration depth of the giomer-based fissure sealant was lower than that of resin-based fissure sealants without filler, and higher than that of glass-ionomer based fissure sealants (Hatırlı et al., 2018). In another study, the retention rates of resin based fissure sealants and giomer were evaluated, and the retention rate of resin based fissure sealants was found to be higher at the end of 18-month follow-up (Güçyetmez & Kırzıoğlu., 2019).

3.2.7. Glass Carbomer-Based Fissure Sealent Materials

Glass carbomers were formed by adding nanosized fluoropatite and hydroxyapatite crystals to glass particles. The liquid of the material is polyacrylic acid, and it contains 20% fluoropatite crystals (Cehreli et al., 2013). Manufacturers aim to create an enamel-like structure with nanoparticle technology. Thus, it is aimed to improve the physical properties of the material and to increase its compression, tensile and abrasion resistance (Capan & Akyüz., 2016).

Glass carbomers also have the ability to release fluoride and be recharged. Researchers show that the fluoride release rate of glass carbomer materials is similar to glass ionomer cement. Glass carbomers can be chemically bonded to dental tissues (Ercan et al., 2019). It is recommended to use a light device to make the curing reaction faster. Depending on the light device, heating takes

place, thereby accelerating the curing reaction, which reduces microleakage and increases compressive strength (Gorseta et al., 2014).

Glass carbomer can be used as an alternative to glass ionomer-based fissure sealant, especially in cases where moisture control cannot be achieved in pediatric dentistry. However, when the retention rates of glass ionomer based fissure sealant, glass carbomer based fissure sealant and resin based fissure sealant were compared, it has been reported that glass carbomer based fissure sealant had the lowest retention rate (Subramaniam et al., 2015). In a study comparing the microleakage and solubility values of glass carbomer-based fissure sealants, no significant difference was found between the microleakage values of resin-based and glass-carbomer-based fissure sealants, and the solubility of glass carbomer was found to be lower than glass ionomer-based fissure sealant when the pH was between 4-6 (Chen et al., 2012).

3.2.8. Amorphous Calcium Phosphate-Based Fissure Sealent Materials

Amorphous calcium phosphate (ACP) is a calcium phosphate compound found in the natural structure of the tooth and leading to the formation of hydroxyapatite. When the pH drops to a critical value, amorphous calcium phosphate neutralizes the acidity of the environment by releasing calcium and phosphate, and also supports the formation of hydroxyapatite and remineralization by ensuring that Ca and P ions dissolved from the enamel layer of the tooth adhere to the tooth surface again (Zawaideh et al., 2016)

With the increase of studies on remineralization, amorphous calcium phosphate compounds have been added to the structure of sugar-free gums, pastil and restorative materials. It is claimed that ACPs, which are claimed to prevent demineralization of teeth and actively stimulate the remineralization process, are added to the structure of fissure sealants and composites, increasing the caries-preventing effects of the materials (Venkatesan & Ranjan., 2014).

3.2.9. Bioactive Resin-Based Fissur Sealents Materials

A bioactive fissure sealant material that can prevent caries and has the requisite physical properties has recently been the subject of numerous experiments. Recently, BioCoat (Premier® Dental Products, PA, USA), a novel bioactive resin-based sealant, was launched to the market (AlQahtani et al., 2022).

Premier BioCoat® is a fissure sealant material with bioactive content developed using SmartCap technology. Smartcap is a microcapsular structure that consists of a semi-permeable membrane and allows active ion exchange. It is claimed that thanks to this structure, it inhibits demineralization and promotes remineralization. The manufacturer claims that Premier BioCoat® fissure sealant material has a high filling content (56%) with high resistance

to chewing forces and abrasion resistance, and low shrinkage values, thus low risk of microleakage, high dimensional stability and long-term durability (Kılınç et al., 2021; AlQahtani et al., 2022)

4. Esterogenicity of Pit and Fissure Sealents

Bisphenol-A (BPA) is the chemical precursor of bisphenol-a dimethacrylate (Bis-DMA) and bisphenol-a glycidyl dimethacrylate (Bis-GMA), the two most often utilized monomers in resin restorations and sealants. It is well known for having estrogenic properties and having the potential to be hazardous to human development and reproduction. Instead of being a raw material in monomers, BPA exists as BPA derivatives, which are occasionally hydrolyzed and discovered in saliva (Dimogerontas et al., 2017).

According to a systematic review, it has been reported that high levels of BPA were discovered in saliva samples that were taken either immediately after resin-based sealant installation or an hour later. Urine samples also contained high levels of BPA (Kloukos et al., 2013). A report from the American Dental Association and the American Academy of Pediatric Dentistry, however, did not support the occurence of negative consequences following the application of sealants and characterized the BPA effect as a minor, temporary effect (AAPD., 2016). Despite the fact that the possible detrimental effects of BPA and its breakdown products have been thoroughly documented, the JADA Special Report concluded that there have been no reports of negative health effects associated with the released components of dental sealants. Therefore, it is debatable whether these substances are actually leached out of dental sealants in such quantities to be dangerous to human health (Simonsen et al., 2002). Using a moderate abrasive, such as pumice, either on a cotton applicator or in a prophy cup on the sealant surface following sealant polymerization is advised for cilinician who seek to reduce patients' exposure to the uncured components in the oxygen-inhibited layer of sealants (Kloukos et al., 2013).

5. Features of Current Pit and Fissure Sealents

Pit and fissure sealant applications are the most essential prophylactic method against caries formation, aside from professional fluoride treatments and regular oral hygiene habits. The choice of pit and fissure sealant material to be used may vary depending on the age of the patient, the eruption time of the teeth, and the child's cooperation. Currently, many materials have been used as pit and fissure sealents. In Table 1, the manufacturer, classification and composition of the used pit and fissure sealants in the market are indicated.

Table 1. Current Pit and Fissure Sealents

Pit and Fissure Sealents	Classification	Composition	Manufacturer
Clinpro Sealent	Resin-based unfilled	Bis-GMA, TEGDMA, EDMAB Difeniliyodonium hekzaflorofosfat, BHT, TBATFB	3M ESPE Saint Paul, MN, ABD
Helioseal F Plus	Resin-based containig Flouride	BIS-GMA, UDMA, TEGDMA, Fluorosilicate glass, highly dispersed silicon dioxide, titanium dioxide, initiators, stabilizers	Ivoclar Vivadent, Schaan, Liechtenstein
Grandio Seal	Resin-based, filled	Bis-GMA, TEGDMA, Nano-filler (%70)	Voco Cuxhaven, Germany
Fissurit Fx	Resin-based, filled, containing Flouride	Bis-GMA, TEGDMA, UDMA, BHT, Benzotriazolderivat, %2 NAF	Voco Cuxhaven, Germany
Ultraseal XT Hydro	Resin-based, Hydrophilic-filled	TEGDMA, DUDMA, Aluminium oxide, methacrylic acid, titanium dioxide	Ultradent Products, South Jordan, Utah, USA
Beauti Sealant	Giomer- based	TEGDMA, UDMA, S-PRG	Shofu Inc., Kyoto, Japan
Dyract Seal	Compomer-based	DGDMA, hydrated silicon dioxide, strontium alumino-fluoro-silicate glass, phosphoric acid modified methacrylate resin ammonium salt, camphoroquinone,	Dentsply, Konstanz, Germany
Aegis	ACP-based	ACP, UDMA, mono and dimethacrylate, Modified-Bis-GMA 38.5% inorganic filler	Bosworth Company, USA
Prevent Seal	Resin-based	Urethanedimethacrylate oligomer, Bis-GMA, TEGDMA, Glass fillers	Itena, France

Table 1. Current Pit and Fissure Sealents (Continous)

Pit and Fissure Sealents	Classification	Composition	Manufacturer
BioCoat	Bioactive resin-based	Bis-GMA, Barrium Aluminoborosilicate ,Triethylene glycol dimethacrylate, Calcium Donor Phosphate, Donor Fumed Silica, Photo-Initiator	Premier® Dental Products, Plymouth Meeting, PA, USA
Teethmate F1	Resin-based, unfilled	TEGDMA, HEMA, MDP-F, colloidal silica, camphoroquinone, methacryloyl-fluoride-methyl methacrylate copolymer, accelerators, initiator, pigment and hydrophobic dimethacrylates	Kuraray, Osaka, Japan

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Chapter 13

PERIANAL REGION MALIGNANCIES

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Introduction:

Anal malignancies are relatively infrequent among gastrointestinal cancers, yet their occurrence has shown a progressive upsurge in recent times. This surge in frequency can be ascribed to several determinants, encompassing female gender, Human Papillomavirus (HPV) infection, polygamous sexual associations, tobacco usage, and Human Immunodeficiency Virus (HIV) infection. Despite being part of the gastrointestinal system, anal region cancers share more similarities in terms of etiology and prognosis with genital cancers. One of the major challenges in diagnosing anal region cancers is their resemblance to benign anorectal conditions, often resulting in delayed presentation at advanced stages. An important aspect of the diagnostic process is the anatomical classification into anal canal and anal margin cancers, as they require different treatment approaches. Effective management of these neoplasms requires a multidisciplinary approach, which has been correlated with favorable therapeutic outcomes.

Embryology, Anatomy, and Tumor Types of the Anal Canal:

Embryology of the Anal Canal:

The upper two-thirds of the anal canal develop from the hindgut during embryogenesis.[1-4] In the early embryonic stages, the primitive intestinal tube has blind ends representing the foregut and hindgut portions. A ectodermal invagination called the proctodeum forms posterior to the rectum. Between this invagination and the rectum, there exists a structure known as the anal membrane. By the ninth week, the rectum moves toward this invagination and perforates the anal membrane, forming the primary anus. With the perforation of the proctodeum, proliferating mesenchymal tissues enter between the epithelial segments. Consequently, the permanent anus develops along with the ectoderm and endoderm, contributing to its formation.[1,3,4] Therefore, the upper portion of the anal canal derives from the hindgut, which is the endoderm, while the distal one-third originates from the proctodeum, which is the ectoderm. In adults, the transition between these two structures is marked by the interlocking of the anal valves, forming the linea pectinata.[1,5,6] Due to this embryological distinction, there are differences in innervation, arterial, venous, and lymphatic circulation above and below the linea pectinata.[1,5,6]

Anatomy of the Anal Canal:

The anorectal canal is a anatomical structure that originates at the level of the puborectal muscles, which constitute a component of the anal sphincter, in the distal segment of the rectum, and extends towards the intersphincteric groove on the perianal integument, measuring approximately 2.5-3.5 cm in length. From a practical standpoint, the puborectal muscle, discernible during

rectal examination, is situated approximately 1-2 cm proximal to the linea pectinata. The segment within the intersphincteric groove is denoted as the anal verge during visual inspection. The region expanding laterally from the anal verge for 5 cm is designated as the anal margin. Based on these anatomical landmarks, the World Health Organization (WHO) and the American Joint Committee on Cancer (AJCC) classify neoplasms of the anorectal region into anal canal and anal margin malignancies.

The vascular supply to the region superior to the linea pectinata is predominantly furnished by the superior rectal artery, a branch of the inferior mesenteric artery. Conversely, the vascular supply to the inferior portion is derived from branches of the internal pudendal artery and the middle rectal artery, originating from the internal iliac artery. There are interconnected blood vessels, known as anastomoses, between these three vascular structures, ensuring collateral circulation and maintaining blood flow to the anal region.[1,5] This vascular network plays a crucial role in providing oxygen and nutrients to the anal canal and surrounding tissues.

Venous drainage within the region superior to the linea pectinata is accomplished via the superior rectal vein, which ultimately converges into the portal system. In contrast, venous drainage in the inferior segment occurs through the inferior rectal vein, which empties into the caval system. This partition of venous drainage reflects the anatomical differentiation between the upper and lower portions of the anal canal and contributes to the intricate venous circulation in the anal region.[1,5]

The lymphatic vessels of the upper division of the anal canal, situated above the linea pectinea, primarily drain into the mesorectum and the internal iliac lymph nodes. Subsequently, they further connect to the common iliac and lumbar lymph nodes. Conversely, the lower section of the anal canal drains into the superficial inguinal lymph nodes.[9-11] When employing the linea pectinata as a reference point, the lymphatic drainage pattern above this demarcation closely resembles that of rectal malignancies. According to the American Joint Committee on Cancer (AJCC) staging system, involvement of any of these lymph nodes is classified as N1, denoting regional lymph node metastasis.

Types of Anal Canal Tumors:

Anal region malignancies arise from three mucosal regions present in this area.[7] These regions are the squamous mucosa, transitional mucosa, and the glandular portions of the mucosa.

-Squamous cell carcinoma: It originates from the squamous or transitional mucosa. Although there are morphological differences among malignancies originating from these zones, they are grouped under a single category in

terms of treatment management, as there is no significant difference in treatment approach.[12,13] The basaloid type is a subtype that develops from the transitional mucosa and accounts for 25% of SCCs.

-Adenocarcinoma: It is a rare malignancy of the anal canal that arises from the glandular portions. The treatments and outcomes are similar to those of rectal malignancies.

Types of Anal Margin Tumors:

Anal margin malignancies are commonly known as perianal skin cancers. With the exception of melanoma, these malignancies share histological similarities with skin cancers and require different management strategies compared to anal canal cancers. Squamous cell carcinoma (SCC) is the most frequent type, but there are also rare types such as basal cell carcinoma, melanoma, Bowen's disease, and extramammary Paget's disease.

In the context of anal margin malignancies, Bowen's disease is referred to as in situ SCC, indicating that the cancer cells are confined to the surface layers of the skin without invading deeper tissues. Paget's disease, on the other hand, represents in situ adenocarcinoma affecting the region.

Epidemiology and Risk Factors:

Anal cancers account for less than 1% of all malignancies, with the most common subtype being squamous cell carcinoma, which has an incidence of 0.5-2.0 per 100,000 individuals.[14] However, the incidence of anal cancer is increasing in Europe, Australia, and America.[14] It is particularly more common in young black males and women in their 6th decade. Individuals infected with HIV and homosexual men are at a higher risk, with anal cancer rates reaching 37 per 100,000 individuals.[15]

Subsets vulnerable to anal malignancies encompass populations afflicted with HIV, male individuals engaging in homosexual practices, females harboring human papillomavirus (HPV), recipients of solid organ transplants, and individuals afflicted with autoimmune disorders. These groups have been empirically identified as high-risk cohorts.

Epidemiological analyses conducted in HIV-infected populations have shown that anal malignancies occur in approximately 3.4% of HIV-infected men and 1.7% of non-infected individuals. No significant difference has been observed among women with or without HIV infection.[16] Cohort studies have demonstrated that the incidence of anal malignancies in HIV-infected homosexual men is 131 per 100,000 individuals, compared to 2 per 100,000 individuals in non-infected homosexual men.[17] Additionally, HIV-infected populations have an increased incidence of HPV and HPV-related malignancies.[18] The relationship between HIV and HPV complicates

the determination of the independent effect of HIV on anal malignancies. However, it is evident that anal malignancies are more common in HIV-infected men, regardless of their sexual orientation.

HPV infection exerts its influence on the development of malignancies not only within the genital tract, but also in the rectum, oral cavity, and oropharynx.[19,20] It has been observed that around 80-85% of individuals harboring precursor lesions like squamous cell carcinoma and anal intraepithelial lesions exhibit HPV infection, with HPV 16 and HPV 18 being particularly prevalent.[21] The existence of HPV 16 has been linked to a more favorable prognosis in these cases.[22]

In a study investigating the efficacy of quadrivalent HPV vaccine against anogenital squamous intraepithelial lesions (SIL) in both males and females, a 78% reduction in SIL incidence was observed in 602 homosexual males.[23] Therefore, HPV vaccination is recommended for homosexual males and females.

Chronic immunosuppression conditions such as solid organ transplantation can lead to high-grade SIL and invasive anal carcinoma.[24] Meta-analyses have shown that in patients who have undergone solid organ transplantation and were followed up for 10 years, anal malignancies developed in 24.5 per 100,000 males and 49.6 per 100,000 females.[24]

In autoimmune diseases, anal malignancies can occur due to chronic glucocorticoid use. In this meta-analysis, the incidence of perianal malignancies was found to be 10 per 100,000 patients with systemic lupus erythematosus and 6 per 100,000 patients with ulcerative colitis.[25,26]

Diagnosis:

In squamous cell carcinomas (SCCs), hemorrhage emerges as the prevailing symptomatology. However, perianal mass, itching, pain, non-healing ulcers, fecal incontinence, and fistula can also occur. Patients frequently exhibit symptoms and clinical manifestations resembling benign anorectal ailments. Consequently, when there are suspicions of anal canal malignancies, it is advisable to conduct rectal examination and even anoscopy for the purpose of evaluating and procuring a biopsy. Subsequent to the diagnosis, it is essential to undertake pelvic magnetic resonance imaging (MRI) and thoracoabdominal computed tomography (CT) to facilitate staging endeavors. MRI can demonstrate the relationship between the internal and external sphincters and assess neighboring organ invasion, aiding in the staging process. It also allows evaluation of regional lymph nodes, guiding the planning of radiotherapy. Thoracoabdominal CT is used to assess potential distant metastatic sites. While PET-CT is useful for detecting metastatic foci, it is primarily recommended for monitoring treatment response. A diagnostic algorithm for anal region malignancies is provided (Figure 1, Table 1).[27]

Figure 1. Anal Cancer Diagnostic Algorithm

MRI: Magnetic Resonance Imaging, CT: Computed Tomography, HIV: Human Immunodeficiency Virus, HPV: Human Papilloma Virus, PET-CT: Positron Emission Tomography

Table 1. SCC Diagnostic Algorithm

Essential Tasks	Recommended	Optional
Biopsy	HIV test	Endo-anal USG
Rectal Examination	PET-CT	FNA to Inguinal Lymph Nodes
Medical History	p16/HPV test	Examination under anesthesia
Clinical Examination		
High Resolution MRI		
Thorax, Abdomen and Pelvic CT		
Endoscopic Examination		
Gynecological Examination		

MRI: Magnetic Resonance Imaging, CT: Computed Tomography, HIV: Human Immunodeficiency Virus, HPV: Human Papilloma Virus, PET-CT: Positron Emission Tomography

Treatment Management

The treatment algorithms for anal region malignancies vary depending on whether it involves anal canal or anal margin cancers, and there are also differences in the management of localized and metastatic diseases. Nevertheless, the primary objective of therapy continues to be the attainment of locoregional control, preservation of anorectal function, and optimization of overall quality of life.

Table 2. Anal cancer TNM staging AJCC 8th edition

Primary Tumor (T)	
T Category	**T Criteria**
TX	Primary tumor could not be evaluated
T0	No evidence of primary tumor
Tis	High-grade squamous intraepithelial lesion , carcinoma in-situ
T1	Tumor ≤ 2cm
T2	Tumor > 2cm, ≤ 5cm
T3	Tumor > 5cm
T4	Invasion regardless of Tumor Size (vagina, urethra, or bladder)
Regional Lymph Nodes (N)	
N Category	**N Criteria**
NX	Regional Lymph Node could not be evaluated
N0	No Regional Lymph Nodes
N1	Inguinal, Mesorectal, Internal iliac, or external iliac nodes
N1a	Inguinal, Mesorectal, or Internal iliac lymph nodes
N1b	External iliac lymph nodes
N1c	External iliac lymph nodes with N1a
Distant Metastasis (M)	
M Category	**M Criteria**
M0	Distant metastasis absent
M1	Distant metastasis present

Table 3. TNM Staging

	N0	N1
T1	Stage I	Stage IIB
T2	Stage IIA	Stage IIB
T3	Stage IIIA	Stage IIIA
T4	Stage IIIB	Stage IIIC

For localized disease, in Stage I anal margin malignancies, local excision is recommended, while for Stage II-III anal margin and Stage I-II-III anal canal malignancies, chemoradiotherapy (CRT) is recommended (Table 2, 3). In Stage I anal margin malignancies, after local excision, if the surgical margin is close to 1 mm, postoperative low-dose CRT is administered. For surgical margins greater than 1 mm, close monitoring is advised, and if recurrence occurs, surgical intervention is recommended (Figure 2).[27]

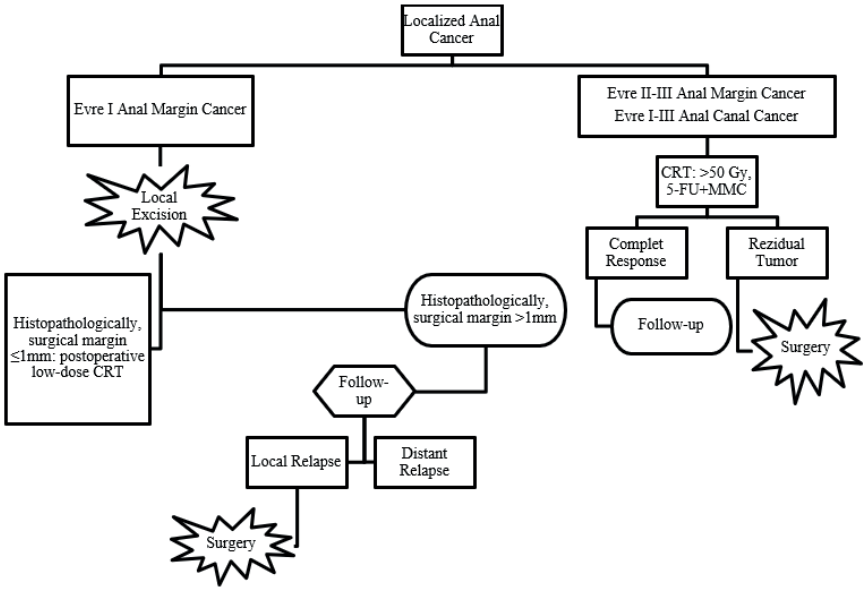
In Stage II-III anal margin and Stage I-II-III anal canal malignancies, if residual tumor is detected during follow-up after CRT, surgery is recommended.[27]

Standard chemotherapy regimens involve the administration of mitomycin C (MMC) and 5-fluorouracil (5-FU). Radiotherapy (RT) achieves complete tumor regression in 80-90% of cases.[28] European-based studies have demonstrated that CRT alone is superior to RT alone in terms of local control and expected life expectancy.[29]

Radiotherapy total dosages exhibit heterogeneity across various investigations. In the ACT II trial, an aggregate dose of 50.4 Gy was employed, whereas the RTOG 98-11 study administered doses ranging from 55-59 Gy for patients harboring T3-4 or lymph node-positive disease. Another expansive investigation conducted in Scandinavian nations utilized a total dose of 60 Gy.[30-32] The irradiation fields encompass the primary neoplasm location, anal canal, regional lymph nodes, and inguinal lymph nodes.

Additionally, HPV-positive anal squamous cell carcinomas are highly radiosensitive.

Figure 2. Anal Cancer Treatment Algorithm



CRT: Chemoradiotherapy, 5-FU: 5-Fluorouracil, MMC: Mitomycin C

In approximately 10-20% of patients with anal region malignancies,

diversion colostomy is performed prior to chemoradiotherapy (CRT) due to reasons such as fecal incontinence, anal fistula, and anorectal pain. Typically, CRT is initiated approximately 2 weeks after surgery. Local excision or radical surgeries are contraindicated in localized anal canal cancers. The primary treatment approach is CRT. Surgical intervention is recommended for cases with recurrence after CRT. Additionally, CRT has reduced the rates of abdominoperineal resection (APR).[33] In anal margin malignancies, only about 5% of cases can achieve cure with local excision. The surgical goal in patients with cT1N0M0 disease and no involvement of the anal sphincter is to obtain a surgical margin greater than 1 mm.[34] Low-dose CRT is administered in cases where the desired surgical margin cannot be achieved. 10-20% of anal region malignancies present with metastatic disease, and the expected 5-year survival rate in these patients is 30%. Para-aortic lymph node metastases are also considered distant metastases. Additionally, although rare, the most common site of distant metastasis is the liver.[35,36]

The first-line treatment for anal region malignancies is carboplatin-paclitaxel. In the second-line treatment, options include cisplatin, 5-FU, cisplatin-carboplatin, doxorubicin, taxanes, and irinotecan. Additionally, in addition to first-line treatments, immune checkpoint inhibitors known as Programmed cell death ligand 1 (PD-L1) inhibitors can be administered as immunotherapy. These include nivolumab and pembrolizumab. When nivolumab is added to the treatment, the median expected survival rate is 11.5 months.[37] For pembrolizumab, the median expected survival rate is 11.9 months.[38] Although there is limited experience with ablative treatments for isolated liver metastases in anal cancer, a multicenter study reported a median expected survival rate of 22.3 months.[39] Factors such as a liver metastasis larger than 5 cm and positive surgical margins are poor prognostic indicators.[39]

After treatment, follow-up for local malignancies typically involves monitoring for tumor response to chemoradiotherapy (CRT) for an average of 26 weeks. In patients who respond to CRT, rectal examination, anoscopy, and palpation of the inguinal region for lymph nodes should be performed every 6 months for a period of 5 years. Thoracoabdominal CT or PET-CT should be conducted annually for 3 years for imaging surveillance.

If there is disease progression or recurrence after completing CRT, salvage treatments such as abdominoperineal resection (APR) may be considered. However, a waiting period of 26 weeks should be observed to assess treatment response.

Anal Melanoma:

Melanomas, arising from melanocytes present in both cutaneous and mucosal structures, predominantly manifest in the gastrointestinal tract, particularly in the anorectal region. Anorectal melanomas constitute approximately 1% of anal cancers.[40] Anal melanomas may exhibit symptoms

such as hemorrhage, neoplasm, pain, or may incidentally manifest in specimens from hemorrhoidectomy. At the time of diagnosis, regional lymph node metastasis is observed in 60% of cases, while distant metastasis is detected in 30%.[41] Prognostic outcomes for anal melanomas are notably inferior compared to squamous cell carcinomas. Even in Stage I malignant melanomas, the 5-year projected survival rate stands at a mere 26.7%.[42]

The primary goal of treatment is achieving tumor-negative surgical margins. Whenever possible, it is important to avoid procedures with high morbidity, such as abdominoperineal resection (APR), which can significantly impact the patient's quality of life. Additionally, studies have shown that the patient's expected survival is more closely related to the stage of the disease rather than the specific surgical technique employed.[43]

Recommendations:

- It is crucial to have a clear understanding of the concepts of anal canal and anal margin.
- It should be remembered that in cases of localized anal canal malignancies, the primary treatment option is chemoradiotherapy (CRT), and surgery is contraindicated.
- Surgery is only performed in Stage I anal margin malignancies. In surgical cases without sphincter involvement, the aim should be to achieve a surgical margin of >1mm. If the surgical margin is histopathologically ≤1mm, postoperative low-dose CRT should be administered.
- In female patients with anal intraepithelial lesions, it should be noted that there may also be synchronous vulvar or cervical intraepithelial lesions. Therefore, a detailed gynecological examination is recommended.
- After diagnosis, pelvic MRI should be used to evaluate adjacent organ invasion and regional lymph nodes, while thoracoabdominal CT should be used to assess the presence of distant metastasis.
- In addition to radiation therapy (RT), 5-FU and MMC should be administered in the treatment.
- In the presence of fecal incontinence and persistent anorectal pain, diversion colostomy should be considered.
- After CRT treatment, a minimum of 26 weeks should be allowed to assess the response.
- Salvage surgery is recommended for residual or recurrent diseases after CRT.
- Carboplatin and paclitaxel are the standard treatment for Stage IV anal cancers.

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Chapter 14

CURRENT REMINERALIZING AGENTS USED IN THE TREATMENT OF INITIAL ENAMEL CARIES

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1. Formation and etiology of dental caries

Dental caries is a multifactorial disease caused by the colonization of bacteria in the oral environment and the disruption of the balance between host biology and oral microflora (Balakrishnan et al., 2000). The pH, which decreases as a result of the acid formed by the fermentation of the carbohydrates that the individual takes with the diet by the bacteria, disrupts the connection between the inorganic Ca-PO_4^{-3} crystals and the organic matrix (Matsui and Cvitkovitch, 2020).

Mineral loss (demineralization) occurs with the dissolution of Ca and PO_4^{-3}, which forms the hydroxyapatite crystals in the enamel structure, and cavitation occurs as this process continues (Aoba, 2004).

Progression, arrest and reversal of dental caries depend on the balance between remineralization and demineralization processes (Haris and Gorcia-Goday, 2004). The demineralization process on the tooth enamel surface can be inhibited in the presence of ions such as Ca and PO_4^{-3}, which are responsible for remineralization in saliva (Selwitz et al., 2007).

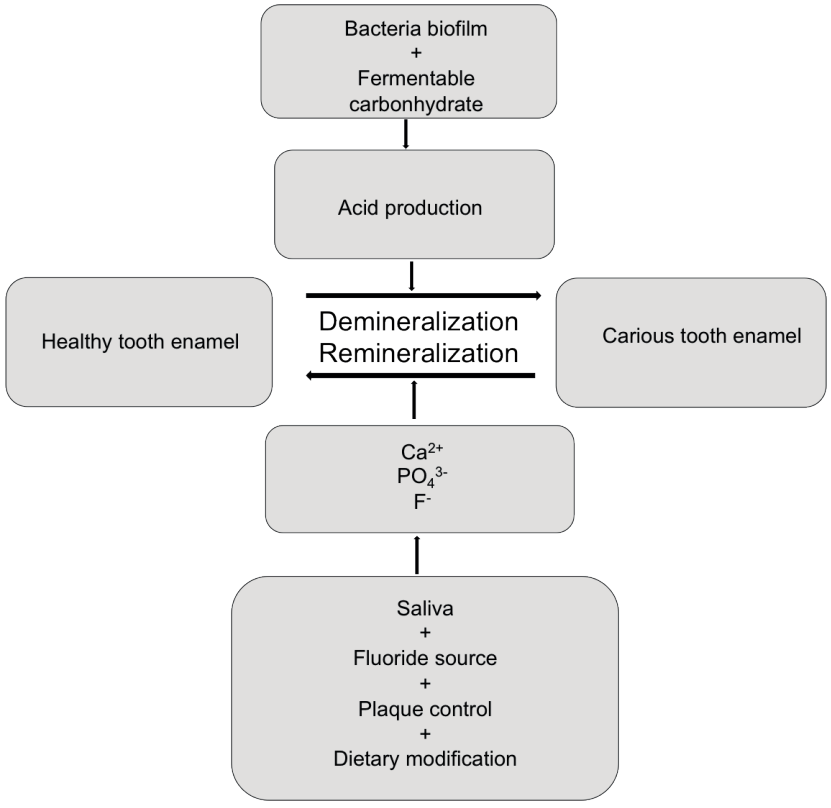

Figure 1: *Remineralization and demineralization balance (Selwitz et al., 2007)*

1.1. Demineralization and remineralization

Demineralization is defined as the decrease in pH as a result of the fermentation of dietary carbohydrates by bacteria and the removal of Ca and PO_4^{-3} ions as a result of dissolution in the dental tissue (Moreno and Zahradnik, 1979).

When the pH value drops below the critical value of 5.5, the demineralization process in the enamel tissue begins with its dissolution in the hydroxyapatite crystals (Chow and Vogel, 2001). The demineralization process begins at the atomic level on the surface of dental tissues, and when the amount of lost mineral is greater than the amount of mineral regained to the tissue, dental caries occurs (Gonzales-Cabezas, 2010).

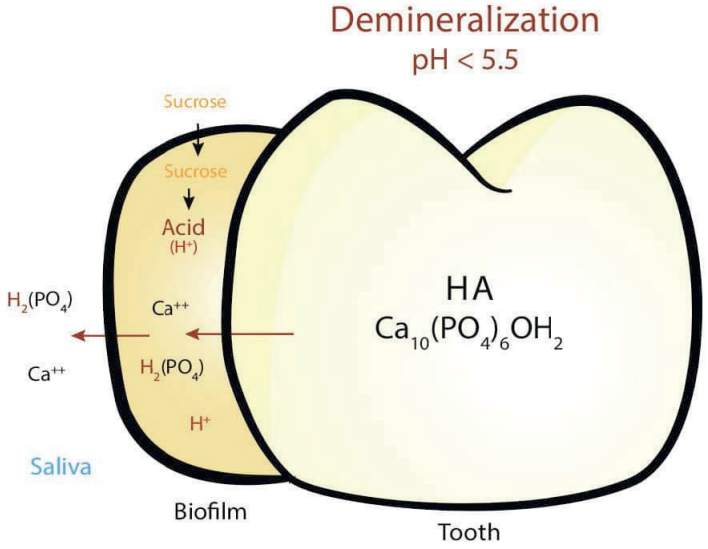

Figure 2: *Process of demineralization (Banchand et al., 2022)*

The first stage of demineralization in the enamel is characterized by interprismatic mineral loss, and in the later stages, the surface layer is formed, which causes the early caries lesion (Kudiyirickal and Ivancaková, 2008). The remineralization process, on the other hand, is defined as a repair process that continues with the accumulation of ions such as calcium and phosphate, which are lost from the enamel tissue after demineralization, on the enamel surface (Tschoppe et al., 2007).

There is a physiological ion exchange between dental hard tissues and saliva (Dönmez et al., 2004). Demineralization-remineralization processes are also determined by the saturation of the ions in saliva and plaque, which can accumulate on the enamel surface when necessary, depending on this ion exchange, and the repair of the carious lesion is achieved by increasing

the calcium, phosphate and fluoride ion concentrations in the oral fluids (Kielbassa et al., 2009; Tschoppe et al., 2007).

2. Initial enamel lesions

Initial caries lesions are the first sign of caries formation on the enamel tissue. In these initial lesions, which are also called white spot lesions, cavitation has not yet occurred, and the textural integrity of the upper surface of the enamel has not been disturbed. They are defined as subsurface lesions characterized by mineral loss just under the enamel layer (Arends and Christoffersen, 1986). In the initial enamel lesion, 4 different layers are histologically observed. From the outer surface to the enamel dentin composition:

- Superficial layer
- Lesion body
- Dark layer
- Translucent layer (Çelik et al., 2011; Tetschke et al., 2020).

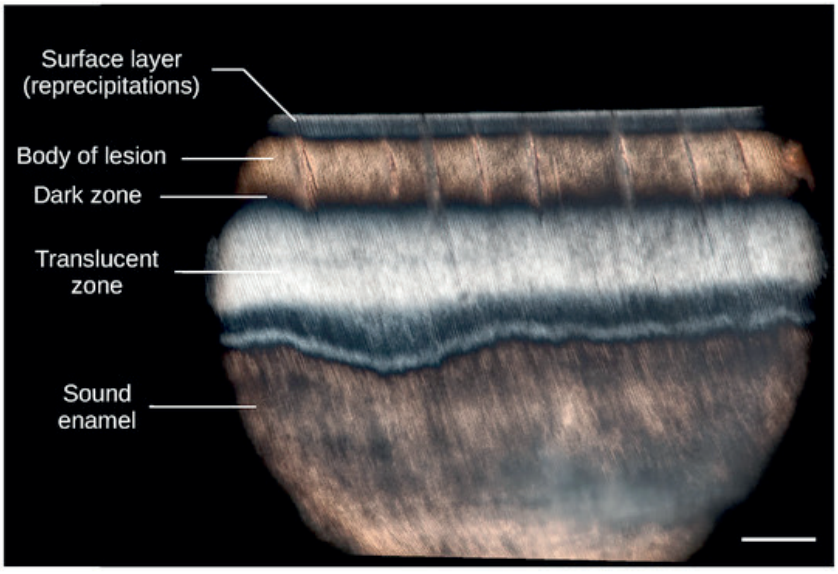

Figure 3: *Polarized light microscopy of an enamel with characteristic zones of lesion formation (Tetschke et al., 2020)*

Studies have reported that the superficial layer and the dark layer are formed as a result of remineralization, while the body of the lesion and the translucent layer are formed as a result of demineralization (Frank, 1990).

The superficial layer is the hypermineralized layer located at the outermost layer of enamel (Çelik et al., 2011). The body of the lesion, on the other hand, forms the center of the caries lesion and forms the layer with the highest demineralization (Çelik et al., 2011). Crystal dissolution center occurs in the dark layer region (Frank, 1990). In this layer, the pores remineralized and took the form of micropores (Çelik et al., 2011). The translucent layer at the bottom is the deepest layer where the caries lesion can progress and separates the healthy enamel tissue from the carious enamel tissue (Çelik et al., 2011).

Initial enamel caries is the earliest stage of dental caries formation and at this stage the caries lesion can be stopped or treated (Tetschke et al., 2020). In enamel lesions without cavities, the crystal structure that is not affected by demineralization in enamel prisms or is affected but not completely structurally intact acts as a nucleation center for remineralization. Calcium and phosphate ions in the saliva penetrate the enamel surface and accumulate on the highly reactive crystal surfaces within the lesion. The high amount of calcium and phosphate ions in the saliva strengthens the remineralization (Meyer-Lueckel et al., 2006)

3. Remineralization materials and classification

In today's dentistry, many methods are recommended and used to prevent the formation of non-cavitated caries lesions, strengthen the tooth structure, and provide aesthetics and function with non-invasive methods including remineralization (Gjorgievska et al., 2013; Zheng et al., 2013; Narayana et al., 2014). The remineralization agents used for this purpose should diffuse under the enamel surface, have the ability to release calcium and phosphate, and be effective at acidic pH levels (Rethman et al., 2011).

Classification of remineralization agents can be made in 5 main groups according to their properties and technologies (Sezer and Kargül, 2020) (Table 1).

a) Mineral and ion technologies

b) Sugar alcohols

c)Herbal products

d)Bioactive materials and nanotechnological products

e) Other calcium and phosphate products

Table 1: *Classification of remineralization agents*

Mineral and ion technologies	Sugar alcohols	Herbal products	Bioactive materials and nanotechnological products	Other calcium and phosphate products
Fluoride ion Silver ion Iron ion	Xylitol Isomalt Sorbitol	Chitosan Glycyrrhiza Glabra Galla Chinensis Grape Seed Extract (Polyphenols) Theobromine	Calcium Sodium Phosphosilicate/ Bioactive Glass Tricalcium Silicate Nanohydroxyapatite Casein Phosphopeptide Amorphous Calcium Phosphate Casein Phosphopeptide Amorphous Calcium Fluoride Phosphate Amorphous Calcium Phosphate Tricalcium Phosphate Self Assembling Peptides	Dicalcium Phosphate Dihydrate Calcium Phosphoryl Oligosaccharides Calcium Carbonate SodiumTrimetaphosphate Calcium Glycerophosphate

4. Current remineralization materials used in the treatment of initial enamel lesions

4.1. Fluoride

Fluoride applications are frequently used methods that have been proven to be effective in preventing dental caries and providing remineralization (Buzalaf et al., 2010). The presence of fluoride in the oral cavity causes a reservoir to form in plaque and saliva and binds to the enamel by slow release from the reservoir it forms. In this way, it takes part in the formation of remineralization by preventing demineralization by affecting the structure of the enamel (Tyagi et al., 2013).

The solubility of the hydroxyapatite structure of tooth enamel is high in the face of acid attacks, and therefore, it may show structural deterioration with the decrease in the pH value of the oral environment during feeding. As a result of fluoride applications, the fluoride ion that enters the hydroxyapatite structure of the enamel replaces the hydroxyl ion, resulting in the formation of fluoroapatite $Ca_{10}(PO_4)_6(F_2)$, a structure that is less soluble against acid attacks (Ten Cate, 2001; Featherstone, 2000). This newly formed crystal form exhibits a more resistant structure against acids (Lata et al., 2010).

Fluoride also prevents caries formation by affecting the activities of cariogenic bacteria. The fluoride ion binds to microorganisms that play a role in carbohydrate metabolism, prevents glucose breakdown and acts on bacteria by preventing the

conversion of sugar to acid. It also reduces adhesions to the enamel surface by lowering the surface energy of the enamel and changing the charge on the bacterial surface (Featherstone, 2000). Due to these mechanisms of action, the presence of fluoride in saliva and plaque is very important in preventing caries formation and ensuring remineralization of existing demineralized areas (Sezer and Kargul, 2020).

Fluoride treatments are of two types, systemic and topical, according to the way they are applied. Systemic fluoride applications are used to strengthen the structure during organic matrix formation and mineralization of enamel. Systemic fluoride applications; fluoridation of drinking water, adding fluoride to table salt, multi-vitamin fluoride combinations, fluoride-containing lozenges, drops and tablets (Ellwood and Fejerskov, 2003; Ercan et al., 2010).

The aim of topical fluoride applications is to create a caries preventive effect by providing enamel remineralization. Topical fluoride application methods include gels and solutions, prophylactic pastes, varnishes, fluoride-containing cements and restorative materials, mouthwashes, toothpastes, fluoride chewing gums, and dental floss (Ellwood and Fejerskov, 2003; Ercan et al., 2010; Featherstone, 2004). When fluorides are applied topically, they form a calcium fluoride (CaF_2)-like structure in plaque, tooth surface and initial caries lesions. This structure contributes to remineralization by serving as a reservoir for fluoride ion release during acid attacks (Ogaard ,1990).

4.2. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)

Casein is naturally present in foods such as milk, cheese, and yogurt, and is the most important of the phosphoproteins, which make up 80% of the proteins in cow's milk (Aimutis, 2004). There are many different types of casein protein, and the αs1, αs2 and β subtypes contain tryptic phosphopeptide bonds. These bonds allow calcium and phosphate to bind to casein. As a result of this important feature, it can stabilize calcium and phosphate as casein phosphopeptide-amorphous calcium phosphate nanocomplex (smaller peptides) (Salman et al. 2019).

The caries preventive effect of casein phosphopeptide amorphous calcium phosphate occurs through 3 different mechanisms. When CPP-ACP is exposed to the effect of acid attacks, ACP is released to the environment. Calcium and phosphate ions released into the environment, participate in the structure of the dental plaque, buffer the acidic environment of the plaque and balance the pH of the plaque (Çetin et al., 2011). This is a very important mechanism in the prevention of demineralization. The increased level of calcium phosphate in the plaque has a supporting effect on the activity of free calcium and phosphate ions. CPP-ACP, which is localized in the part of the plaque close to the tooth, also binds the free calcium and phosphate in the plaque, making the tooth surface supersaturated, thus preventing demineralization and increasing remineralization (Srinivasan et al., 2010; Sathyakumar et al., 2011). It also binds to the surfaces of bacterial cells

in plaque and prevents them from colonizing the tooth (Sudjalim et al., 2006; Ardu et al., 2007).

In a study examining the effect of gels and toothpastes containing CPP-ACP, tricalcium phosphate and fluoride on initial enamel caries in primary dentition, it was shown that gels containing CPP-ACP were more effective in remineralization of initial caries (Memarpour et al., 2015). In another study evaluating the effects of varnishes containing 5% NaF and products containing CPP-ACP on remineralization in primary teeth, it was found that the product containing 5% NaF and 2% CPP-ACP was significantly more effective in remineralization (Salman et al., 2019).

Today, CPP-ACP nano-complex is included in various products such as mouthwashes, chewing gums, lozenges, polishing pastes in order to benefit from its topical effect (Pai et al., 2008; Adebayo et al., 2009). GC Tooth Mousse is a cream product containing CPP-ACP, which has recently been recommended by dental professionals. This product is used as a caries preventive treatment in individuals with high caries risk, in the prevention and treatment of dental erosion, in the treatment of white spot lesions in orthodontic patients and in the removal of dentin sensitivity (Çetin et al., 2011).

4.3. Casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP)

Casein phosphopeptide amorphous calcium fluoride phosphate has a synergistic effect in preventing caries formation (Reema et al., 2014). Adding fluoride to products containing CPP-ACP contributes to remineralization by increasing the level of fluoride ions along with calcium and phosphate ions in the plaque (Li et al., 2014). The advantage of casein phosphopeptide amorphous calcium fluorophosphate is to have both calcium, phosphate and fluoride in one product at the same time (Reema et al., 2014).

CPP-ACFP is localized on tooth surfaces, can release calcium, phosphate, and fluoride during acidogenic exposure, and maintain a supersaturation of calcium and phosphate ions of the tooth surface (Cochrane et al., 2008). Thus, it can suppress the demineralization and increase the remineralization (Walsh, 2009).

The combination of CPP-ACP with fluoride improves the surface microhardness of enamel and its long-term use reduces the incidence of caries in children (Huang et al., 2013; Memarpour et al., 2015) GC MI Paste plus is a preparation that has been marketed to take advantage of the synergistic effect of fluoride and CPP-ACP. This preparation is frequently recommended and used in caries prophylaxis, as a preventive treatment in individuals at high caries risk, in the prevention of erosions, in the treatment of white spot lesions and in the treatment of dentin sensitivity (Azarpazhooh and Limeback, 2008).

4.4. Amorphous calcium phosphate (Enamelon)

Amorphous calcium phosphate (ACP) is a tricalcium phosphate with a molecular formula of [$Ca_3(PO_4)_2$-nH_2O]. ACP is not the basic structure of hard tissues. However, it has a special role as a precursor of bioapatite and constitutes a temporary phase for biomineralization. ACP contains Ca and P ions in an amorphous structure, and this combination reduces enamel demineralization during acid attacks (Azarpazhooh and Limeback, 2008; Cross et al., 2005). In this remineralization technology, calcium ions in the form of calcium sulfate and phosphate ions in the form of ammonium phosphate are applied separately to the oral environment, resulting in the formation of amorphous calcium phosphate (ACP) nanocomplex in the mouth. Acting as a calcium and phosphate ion reservoir on the tooth surface, ACP only comes into contact with saliva, resulting in the dissolution of calcium and phosphate salts, and calcium and phosphate ions are released into the environment (Tung and Eichmiller, 2004).

Although ACP technology is included in toothpastes (Enamelon TM, Enamel care), it has also been used as a whitening agent (Discus Dental's Nite White Bleaching Gel) of some companies, in pits and fissure sealants (Aegis) and polishing paste (Premier Dental's Enamel Pro Polishing Paste) (Goswami et al., 2012).

Enamelon™ toothpaste has been reported to be effective in reducing and repairing enamel white lesions and remineralizing erosions caused by soda (Pradeep and Rao, 2011).

4.5. Tricalcium phosphate

Calcium phosphate is the principal form of calcium found in bovine milk and blood (Li, 2014). As the main components of hydroxyapatite (HA) crystals, calcium and phosphate concentrations in saliva and plaque play an important role in influencing dental demineralization and remineralization processes (Rirattanapong et al., 2015).

When tricalcium phosphate comes into contact with the tooth surface and is wetted with saliva, it dissolves, releasing calcium, phosphate and fluoride ions. Calcium and fluoride then react on the weak enamel surface to form nuclei to increase mineral growth (Karlinsey et al., 2010a).

Studies have shown that the combination of TCP with fluoride can provide greater enamel remineralization and form a more acid-resistant mineral than fluoride alone (Li, 2014). When used in toothpaste formulations, it forms a protective barrier around calcium, allowing calcium to be combined with fluoride ions. During brushing, TCP comes in contact with saliva, causing the barrier to dissolve and release calcium, phosphate and fluoride (Hemagaran, 2014).

There are two forms of tricalcium phosphate (TCP). These are: alpha-tricalcium phosphate (α-TCP) and beta-tricalciumphosphate (β-TCP). β-TCP is a rare form of calcium phosphate and is the precursor of hydroxyapatite formation (Karlinsey et al., 2010b).

The precursor of β-TCP in hydroxyapatite formation is due to its being a bioactive source with an important calcium-phosphate system and mineralizing components (Ekambaram et al., 2017). Modification of the β-TCP crystalline system (reaction with sodium lauryl sulfate) yields functionalized β-tricalcium phosphate (fTCP). As a result of this reaction, a hybrid layer containing organic Ca-PO_4 is formed. The resulting hybrid layer prevents undesirable interactions between calcium and fluoride (Ekambaram et al., 2017; Karlinsey & Pfarrer, 2012). When necessary, it contributes to remineralization by releasing Ca and PO_4^{-3} ions to the tooth tissue in the face of acid attacks (Rehder et al., 2009).

Clinpro Tooth Creme has been marketed as a β-TCP-containing product and has been reported to be more effective than other CPP-ACP-containing agents in healing white spot lesions (Jo et al., 2014).

4.6. Nanohydroxyapatite

Nanohydroxyapatite is a bioactive and biocompatible agent consisting of nano-sized hydroxyapatite particles. Nano-sized hydroxyapatites can penetrate into the enamel tissue, form a remineralization layer on the enamel surface, and protect the tooth tissue against acid attacks. Nanohydroxyapatite increases surface hardness and is defined as the least soluble agent compared to other calcium phosphate compounds.

The caries prevention potential of hydroxyapatite has been shown to be based on multiple mechanisms (Schlagenhauf et al., 2019). It has been reported that hydroxyapatite increases the calcium and phosphate ion concentrations in saliva, plaque and tooth surfaces, and thus acts as a calcium and phosphate reservoir, supporting the local saturation of these ions (Schafer et al., 2009, Huang et al., 2011). It has been reported that the high potential of hydroxyapatite to be adsorbed on the bacterial cell wall causes an antibiofilm effect by inducing coagulation of bacteria within the hydroxyapatite particles, thus preventing oral biofilm formation (Kensche et al., 2017, Palmieri et al., 2013). In addition, it has been shown that hydroxyapatite taken from the outside binds tightly to the enamel and dentin surface, forming layers that can protect the tooth surface from erosion and acid attacks, and additionally acts as a reservoir for the continuous release of calcium and phosphate ions (Fabritius-Vilpoux et al., 2019).

In a study investigating the effect of nano-hydroxyapatite concentrations on initial enamel lesions under varying pH conditions, nano-hydroxyapatite

has been reported to have the potential to remineralize initial enamel lesions (Goswami et al., 2012).

Nanohydroxyapatite, which is known to have high positive properties, has been added to oral care products such as toothpastes and mouthwashes in order to reduce tooth sensitivity by blocking open dentinal tubules, and to increase enamel remineralization (Vano et al., 2014; Vano et al., 2018; Jena et al., 2017).

4.7. Calcium sodium phosphosilicate (Novamin)

Bioactive glass, defined as calcium sodium phosphosilicate, can be used as anti-caries agents in preventive dentistry (Kes and Başeren, 2022). Its most important feature is the formation of a "hydroxycarbonapatite" layer that provides bond formation between the surface and the tissues. Thanks to these properties, bioactive glasses can be chemically bonded to hard and soft tissues (Ceyhan et al., 2007).

As a result of the contact of the nanoparticles in the material with saliva, calcium, sodium and phosphate are released. These materials with high bioactive properties are used in the treatment of dentin hypersensitivity and to increase enamel remineralization (Cury and Tenuta, 2009).

NovaMin is one of the materials developed in the bioactive glass technology containing calcium sodium phosphosilicate. NovaMin, like other remineralizing agents, aims to protect the tooth in a conservative way (Burwell et al., 2010). The remineralization effect occurs by dissolving in the oral environment and increasing the pH. At rising pH, calcium and phosphate ions are released by hydrolysis reaction and provide a substrate for mineralization (Dai et al., 2020).

It is stated that they create successful results in toothpastes due to their high biocompatibility and remineralization properties. Bruwell et al. reported that toothpaste containing bioactive glass (calcium sodium phosphosilicate) protects the dentin from demineralization against repetitive acidic and mechanical changes by forming a strong layer, and when calcium sodium phosphosilicate is added to a fluoride-containing toothpaste, the paste hardens white opaque lesions (Burwell et al., 2009). In a study by Job et al., the effects of NovaMin®-containing toothpastes and CPP-ACP and NaF-containing toothpastes were compared, and it was reported that NovaMin®-containing toothpastes provide a higher level of remineralization (Job et al., 2018).

4.8. P11-4 peptide

Peptide monomers applied to the initial caries lesions for biomimetic remineralization form a three-dimensional enamel matrix, and hydroxyapatite crystals are formed by biomineralization 'biomimetic remineralization' with

the support of calcium phosphate from saliva (Yang et al., 2014). The formation of regeneration of hydroxyapatite crystals in the lesion body of subsurface caries is highlighted as an important advantage of the strategy of biomimetic remineralization (Brunton et al., 2013)..

The self-assembled peptide P_{11}-4 is able to form a 3D biomimetic scaffold structure within the subsurface body of the initial enamel caries lesion, controlling the precipitation and growth of hydroxyapatite crystals (Dawasaz et al., 2022). The success of P_{11}-4, which can mimic biological macromolecules such as enamel matrix molecules, in enamel remineralization has been reported as a promising agent in clinical studies (Akal, 2021).

It has been stated that the P_{11}-4 peptide creates an enamel texture similar to the natural structure in subsurface enamel remineralization (Kind et al., 2017). In a study investigating the effect of P_{11}-4 peptide on enamel remineralization with other remineralization agents, it was stated that P_{11}-4 peptide showed higher remineralization ability than CPP-ACFP and NAF (Üstün and Aktören, 2019).

Self-assembled peptide P_{11}-4 is a recommended therapeutic approach for the remineralization of tooth enamel and for the treatment of cavitation-free early carious lesions using a fluoride-free biomimetic regeneration system (Shetty and Nekkanti, 2023).

The self-assembled peptide P_{11}-4, marketed as Curodont™ Repair, is used in the regenerative biomimetic remineralization of early enamel lesions. For children and adolescents with early caries, this technique is reported as a safe and effective preventive and less intrusive treatment (Kind et al., 2017).

5. Commercial current remineralization agents

There are many materials developed in recent years in addition to fluoride applications, which have been accepted as the gold standard until today, in order to prevent demineralization in dental hard tissues and to remineralize demineralized areas. Although research and studies on the use of these biocompatible caries preventive and remineralizing agents, which are offered for individual and professional applications, are continuing, there are many new agents available in the market. Some of these agents are shown in the Table 2. Studies and researches on remineralization agents are very important, especially in minimal intervention dentistry, and are promising in the treatment of initial caries.

Table 2: Current remineralization agents

Remineralization material	Classification	Product type	Manufacturer
Tooth Mousse	Casein phosphopeptide-amorphous calcium phosphate	Topical gel	GC Corp., Japan
Recaldent	Casein phosphopeptide-amorphous calcium phosphate	Chewing gum	GC Corp., Japan
Trident White	Casein phosphopeptide-amorphous calcium phosphate	Chewing gum	Cadbury Adams USA
Recaldent Mints	Casein phosphopeptide-amorphous calcium phosphate	Dragee	GC Corp., Japan
MI Paste	Casein phosphopeptide-amorphous calcium phosphate	Topical gel	GC Corp., Japan
MI Paste plus	Casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP)	Topical gel	GC Corp., Japan
Tooth Mousse Plus	Casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP)	Topical gel	GC Corp., Japan
Enamelon	Amorphous calcium phosphate	Toothpaste	Cranbury, NJ, USA
Clinpro Tooth Creme	Tricalcium phosphate	Toothpaste	3M ESPE, Saint Paul, MN, USA
Apagard	Nanohidroksiapatit	Toothpaste	Sangi Co., Ltd, Tokyo, Japan
Oravive	Calcium sodium phosphosilicate	Toothpaste	Glaxo Smith Kline, Weybridge, Surey, UK
Novamin	Calcium sodium phosphosilicate	Toothpaste	Glaxo Smith Kline, Weybridge, Surey, UK
Curodont Repair	Self Assembling Peptides	Topical gel	Credentis AG, Switzerland

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Chapter 15

NATURAL NANOANTIOXIDANT SYSTEMS: RECENT DEVELOPMENTS AND FUTURE PROSPECTS

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1. INTRODUCTION

Free radicals are unstable molecules that can damage cells and tissues through a process called oxidative stress (Santos-Sánchez, et al., 2019). Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify or repair the damage caused by these ROS. However, excessive ROS production can lead to cellular damage, including oxidative damage to DNA and contribute to the development of various diseases such as cancer, cardiovascular diseases, neurodegenerative disorders and diabetes (Gupta et al., 2015; Tijjani et al., 2020). Antioxidants play a crucial role in preventing and combating the damaging effects of ROS. They work by neutralizing ROS, thus reducing oxidative stress and its detrimental effects on the body (Khalil et al., 2020).

In the body, there exists a natural equilibrium between the production of free radicals and the presence of antioxidants, which serve to safeguard the body against harmful effects. However, the quantity of antioxidant compounds available under normal physiological conditions may often be inadequate to counteract the generation of free radicals. Consequently, the food industry and preventive medicine have shown a growing interest in the creation of "natural antioxidants" derived from plant materials (Nantz et al., 2006).

Nanoantioxidants are modified forms of antioxidants encapsulated with nanomaterials to provide altered pharmacokinetic and tissue distribution properties. In addition, nanoantioxidants improve the cellular penetration and distribution of the targeted compound (Sanjay et al., 2021). Recent scientific research has evaluated the potential of plant-derived nanooxidants to increase antioxidant activity and combat oxidative stress associated with health problems such as cancer and heart disease. Some of the key findings from these studies include: Natural nanoantioxidant delivery systems refer to delivery systems that utilize plant-based bioactive compounds as the main components. These bioactive compounds possess effective oxidative stress-neutralizing properties and exhibit similarities in addressing chronic inflammation (Akbari et al., 2022).

In this chapter, the aim of the first section is to provide a comprehensive overview of antioxidant activity, laying the foundation for the subsequent discussion on plant-derived nanooxidant systems and their efficient and enhanced antioxidant activities. The second section summarizes the plant derived natural compounds and extraction methods. The third chapter provides information about the importance of nanoencapsulation for antioxidant. This section is splitted into three subsections that intend to highlight the use of antioxidant methods, spectroscopic, electrochemical and biosensor technic. Finally, the fourth section is the nanophytoantioxidant studies and information about the antioxidan activity.

2. PLANT-DERIVED NATURAL COMPOUNDS

Plant-derived natural compounds are bioactive molecules that are extracted from various parts of plants, such as leaves, flowers, roots, stems, and fruits (Figure 1). These compounds have been used in traditional medicine systems for centuries and have therapeutic properties due to antioxidant activity (Abdin et al., 2007).

The antioxidant effects of herbal products are mainly due to the phenolic compounds. A phenolic molecule is often characteristic of a plant species or even of a particular organ or tissue of that plant Polyphenols, mainly flavonoids, are secondary plant metabolites contained in fruits and vegetables. They can be divided into several classes according to the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and flavanones (Teng et al., 2023).

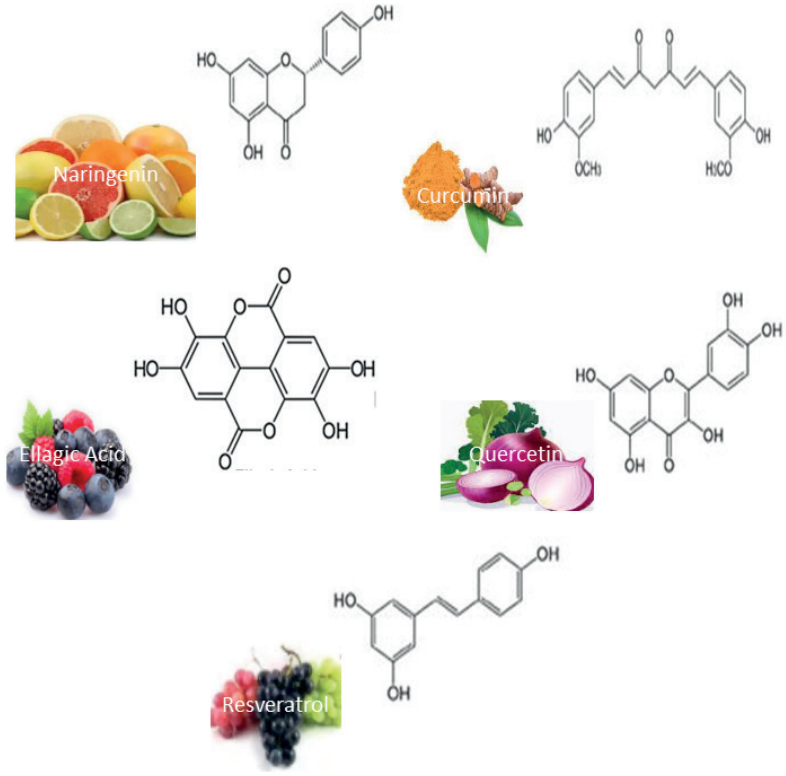

Figure 1: Chemical structure of some polyphenolic medicinal plants

2.1. Extraction Methods and Their Applications for Extracting Phytochemicals from Plants

Two types of extraction methods are used to obtain bioactive compounds from plants: *conventional*, which uses simple and low-cost equipment, large amounts of solvent, and extended extraction times working at atmospheric pressure and at relatively higher temperatures, and *nonconventional*, which are modern, ecological, and use more expensive. Equipment save extraction time and generally can work at higher pressure and temperature values (Chibuye et al., 2023. Ingle et al., 2017; Rodríguez De Luna, et al., 2020).

Maceration is a commonly used cnventional extraction method in which the crude extract is placed in a container containing a solvent. During the process, the polyphenolic components are dissolved in the solvent. After the desired extraction time is complete, the mixture is filtered to separate the liquid extract from the solid residue and finally the combined liquids are clarified by filtration or decantation to remove particulate matter or impurities after a settling time.

Soxhlet extraction is a method used for the extraction of plant based compounds. The crude substance in the thimble shaped filter paper, positioned into the Soxhlet extractor, and the device is assembled. The solvent is added to the solvent reservoir flask and mounted onto a heating mantle. After heating, the condensed vapors of the solvent come in contact with the sample powder, and the soluble part of the powder gets mixed with the solvent for extraction. When the solvent surface exceeds the maximum height of the siphon, the solvent containing the extract is siphoned back. The process is repeated until the extraction is complete and the desired compounds are efficiently extracted from the sample.

Microwave-assisted extraction is an alternative method commonly used for extracting active components from medicinal plants. It involves the application of microwave radiation which enhances the evaporation of raw material residual water and eventually breaks the plant cell walls to promote the extraction through internal diffusion.

Ultrasound extraction is a non-conventional method that uses ultrasound waves with frequencies typically ranging from 20 kHz to 2000 kHz to facilitate the extraction process. The use of ultrasound waves during extraction disrupts the cell structure, allowing for the release of intracellular compounds into the extraction solvent induce the plant material to brake facilitating the release of the extracts.

Supercritical fluid extraction is a sample preparation method designed to minimize the use of organic solvents and enhance the efficiency of sample processing. Supercritical fluid is used to extract the target compounds from

the mixture. It refers to a substance that is above its critical temperature and pressure, as the extracting medium to isolate analytes from a sample.

3. DETERMINATION OF ANTIOXIDANT ACTIVITY

Several instrumental methods utilize to measure antioxidant activity, including spectrometry, electrochemical assays, chromatography, and biosensor techniques (Maqsoudlou et al. 2020; Moharram, et al., 2014). When screening the antioxidant properties of plant-derived compounds and plants, it is crucial to employ suitable methods that investigate the mechanism of antioxidant activity and consider the kinetics of the reactions involving antioxidants (Gulcin 2020). Obtaining accurate conclusions from the results requires a clear understanding of the underlying mechanisms employed in these assays to avoid misleading interpretations (Munteanuet al., 2021).

3.1. Electrochemical Techniques

Electrochemical methods involve the application of an electrical potential to a working electrode in the presence of an antioxidant (Povlich, et al., 2017). These methods based on differential pulse, cyclic, square wave voltammetry and coulometry allow direct and rapid screening of antioxidant activity.

3.2. Spectroscopic Techniques

Antioxidant capacity determination methods, in terms of the chemical reaction used. It can be grouped into two classes as the hydrogen atom transfer reaction (HAT) and single electron transfer reactions (ET). Most of the analysis methods based on the HAT reaction are the degradation of azo compounds. Peroxyl radicals formed as a result of a competitive action by the antioxidant and the substrate based on the principle of elimination. HAT analysis methods can be listed as: Induced low-density lipoprotein autoxidation, Oxygen radical absorbance capacity (ORAC) Total radical scavenging antioxidant capacity (TRAP) and Crocin bleaching experiments.

ET-based analysis methods show that the antioxidant substance changes color when reduced. It is based on the measurement of the capacity to reduce an oxidant substance color change degree is correlated with the antioxidant concentration in the plants and plant-based foods sample (Sign et al, 2008; Koksal et al., 2009).

ET based analysis methods:

a) **Total phenolic substance analysis with Folin-Ciocalteu reagent (FCR):** This method is commonly employed to determine the overall polyphenol content in plant samples. In this technique, the FCR reacts with phenolic compounds, causing the reduction of Mo^{6+} to Mo^{5+}. The reduced form, which is blue, can be quantitatively measured optically at a wavelength of 730 nm.

b) Trolox equivalent antioxidant capacity (TEAC) measurement: This assay evaluates the antioxidant potential of compounds by measuring their ability to scavenge the stable radical cation $ABTS^{+}$ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)). $ABTS^{+}$ is a blue-green chromophore with its maximum absorption occurring at 734 nm. The assay quantifies the capacity of antioxidants to neutralize $ABTS^{+}$ and provides a measure of their antioxidant activity, often expressed in terms of Trolox equivalents.

c) Measurement of ferric ion- reducing antioxidant power (FRAP): This assay measures the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} ions. The antioxidant activity is measured by the increase in the absorbance of Fe^{2+} at 593 nm.

d) Total antioxidant potential measurement method using DPPH: (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is a spectrophotometric antioxidant determination method based on electron transfer. At room temperature condition, this free radical is reduced in the presence of an antioxidant molecule, resulting in a colorless ethanol solution.

e) CUPRAC (Copper(II) Reducing Antioxidant Capacity) Method: Using Cu(II)-Using the neocuproine reagent, plasma antioxidants, flavonoids, food polyphenols, a simple, broadly applicable antioxidant capacity determination of polyphenol has been developed (Apak et al., 2008). In this method, the reduction of Cu^{2+} in the presence of neocuproine, facilitated by a reducing agent, forms a Cu^{+} complex. This complex exhibits a maximum absorption peak at 450 nm, which can be measured to assess the antioxidant capacity of the sample.

3.2. Biosensors

Biosensors are analytical devices that use biological components, such as enzymes, antibodies, or living cells, to detect and quantify the presence of a target molecule or activity in a sample. Biosensors can also be used to measure antioxidant activity by incorporating antioxidants or free radicals as the target molecule in the biosensor. There are several types of biosensors that can be used to measure antioxidant activity, including; Enzyme-based biosensors, Antibody-based biosensors, living cell-based biosensors (Munteanu et al., 2022; Prieto-Simón et al., 2008; Mello et al., 2006; Singh et al., 2021).

4. IMPORTANCE OF NANOENCAPSULATION FOR ANTIOXIDANT

When phenolic compounds are exposed to high temperatures, oxidative conditions, and intense light, their antioxidant activity decreases. Encapsulation technology serves as a viable approach to ensure the effective distribution of antioxidant components in food and pharmaceutical products (Figure 2). This technology also facilitates the controlled release of antioxidant

components, thereby extending the shelf life of foods and improving their bioavailability after ingestion in the gut (Maqsoudlou et al., 2020; Magne et al., 2022).

Figure 1: How to increase phytoantioxidant activity?

4.1. Nano-Phytoantioxidants:

Significant advances have been made in the field of nanotechnology to improve the delivery of phytochemicals with advancing technology. These benefits include improved solubility and stability in the body, enhanced absorption in the gastrointestinal tract, protection against premature enzymatic degradation and metabolism, prolonged circulation time, and reduced side effects. In addition, research shows that nano delivery systems containing phytochemicals have the potential to modulate oxidative stress and inflammation. In recent years, studies have been conducted showing the synergistic effects of plant extract-loaded nanocarriers in the fight against diseases such as cardio-metabolic and neurodegenerative disorders, rheumatoid arthritis and osteoporosis (Vaiserman e al., 2021).

One example of plant extract loaded nanofibers with antioxidant activity is green tea extract-loaded electrospun nanofibers. Green tea extract is rich in polyphenols, particularly catechins, which have potent antioxidant activity. Electrospinning is a technique used to produce nanofibers, and it has been used to encapsulate green tea extract into nanofibers. The resulting nanofibers have been shown to have high antioxidant activity, with potential applications in wound healing and tissue engineering (Sadri et al., 2015; Himadri et al.,

2022). Also, Pusporini et all reported the size of nanoantioxidants, such as in the case of PVP /green tea extract composite nanofiber mat, can impact their antioxidant activity. When the average fiber diameter of a composite nanofiber mat is reduced, it results in an increase in the surface area of the fibers. This increased surface area allows for a higher exposure of the composite material to the surrounding environment. The smaller fiber diameter not only contributes to an increased surface area but also improves the accessibility and efficiency of antioxidant molecules in the composite (Pusporini et al., 2018). In addition to green tea extract other plant extracts such as grape seed extract, pomegranate extract, and aloe vera extract have also been used to produce antioxidant-loaded nanofibers (Leyva-Porras et al., 2021). Natural nanoantioxidant delivery systems often utilize plant-based bioactive compounds that possess effective oxidative stress- neutralizing properties and can help modulate chronic inflammation. For this, the application of nanotechnology to herbal antioxidants has the potential to improve the efficacy and delivery of phytomedicine (Salehi et al., 2019).

Most phytochemicals, including herbal antioxidants have been extensively studied for their health benefits, including their antioxidant, anti-inflammatory, and anticancer properties (Pereira & Cotas, 2023). However, the poor solubility and low bioavailability of these compounds pose challenges to their effectiveness in the body. In particular, the acidic environment of the stomach can further degrade these compounds and prevent their absorption. Therefore, absorption occurs in the small intestine. However, many herbal antioxidants have limited solubility in the aqueous medium of the small intestine, resulting in poor absorption. To overcome these, antioxidants have been turned into their nanoencapsulation forms such as nanoparticles, nanofibers or micelles. Thus, they increased the stability of herbal antioxidants and their absorption in the gastrointestinal tract (Schulz et al., 2016).

4.1.1. Curcumin

Curcumin a bioactive compound found in turmeric, has antioxidant and anti-inflammatory properties (Gera et al, 2017; Pizzo et al, 2010; Saghir et al, 2020; Al-Dossari et al, 2020). However, its effectiveness is limited due to its low solubility in water, rapid metabolic activity and rapid excretion from the body. In response to these limitations, researchers have explored the use of curcumin nanoparticles (Bisson et al., 2007; Sheikh et al., 2017; Dong et al., 2020; Karnawat & Tukur, 2021; Ganugula et al., 2017; Ranjbar et al., 2019, Hanna, & Saad, 2020; Yadav et al., 2012).

4.1.2. Naringenin

Naringenin is an antioxidant, anti-inflammatory, anticancer and cardioprotective polyphenolic phytochemical (Kumar et al., 2015). However, since its hydrophobic nature tends to exhibit low solubility and absorption

in the aqueous environment of the gastrointestinal tract, decreased efficacy is observed (Bhia et al., 2021). Shadab et al. applied the neuroprotective and antioxidant properties of naringenin to the treatment of Parkinson's (Shadab et al., 2019). Also, it was reported Naringenin has antiviral and anti-inflammatory effects (Tunchiand et al., 2020).

Naringenin-loaded nanostructures, on the other hand, are a promising area of research to increase the bioavailability and therapeutic potential of naringenin. These structures can increase the solubility and stability of naringenin by increasing its absorption and distribution in the body (Fuster et al., 2020; Parasharand et al., 2018; Chaurasia et al., 2020; George et al., 2020). Such that naringenin has shown that charged nanostructures exhibit improved bioavailability and enhanced pharmacological activity compared to free naringenin (Smruthi et al., 2022). The development of a naringenin-loaded nanoemulsion is an interesting approach to improve the bioavailability of naringenin, especially for the treatment of cerebral ischemia (Ahmad et al., 2018).

4.1.3. Ellagic acid

Ellagic acid is a polyphenol that occurs naturally in a variety of fruits and vegetables. It offers several health benefits attributed to its antioxidant, anti-inflammatory, and anticancer properties. Ellagic acid helps reduce oxidative stress and protect cells from damage. Moreover, it exhibits anti-inflammatory effects by inhibiting the expression of pro-inflammatory cytokines and enzymes. Ellagic acid is insoluble in water, therefore, due to its metabolites, its bioavailability is low and is rapidly eliminated. Since most clinical drugs are given orally and their therapeutic advantage is limited, their bioavailability has been increased by converting them into nanoformulation form. Ellagic acid has garnered interest as a promising natural nanoantioxidant compound, holding potential for various health benefits (Yaşar et al., 2022; EL Barky, & Ali, 2020).

4.1.4. Quercetin

Quercetin is a flavonoid, a type of plant pigment, that is known for its strong antioxidant and anti-inflammatory properties, as well as its potential therapeutic effects on various health conditions (Boots et al. 2008). Quercetin, being a hydrophobic compound, exhibits very poor solubility in water. Moreover, it undergoes rapid metabolism within the human body, leading to low circulation time and concentrations. The utilization of biocompatible and biodegradable nanoparticles can effectively address these challenges, enhancing the bioactivities of quercetin while reducing its toxicity and side effects (Wang & Zhang, 2015).

According to Kaya and Derman Quercetin PLGA-nanofiber has high antioxidant activity and may scavenge free radicals that delay healing in the wound area (Kaya and Derman 2022). Also, the researcher reported thatt heat treatment blended nanofiber films to improve water resistance while preserving antioxidant activity is an interesting finding (Li et al., 2020).

4.1.5. Resveratrol

Resveratrol has been found to have potent antioxidant effects, as it can scavenge free radicals and reactive oxygen species, thereby reducing oxidative stress and preventing cellular damage (Kim et al., 2016). It has also been found to have anti-inflammatory effects by inhibiting the production of pro-inflammatory enzymes (Karakaya, et al. 2019). Resveratrol has demonstrated significant anticancer effects against various types of cancers. Pharmacological effects, resveratrol faces certain physiological and pharmacokinetic constraints. In spite of its pharmacological effects, resveratrol faces certain physiological and pharmacokinetic constraints. Bioavailability, poor solubility, and various other factors that restrict its potential effect. To overcome these challenges, researchers are focusing on developing nanotechnology-based drug delivery systems to enhance the stability of resveratrol (Houacine & Singh, 2018; Balasubramanian & Girigoswami, 2023; Rostami et al., 2019).

In addition, nanotechnology has played a crucial role in increasing the synergistic effect in the treatment by loading two or more phytoantioxidants together A review of several studies shows that co-loaded phytoantioxidant nanoformulations can enhance therapeutic benefits by improving bioavailability and pharmacokinetics, which in turn improves binding, internalization (Haghi., et al., 2017; Shabbir, et al., 2021; Mansourizadeh et al., 2020; Ghayour, Eskandari, Esteghlal, Nekoei, Gahruie,… & Naghibalhossaini, F. 2019; Kuo, et al., 2020; Sheik & Huh 2022; Ouni et al., 2021).

5. FUTURE PROSPECTS AND CONCLUSION

In conclusion, nanoantioxidant systems hold promise for humanity. The creation of new drug systems loaded with plant extracts to control effective dose and release timing by designing biocompatible natural materials with contributions from various disciplines such as polymer chemistry, materials science, biology and pharmaceutical absorption are techniques to increase the antioxidant properties of phytochemicals.

It is also highly desirable to support clinical trials. Plant-derived nanooxidants may exhibit high antioxidant activity due to their penetration into cell membranes and scavenging free radicals more effectively than conventional antioxidants. Plant-derived nanooxidants can be synthesized using environmentally friendly methods that reduce their environmental impact and increase their sustainability. With the nanoformulation of

antioxidants, stability increased half-life, improved pharmacokinetics, improved tissue targeting, increased bioavailability by minimizing side effects. However, about the biochemical processes in the human body, nanoantioxidant structures.

In addition, appropriate characterization techniques and standardized experiments are required to evaluate the antioxidant capacity, stability and other relevant properties of nanoantioxidant formulations. Therefore, in order to maximize the potential benefits of nanoformulations for antioxidants, ongoing research to better understand the effects of nanoparticles on biochemical pathways is crucial.

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Chapter 16

THE MANAGEMENT OF HELICOBACTER PYLORI INFECTION INVOLVES A COMBINATION OF PHARMACOLOGICAL THERAPY AND LIFESTYLE MODIFICATIONS

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Introduction

Helicobacter pylori (H. pylori), a spiral-shaped gram-negative bacterium, is recognized as one of the most prevalent causes of chronic bacterial infections worldwide. H. pylori infection has been extensively linked to the etiology of various conditions, including chronic gastritis, peptic ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma (1). In 2020 alone, the global burden of H. pylori-related diseases resulted in over 1 million new cases of gastric cancer and nearly 800,000 deaths, making it the third leading cause of cancer-related mortality worldwide (2).

H. pylori infection is classified as an infectious disease, and it is recommended to initiate treatment irrespective of symptoms to prevent serious complications and transmission (3). However, the emergence of antibiotic-resistant strains of H. pylori has become a significant global threat, significantly impacting treatment outcomes. Resistance rates to antibiotics such as clarithromycin, metronidazole, and levofloxacin have exceeded 15% in almost all World Health Organization (WHO) regions (4). Consequently, clinical guidelines suggest different first-line treatment options based on regional patterns of antimicrobial resistance (5–7). Successful eradication of H. pylori depends on appropriate antibiotic selection, adequate acid suppression, and patient adherence to therapy. Genetic polymorphisms in the host's CYP2C19 enzyme have been associated with reduced effectiveness of certain proton pump inhibitors (PPIs). As a result, second-generation PPIs like rabeprazole and esomeprazole, which are less affected by CYP2C19, are recommended. Additionally, novel potent acid blockers such as vonoprazan, unaffected by CYP2C19, have been introduced (9).

This review aims to provide an overview of the prevalence, disease spectrum, diagnostic tests, and treatment options for H. pylori infection, considering the latest global antibiotic resistance patterns. It includes recommendations for first-line therapy, second-line therapy, and cases of multiple treatment failures.

Prevalence of H. pylori infection

The global prevalence of H. pylori infection exhibits wide variations, ranging from 18.9% to 87.7%. In 2015, an estimated 4.4 billion individuals worldwide were infected with H. pylori. Regions with the highest prevalence include Africa (70.1%) and South America (69.4%), while Oceania (24.4%) and Western Europe (34.3%) have the lowest prevalence (10). Notably, Nigeria had the highest prevalence at 87.7%, while Switzerland had the lowest at 18.9% (10). The burden of gastric cancer associated with H. pylori infection is particularly high in Asia, where over 2 billion H. pylori-positive individuals and three-quarters of global gastric cancer cases are concentrated. Among Asian countries, Mongolia and Japan have the highest age-standardized incidence

rates of gastric cancer (11). It is interesting to note that Africa has high H. pylori prevalence but low incidence of gastric cancer, which is often attributed to limited healthcare resources in many parts of the continent (11). The prevalence of H. pylori infection also varies among countries in the Association of Southeast Asian Nations (ASEAN), with Vietnam, Myanmar, Laos, and Thailand exhibiting varying prevalence rates (12). Factors influencing the prevalence of H. pylori infection include age, ethnicity, and socioeconomic status. The routes of transmission can be fecal-oral, oral-oral, or gastric-oral (14). Children acquire H. pylori infection at a young age, with the mean age of acquisition reported as 32.78 months (15). Intrafamilial clustering suggests person-to-person transmission or exposure to a common contaminated source (16). The prevalence of H. pylori infection gradually increases with age, varying between different ethnic groups and socioeconomic classes (17-19). Asian countries generally exhibit higher prevalence compared to Western countries, which may be attributed to lower socioeconomic status in many Asian nations (10, 20).

H. pylori and the Gastric Microbiome

The human stomach has lower microbial density compared to the colon due to its highly acidic environment (21). The gastric microbiota primarily consists of dominant phyla such as Actinobacteria (genus Bifidobacterium), Bacteroidetes (genus Prevotella), Firmicutes (genera Lactobacillus, Streptococcus, Clostridium, Veillonella), Fusobacteria, and Proteobacteria (22). Notable genera include Streptococcus, Prevotella, Neisseria, Hemophilus, Fusobacterium, and Veillonella (22). Recent studies have demonstrated strong co-exclusion interactions between H. pylori and other bacteria such as Fusobacterium, Neisseria, Prevotella, Veillonella, and Rothia, particularly in patients with advanced gastric lesions (23). H. pylori infection can lead to gastric dysbiosis, reducing microbial diversity. Successful eradication of H. pylori can restore the gastric microbiota to a state resembling that of H. pylori-negative individuals, including an increased abundance of Bifidobacterium and hypothetical downregulation of drug-resistance mechanisms (23). Dysbiotic gastric microbiota profiles, decreased H. pylori abundance, and an enrichment of oral or intestinal bacteria have been observed in patients with gastric cancer (24).

Disease Spectrum of H. pylori Infection

H. pylori infection presents a broad spectrum of clinical manifestations, including gastrointestinal diseases and extragastric manifestations.

Gastrointestinal diseases

Dyspepsia: Young dyspeptic patients without alarm symptoms and with no risk of gastric cancer are advised to undergo noninvasive testing for H.

pylori infection, while older patients should undergo upper gastrointestinal (GI) endoscopy due to the increased risk of gastric cancer. The age cutoffs for performing endoscopy vary between countries based on the prevalence of gastric cancer. For high-prevalence countries such as China, Korea, Japan, and Taiwan, the optimal cutoff is 40 years, while for intermediate or low-prevalence countries such as India, Malaysia, Singapore, Thailand, Africa, North America, and Western Europe, the cutoff is 45 or 50 years. In Vietnam, the age cutoffs are even lower, set at 30 years for women and 35 years for men, in order to detect 98.2% of upper GI malignancies. Gastric biopsies should be obtained in dyspeptic patients undergoing gastroscopy as H. pylori can be detected even in visibly normal gastric mucosa. Eradication of H. pylori is recommended for symptomatic relief in dyspepsia (28).

Gastritis: Persistent H. pylori infection leads to chronic gastritis of varying severity. H. pylori typically causes antral-predominant gastritis, with continued inflammation resulting in parietal cell destruction and decreased acid secretion. Atrophic gastritis and intestinal metaplasia, precancerous lesions, can subsequently develop.

Peptic ulcer disease: H. pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) are common causes of peptic ulcers. Testing for H. pylori infection is recommended in patients with active or past history of peptic ulcer disease. The prevalence of H. pylori infection in duodenal ulcers ranges from 90% to 100%, and in gastric ulcers, it ranges from 60% to 100%. However, there has been a decreasing trend of H. pylori infection in patients with peptic ulcer disease in the United States (30).

Gastric cancer: H. pylori can progress chronic gastritis to atrophic gastritis, intestinal metaplasia, dysplasia, and eventually adenocarcinoma through Correa's precancerous cascade. H. pylori-induced chronic gastritis leads to increased epithelial cell turnover, reactive oxygen species, DNA damage, and microRNA dysregulation. Furthermore, H. pylori can induce DNA methylation changes, causing epigenetic aberrations in gastric epithelial cells. Promoter hypermethylation of E-cadherin, a tumor suppressor gene, is associated with gastric cancer development. Certain factors, such as gender, race, ethnicity, and smoking, are associated with a higher risk of gastric cancer in H. pylori-infected individuals. A family history of gastric cancer in first-degree relatives is also linked to an increased risk. Eradication of H. pylori not only decreases the risk of gastric cancer development but also reduces the progression to gastric dysplasia. Testing for H. pylori is recommended in patients with a history of endoscopic resection of early gastric cancer (37).

Mucosa-associated lymphoid tissue (MALT) lymphoma: Over 90% of MALT lymphomas are associated with H. pylori infection. The pathogenesis of MALT lymphoma may involve direct antigenic stimulation of B-cell prolif-

eration by H. pylori. Eradication of H. pylori is recommended as it can induce complete remission in a significant proportion of patients with low-grade gastric MALT lymphomas. Even in H. pylori-negative cases, eradication therapy has shown positive outcomes (40).

These findings provide insights into the prevalence, disease spectrum, diagnostic tests, and treatment options for H. pylori infection, taking into account the latest global antibiotic resistance patterns. Regimens for first-line therapy, second-line therapy, and multiple treatment failures are recommended based on the respective resistance patterns.

Extragastric diseases

Iron deficiency anemia. H. pylori infection can induce depletion of iron stores by several mechanisms including chronic occult gastrointestinal blood loss, impaired iron absorption due to decreased intragastric acidity and ascorbic acid concentration (41), and enhanced iron uptake for bacterial growth (42). The previous study reported that the prevalence of H. pylori-related chronic gastritis was 60% in patients with unexplained iron deficiency anemia (IDA) compared with 43% of the control group without IDA ($P < 0.01$) (43). H. pylori infection was associated with IDA with a pooled odds ratio (OR) of 2.22 (95% CI 1.52–3.24, $P < 0.0001$) (44). The meta-analysis demonstrated significant improvement in IDA after combination of H. pylori eradication and oral iron compared with oral iron alone (45).

Idiopathic thrombocytopenic purpura. Potential mechanisms of H. pylori-related idiopathic thrombocytopenic purpura (ITP) might be caused by molecular mimicry between H. pylori-induced antibodies and platelet glycoprotein antigens (46). Moreover, antibodies to cytotoxin-associated gene A protein (CagA), one of H. pylori virulence factors, can cross-react with specific peptides expressed by platelets of ITP patients (46). The prevalence of H. pylori infection was significantly higher in patients with ITP than in controls (90.6% vs. 43.8%, $P = 0.00006$) in the Colombian study (47). The systematic review reported that among 696 ITP patients receiving H. pylori eradication, 42.7% achieved complete response (platelet count ≥100 10^9/L) (48). A higher response rate was observed in patients with a milder degree of thrombocytopenia and in countries with a high prevalence of H. pylori infection (48). The American Society of Hematology recommends H. pylori eradication for infected patients with ITP (grade 1B) and also suggests H. pylori screening in patients with ITP (grade 2C) (49).

Diagnostic tests for H. pylori infection

Clinical guidelines recommend diagnostic testing for H. pylori infection in patients with the following conditions: (1) active or past history of peptic ulcer, (2) chronic dyspepsia, (3) chronic NSAID or aspirin use, (4) precancer-

ous gastric lesions, (5) gastric cancer, (6) MALT lymphoma, (7) family history of gastric cancer in a first-degree relative, (8) family history of peptic ulcer, (9) having a household family member with active H. pylori infection, (10) unexplained IDA, (11) ITP, and (12) vitamin B12 deficiency (5–7, 50). As gastric cancer prevalence is relatively high in Asia, the Bangkok consensus recommends community screening and treatment for H. pylori infection to prevent gastric cancer, especially in areas with a high cancer burden (6).

Current diagnostic tests for H. pylori infection are classified into two groups, which are noninvasive and invasive methods. Invasive diagnostic tests are endoscopy-based tests including histology, rapid urease test, culture, and polymerase chain reaction. Noninvasive tests are the urea breath test, stool antigen test, and antibody tests in serum, urine, and saliva (51). A patient should discontinue PPI for at least 2 weeks, antibiotics and bismuth compounds for at least 4 weeks before testing by histology, culture, rapid urease test, urea breath test, or stool antigen test to avoid a false negative result.

Invasive tests

Histology. Histologic examination is considered to be the gold standard in H. pylori detection with high sensitivity and specificity of 95% and 99%, respectively (52). Histology can provide information about associated lesions such as atrophic gastritis, intestinal metaplasia, dysplasia, and MALT lymphoma.

Rapid urease test. Gastric biopsy specimens are placed in a medium containing urea and pH indicator. Urease from H. pylori converts urea to ammonia, which increases the pH resulting in a color change of the pH indicator. Sensitivity and specificity are approximately 85%–95% and 95%–100%, respectively (51).

Culture. Culture has a lower sensitivity (85%–95%) than the rapid urease test or histology, but the specificity is as high as almost 100% (51). This test provides essential information about antimicrobial susceptibility.

Polymerase chain reaction. H. pylori can be detected by polymerase chain reaction (PCR) in several types of specimens such as gastric juice, gastric biopsies, stool, and saliva. PCR had a high sensitivity of 100% and 98% for gastric biopsy and stool specimens, respectively, while the specificity was 98% for both specimens (53). Antibiotic resistance can also be determined by amplification of resistance-associated genes using real-time PCR.

Non-invasive tests

Urea breath test. 13C-labeled urea is administered orally and hydrolyzed by H. pylori's urease producing ammonia and labeled CO2, which is subsequently exhaled and collected as a breath sample. Until now, the urea breath

test (UBT) has been the most popular and accurate noninvasive test for the diagnosis of H. pylori infection with high sensitivity (96%) and specificity (93%) (54). In patients with upper gastrointestinal bleeding, the diagnostic accuracy of UBT is still high with sensitivity and specificity of 93% and 92%, respectively (55). UBT can also be used after treatment to confirm H. pylori eradication.

Stool antigen test. There are two types of stool antigen tests that detect H. pylori antigens by using monoclonal or polyclonal antibodies. The monoclonal stool antigen test had a higher pooled sensitivity (94% vs. 83%) than the polyclonal stool antigen test but comparable specificity (97% vs. 96%) (56). Therefore, the monoclonal stool antigen test should be used for the diagnosis or confirmation of eradication. PPI can decrease the sensitivity of the stool antigen test from 95.2% to 88.9% and should be discontinued at least 2 weeks before the test (57).

Antibody tests in serum, urine, and saliva. Serologic tests are inexpensive and noninvasive. The serological assay detects anti-H. pylori IgG in serum, which generally becomes positive 3 weeks after H. pylori infection and persists up to 6–12 months after eradication (55). The serologic test had lower sensitivity and specificity of 85% and 79%, respectively (58). Most serologic tests cannot differentiate past infection from current infection except for rapid tests with a current infection marker (CIM) test, providing sensitivity and specificity of 93.2% and 96.2%, respectively, compared with UBT (59). Upper gastrointestinal bleeding did not affect the accuracy of serologic tests. There are less commonly used urine and saliva antibody tests. Urine-based tests had fair sensitivity (84.7%), specificity (89.9%), and accuracy (87%) (60). Salivary tests provided unsatisfactory results with fair sensitivity (81%) and specificity (73%) (61). Urine and saliva antibody tests still have a high variability of diagnostic accuracy; therefore, test usage is found only in the research field.

Confirmation of cure

Confirmation of successful eradication should be performed to avoid further complications of H. pylori infection and prevent transmission to other family members. Posttreatment tests are UBT, stool antigen test, or biopsy-based test. UBT is recommended as the best option for confirmation of cure. The suitable timing for testing should be at least 4 weeks after treatment completion.

Antibiotic resistance patterns

Antibiotic resistance patterns are determined by H. pylori culture and antimicrobial susceptibility testing (AST). This test requires proper gastric biopsy handling, efficient specimen transportation, and microbiological expertise (62). Moreover, traditional H. pylori culture is time-consuming and unavailable in some areas. Next-generation sequencing (NGS) is a new mo-

lecular-based test, which determines antibiotic resistance by identifying mutations or variances of the H. pylori DNA (62). Fresh or formalin-fixed paraffin-embedded gastric biopsies, gastric juice, or feces can be used for NGS. Targeted genes associated with antibiotic resistance are as follows: 23S rRNA for clarithromycin, 16S rRNA for tetracycline, gyrA for fluoroquinolones, rdxA for metronidazole, pbp1 for amoxicillin, and rpoB for rifabutin (62). NGS reliably determined clarithromycin and levofloxacin resistance from culture isolates and formalin-fixed gastric tissue compared with agar dilution (62). Most of the WHO regions had a high pooled prevalence of primary and secondary resistance to metronidazole, clarithromycin, and levofloxacin (>15%) (4). The most common antibiotic resistance in all regions was metronidazole (12, 63–66). The ASEAN, the United States, and Europe had almost the same antibiotic resistance patterns, while extremely high resistance rates to all antibiotics were demonstrated in Africa (66). Amoxicillin and tetracycline resistance rates were low in all regions except Africa.

Treatment

The treatment goal for H. pylori infection is an intention-to-treat eradication rate of at least 90% (67). Choosing the suitable first-line regimen depends on known or anticipated patterns of regional antibiotic resistance (5–7). Treatment regimen comprises anti-secretory drugs and antibiotics. The choice of antisecretory drugs is either PPIs or potassium-competitive acid blockers (P-CABs). At least two antibiotics are generally chosen in regimens.

Antisecretory drugs

Proton pump inhibitors. Proton pump inhibitors (PPIs) irreversibly bind and inhibit H+, K+-ATPase on parietal cells, causing effective gastric acid secretion blockage. PPIs are mainly metabolized by the CYP2C19 enzyme. The meta-analysis reported that using a high-dose PPI was more effective in H. pylori eradication than using a standard-dose PPI (82% vs. 74%, RR 1.09; 95% CI 1.01–1.17) (68). High-dose PPI is defined as a double dose of 40 mg of omeprazole or equivalent (7, 69). The potency of PPIs to inhibit CYP2C19 varies among agents (esomeprazole > lansoprazole > omeprazole > rabeprazole > pantoprazole) (70). CYP2C19 polymorphisms affect PPI metabolism. Poor metabolizers had a higher eradication rate in the rabeprazole-based regimen, but a lower eradication rate in the omeprazole-based regimen compared with extensive metabolizers (71).

Potassium-competitive acid blockers. Vonoprazan and revaprazan are potassium-competitive acid blockers (P-CABs), a new class of anti-secretory drugs. They reversibly bind to the H+, K+-ATPase pump and inhibit acid secretion. P-CABs are effective in patients with PPI-resistant H. pylori infection. A Japanese study reported that vonoprazan achieved a higher eradication rate than PPI-based triple therapy (92.7% vs. 78.5%, $P < 0.0001$) (72). A Korean

study demonstrated that revaprazan-based triple therapy achieved a higher eradication rate than esomeprazole-based triple therapy (90.7% vs. 83.3%, P = 0.046) (73). The efficacy of P-CABs in patients with clarithromycin resistance remains unclear. Vonoprazan, not revaprazan, is used in the eradication therapy of H. pylori infection.

Antibiotics

Clarithromycin. Clarithromycin, a macrolide antibiotic, inhibits protein synthesis by binding to the 50S ribosomal subunit of H. pylori. Resistance to clarithromycin is mainly caused by point mutations in the 23S rRNA gene of H. pylori. High resistance rates to clarithromycin are found in most countries and regions (4). Clarithromycin resistance is a key factor in the failure of standard triple therapy (74). Clarithromycin-based triple therapy is recommended in areas with a clarithromycin resistance rate of <15% (5, 6).

Amoxicillin. Amoxicillin is a β-lactam antibiotic, inhibiting peptidoglycan synthesis by binding to penicillin-binding proteins. H. pylori resistance to amoxicillin is rare (75). In most areas, amoxicillin-based triple therapy is recommended as a first-line treatment regimen (5, 6).

Metronidazole. Metronidazole is a nitroimidazole antibiotic, which disrupts the DNA helical structure of H. pylori. Metronidazole resistance occurs by mutation in the rdxA gene, which encodes oxygen-insensitive NADPH nitroreductase, producing active metabolites that damage DNA. High resistance rates to metronidazole are found in most countries and regions (4). Metronidazole-based triple therapy is recommended in areas with a metronidazole resistance rate of <15% (5, 6).

Tetracycline. Tetracycline is a bacteriostatic antibiotic, binding to the 30S ribosomal subunit and inhibiting protein synthesis. Tetracycline resistance in H. pylori is extremely rare (75). Tetracycline-based triple therapy is recommended in areas with a tetracycline resistance rate of <3% (5, 6).

Levofloxacin. Levofloxacin is a fluoroquinolone antibiotic, inhibiting DNA gyrase and topoisomerase IV, leading to DNA breakage and inhibiting DNA synthesis. Levofloxacin resistance in H. pylori is increasing worldwide. High resistance rates to levofloxacin are found in most countries and regions (4). Levofloxacin-based triple therapy is recommended in areas with a levofloxacin resistance rate of <15% (5, 6).

Bismuth-containing quadruple therapy. Bismuth-containing quadruple therapy consists of a PPI, bismuth, tetracycline, and metronidazole. Bismuth salts have antimicrobial effects, anti-secretory effects, and mucosal protection (76). Bismuth-based quadruple therapy is recommended as a first-line treatment regimen in areas with a clarithromycin resistance rate of ≥15% (5, 6). The efficacy of bismuth quadruple therapy is higher than that of clarithromy-

cin-based triple therapy (77). However, the use of bismuth therapy is limited due to adverse events, including a metallic taste, darkening of the tongue and stool, and drug interactions (76).

Sequential therapy. Sequential therapy consists of a PPI and amoxicillin for the first 5 days, followed by a PPI, clarithromycin, and metronidazole for the next 5 days. Sequential therapy is recommended as a first-line treatment regimen in areas with a clarithromycin resistance rate of ≥15% and a metronidazole resistance rate of <40% (5, 6). Sequential therapy is more effective than standard triple therapy and may achieve eradication rates comparable to bismuth quadruple therapy (78).

Concomitant therapy. Concomitant therapy consists of a PPI, amoxicillin, clarithromycin, and metronidazole, all given together for 10–14 days. Concomitant therapy is recommended as a first-line treatment regimen in areas with a clarithromycin resistance rate of ≥15% and a metronidazole resistance rate of ≥40% (5, 6). Concomitant therapy achieves high eradication rates comparable to or even higher than bismuth quadruple therapy (79).

Hybrid therapy. Hybrid therapy consists of a PPI and amoxicillin for the first 7 days, followed by a PPI, amoxicillin, clarithromycin, and metronidazole for the next 7 days. Hybrid therapy is recommended as a first-line treatment regimen in areas with a clarithromycin resistance rate of ≥15% and a metronidazole resistance rate of ≥40% (5, 6). Hybrid therapy achieves high eradication rates comparable to or even higher than bismuth quadruple therapy (80).

Culture-guided therapy. Culture-guided therapy is an individualized approach based on the results of H. pylori culture and antimicrobial susceptibility testing. It allows the selection of appropriate antibiotics for eradication based on individual antibiotic resistance patterns. Culture-guided therapy is recommended when H. pylori culture and antimicrobial susceptibility testing are available (5, 6). This approach can achieve high eradication rates but is limited by the availability of culture facilities.

Rescue therapy. Rescue therapy is used when initial treatment fails. It involves a different combination of antibiotics and anti-secretory drugs than the initial treatment regimen. The choice of rescue therapy depends on the antibiotics previously used and local antibiotic resistance patterns. Rescue therapy should aim for the highest eradication rate possible (5, 6).

It is important to note that treatment regimens should be tailored to individual patients based on local antibiotic resistance patterns, patient characteristics (such as age, comorbidities, and medication use), and treatment history. Consultation with a healthcare provider is essential to determine the most appropriate treatment strategy for H. pylori infection.

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Chapter 17

GESTATIONAL DIABETES MELLITUS (GDM): PREVALENCE, LIFESTYLE MODIFICATIONS, AND FUTURE DIRECTIONS

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Introduction

Gestational Diabetes Mellitus (GDM) is recognized as one of the most frequent endocrinopathies related to pregnancy, impacting as many as 25% of pregnancies around the world[1]. This condition enhances the potential of perinatal and delivery complications, as well as the development of type 2 diabetes mellitus and its cardiovascular complications[2]. This heightened risk is subsequently transmitted to future generations, setting a trans-generational cycle of metabolic disorders into motion. Even though GDM preventive strategies have previously shown inconsistent outcomes, recent systematic reviews and meta-analyses have shed light on new promising preventive measures[1]. This review's goal is to compile the evidence that evaluates the effectiveness of lifestyle changes in preventing GDM and to summarize the impacts of two primary lifestyle alterations: physical exercise and dietary modifications[2]. According to existing research, future inquiries should aim to assess whether initiating lifestyle interventions during the preconception phase can be more advantageous in preventing GDM. Moreover, research focusing on pregnancy should be constructed with a personalized design. Thus, studies should adapt intervention methods based on the presence of both modifiable and non-modifiable risk factors at the individual level[1]. GDM is a condition characterized by hyperglycemia due to an increase in placental hormone secretion during pregnancy. This condition occurs when the pancreas is unable to secrete sufficient insulin to counteract the surge in blood sugar and is typically identified between the 13–26 gestational weeks or early in the third trimester of pregnancy[2]. Although GDM often improves after delivery, it is connected to severe maternal consequences, including type 2 diabetes mellitus, cardiovascular disease, and metabolic syndrome[3,4]. Beyond the maternal outcomes, offspring from GDM pregnancies exhibit a higher frequency of childhood overweight/obesity, dysglycemia, dyslipidemia, and metabolic syndrome[5,6]. Furthermore, there is an increased risk of T2DM development in childhood and neurodevelopmental impairments[7,8]. This propensity towards metabolic dysfunction bridging mother and offspring could be the result of a blend of factors, including shared genetic factors, the home and lifestyle environment, and fetal exposure to the altered intrauterine environment during a GDM pregnancy. This exposure can potentially program detrimental developmental pathways according to the Developmental Origins of Health and Disease paradigm[9].

The onset of GDM is linked with both modifiable and non-modifiable risk factors. Non-modifiable factors incorporate inherent genetic risks[10], ethnic background (such as Hispanic or Asian)[12], maternal age, having a first-degree relative with T2DM, and a history of Polycystic Ovarian Syndrome (PCOS)[13]. On the other hand, modifiable risk factors predominantly encompass lifestyle and environmental factors that can influence the develop-

ment of GDM. These can be split into pre-pregnancy and peri-pregnancy risk factors. The former largely involves lifestyle factors like pre-pregnancy obesity and overweight, low-quality diet, and physical inactivity[14], while the latter predominantly refers to excessive weight gain during pregnancy, termed as gestational weight gain (GWG)[15].

Several factors can influence maternal glucose control, including physiological changes during pregnancy, pathological conditions, and maternal nutrition. The types of carbohydrates in the diet significantly impact maternal glucose through their direct effect on glycemia[16]. The carbohydrate type and glycemic index (GI) of the diet can either exacerbate or mitigate hyperglycemia during pregnancy caused by pathological conditions or the mother's inability to handle physiological Insulin Resistance (IR) of pregnancy[16]. Moreover, physical activity has been shown to improve glycemic control, partially through boosting contraction-mediated glucose absorption into skeletal muscle[17].

Post-delivery, pregnancy-related IR typically recedes, and blood glucose levels in women with GDM frequently return to normal, removing the need for continuous glucose-lowering medication[11]. While the recorded temporary hyperglycemia during pregnancy might make GDM seem like a purely medical result of pregnancy, the glycemic effects of this diagnosis reach far beyond pregnancy[11]. The beta-cell dysfunction leading to inadequate compensation for pregnancy IR is both chronic and progressive[11]. Consequently, women who develop GDM often experience a progressive decrease in beta-cell function in the years following the pregnancy, leading to elevated glycemia over time, potentially resulting in pre-diabetes and T2DM[11]. This review's objective is to compile evidence examining the effectiveness of lifestyle interventions for preventing GDM and to outline the impacts of two main lifestyle modifications: physical activity and dietary interventions.

Methodology

A thorough literature search was conducted across PubMed, Cochrane Reviews, Google Scholar, SpringerLink, and Scopus databases for pertinent articles. This review considered articles published in the previous decade, from July 1, 2012, to July 1, 2022. Search terms included 'gestational diabetes' in combination with 'lifestyle interventions', 'physical activity', 'dietary interventions', and 'prevention'. The retrieved articles were assessed for their relevance to the topic and manually reviewed to find additional related publications. Included were randomized controlled trials (RCTs), meta-analyses, systematic reviews, and cohort studies. Only articles involving interventions for adults (18 and older), randomized and non-randomized studies including intervention groups (with diet and/or physical activity) and control groups (without intervention or standard treatment/placebo), and meta-analyses in-

corporating RCTs measuring the effect of physical activity, dietary interventions, combined lifestyle interventions, and supplements on GDM prevention were included. The initial search identified 1284 articles. After excluding 299 duplicates, 985 titles or abstracts were screened, with 840 deemed irrelevant and subsequently excluded. The remaining 145 full-text articles underwent further eligibility review, which resulted in the removal of 115 full-text articles due to the outlined inclusion criteria (as seen in Figure 1). Hence, this review includes 30 articles.

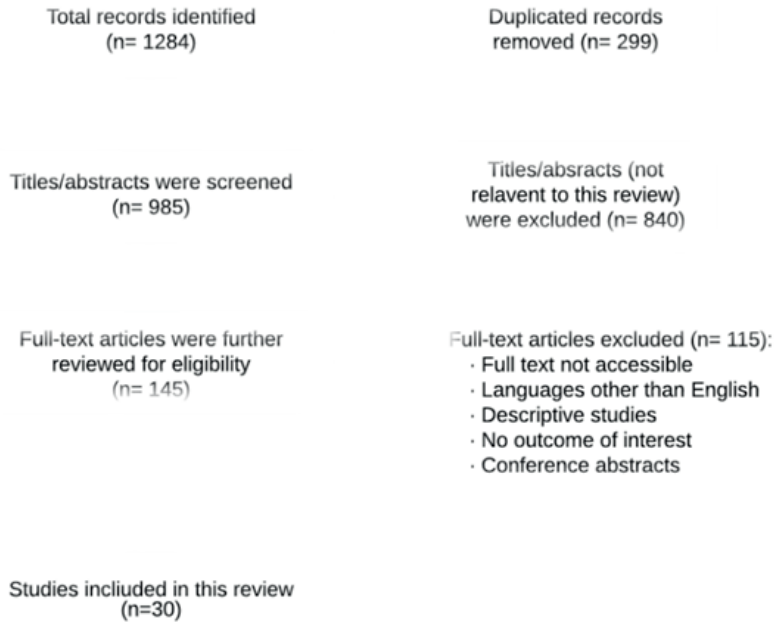

Figure 1. *Flow chart*

According to a meta-analysis conducted by Ying Yu et al. [29], physical activity (PA) during pregnancy significantly reduces the risk of gestational diabetes mellitus (GDM) compared to the control group. However, it does not have an impact on birth weight, gestational age at birth, 2-hour oral glucose tolerance test (OGTT), or preterm birth. Another meta-analysis by Aune et al. [22] found that increased leisure-time PA before, during, and combined before and during pregnancy reduces the relative risk of GDM. Higher total PA before pregnancy is associated with a 34% reduction in the relative risk of GDM, while the association for total PA during pregnancy indicates a reduced risk but is not statistically significant, possibly due to limited studies. The meta-analysis by Davenport et al. [24] reveals that exercise interventions during pregnancy reduce the risk of developing GDM, gestational hyperten-

sion (GH), and pulmonary embolisms (PE) by 38%, 39%, and 41%, respectively. Pregnant women need to engage in at least 600 MET-min/week of moderate-intensity exercise to reduce the chances of developing GDM, PE, and GH by 25%. Brown et al. [35] demonstrate that exercise interventions lead to a reduction in fasting blood glucose and postprandial blood glucose concentration compared to standard treatment.

Da Silva et al. [23] find that participation in leisure-time PA during pregnancy leads to a reduction in weight gain, lower risk of GDM, and decreased chance of delivering a large for gestational-age newborn. Mijatovic-Vukas et al. [25] associate different types of PA interventions with GDM risk and discover that engaging in any type of PA early in pregnancy reduces the risk by 21%, while engaging in any type of PA pre-pregnancy reduces the risk by 30%. Risk reduction is further observed with more than 90 minutes/week of pre-pregnancy PA (46% reduction) and more than 15 MET h/week of pre-pregnancy PA (48% reduction). Russo et al. [28] show that exercise intervention during early pregnancy can slightly reduce the risk of GDM by 28% when implemented through a group exercise plan or an individualized plan. Similarly, Doi et al. [34] find that PA administered in a facility and initiated before the 16-20th week of gestation can prevent the development of GDM in high-risk pregnant women.

Meta-analyses by Ming et al. [26] and Nasiri-Amiri et al. [27] confirm the risk-reducing effects of PA on GDM. Ming et al. categorize the studies based on different diagnostic criteria of GDM and find that PA reduces the risk by 40-42% and decreases gestational weight gain by 1.61 kg. Nasiri-Amiri et al. report a 24% reduction in GDM risk with PA interventions during pregnancy and a 41% risk reduction with engaging in PA three times per week. However, no significant impact on GDM risk is observed during the first and second trimesters, likely due to limited studies. Zheng et al. [30] associate exercise intervention with a significantly reduced incidence of GDM compared to controls, but it does not have a significant impact on other factors such as preterm birth, gestational age at birth, OGTT, birth weight, or preeclampsia. Madhuvrata et al. [31] find no statistical difference in GDM risk between the intervention and control groups.

In conclusion, current data highlights the importance of PA before and during early pregnancy in preventing GDM. Initiating PA early in pregnancy (<20 weeks of gestation) and engaging in organized activities with high adherence and compliance are more effective in reducing the risk of developing GDM. Few studies have explored the dose-response relationship and the effects of different exercise frequencies, intensities, durations, and types on GDM risk.

Current Nutrition Therapy for GDM

Nutritional therapy aims to provide appropriate energy, macronutrients, and vitamin supplements to ensure adequate maternal and fetal nutrition while achieving glycemic goals. A meta-analysis by the Cochrane Database found that following the Dietary Approaches to Stop Hypertension (DASH) diet may reduce the risk of cesarean section deliveries, but the quality of evidence was low [37]. Various dietary interventions have been studied, including low-to-moderate glycemic index (GI) diets, energy-restricted diets, and the DASH diet, but combining the findings to recommend a specific dietary pattern remains challenging. The American Diabetes Association (ADA) recommends a minimum daily carbohydrate intake of 175 g, along with specific recommendations for protein, fiber, and fat intake [20]. The ADA emphasizes the importance of consuming monounsaturated and polyunsaturated fats while avoiding saturated and trans fats. Higher-quality carbohydrates have shown positive effects on glucose levels, insulin action, and infant adiposity. However, there is insufficient data to support any specific dietary regime conclusively.

Effect of Dietary Interventions on the Prevention of GDM

Both the quantity and quality of food consumed play a significant role in maintaining glucose homeostasis and improving insulin resistance. Poor diet quality, even without excessive caloric intake, can have long-term effects on beta cell function and increase the risk of impaired glucose tolerance and type 2 diabetes. Observational studies have shown that dietary habits before and during pregnancy impact the risk of GDM. A meta-analysis revealed that low glycemic index diets and calorie restriction can reduce the risk of GDM by 33%, particularly in overweight and obese women. However, the evidence quality was low, and more research is needed to determine the effectiveness of different dietary interventions [47, 48].

Effect of Combined Interventions (Physical Activity and Dietary Interventions) on the Risk of GDM

Combining dietary changes with physical activity interventions has shown promise in reducing the risk of GDM. Self-monitoring of blood sugar levels, along with lifestyle interventions, has been associated with decreased gestational weight gain and a lower risk of delivering large-for-gestational-age infants. A systematic review found moderate evidence for a possible reduced risk of GDM with lifestyle interventions compared to standard care. Several studies and meta-analyses have demonstrated the effectiveness of lifestyle counseling and interventions in reducing the incidence of GDM. However, the long-term effects and specific outcomes, such as hypoglycemia in newborns and childhood obesity, require further investigation. The timing and intensity of interventions, as well as individual characteristics, may influence the effectiveness of combined interventions [38, 55, 57, 59, 60, 61].

The Effect of Dietary Supplements on the Risk of GDM

Vitamin D: Vitamin D deficiency is prevalent among pregnant women and has been linked to pregnancy complications such as GDM, hypertension, premature birth, and small gestational age [62]. In women diagnosed with GDM, the administration of vitamin D supplements (with doses ranging from 1000 to 4762 IU per day) has been found to improve glucose metabolism by reducing fasting blood glucose (FBG), HbA1c, and serum insulin levels [63]. A study conducted in Australia and New Zealand called the SCOPE study examined the correlation between vitamin D levels in pregnant women at 15±1 weeks of gestation and the risk of developing GDM. The study found that pregnant women with high vitamin D levels (>81 nmol/L) had a lower risk of GDM [64]. The available evidence suggests that vitamin D supplementation in pregnant women significantly reduces FBG (mean difference (MD) = -1.87 mg/dL, 95% confidence interval (CI) -3.39 to -0.35) and the incidence of GDM (odds ratio (OR) = 0.42, 95% CI 0.30 to 0.60) [65]. A meta-analysis published in 2020 reported that vitamin D supplementation was more effective than other supplements, such as omega-3, zinc, and probiotics, in lowering FBG and improving homeostatic model assessment of insulin resistance (HOMA-IR) [66]. Furthermore, studies have investigated the combined effects of vitamin D supplementation with other supplements. A dose of 1000 IU/day of vitamin D3 has been shown to significantly reduce serum triglycerides, very low-density lipoprotein (VLDL), total cholesterol, and low-density lipoprotein (LDL) concentrations. In addition, a combination of vitamin D, magnesium, zinc, and calcium supplementation has demonstrated reductions in inflammation and oxidative stress markers and a potential decrease in birth weight and the rate of macrosomia [67, 68].

Myo-Inositol: Myo-inositol, a naturally occurring sugar, has been found to improve insulin sensitivity and ovulatory function in women with polycystic ovarian syndrome (PCOS). Several studies have examined the effects of myo-inositol supplementation on GDM prevention. In a prospective randomized controlled trial (RCT) involving pregnant women with a family history of type 2 diabetes but excluding those who were obese or had a history of PCOS, GDM, or pre-gestational diabetes, myo-inositol supplementation (2.0 g) plus folic acid twice a day significantly reduced the incidence of GDM and macrosomia when compared to folic acid alone [69]. Another RCT among obese pregnant women showed that myo-inositol supplementation (2.0 g) at the end of the first trimester significantly decreased the incidence of GDM compared to a placebo [70]. A recent systematic review and meta-analysis of five RCTs further supported the role of myo-inositol in reducing the incidence of GDM and preterm birth [71]. These findings suggest that myo-inositol supplementation may be beneficial in improving insulin sensitivity and preventing GDM, particularly in obese or overweight women, women with PCOS, or women with a family history of type 2 diabetes.

Effect of Combined Interventions (Physical Activity and Dietary Interventions) on Outcomes in Patients with GDM

Glycemic Control: Meta-analyses of randomized trials have shown that combined lifestyle interventions, incorporating physical activity and dietary changes, can improve glycemic control in pregnant women with GDM. These interventions have been found to significantly reduce postprandial blood glucose levels (mean difference: -27.11 mg/dL, 95% CI -44.62 to -9.61) [72]. In terms of glycated hemoglobin (HbA1c) levels, lifestyle interventions have led to a reduction in HbA1c levels at the end of the intervention period (mean difference: -0.33 mmol/mol, 95% CI -0.47 to -0.19) [72]. However, no significant effects on fasting blood glucose levels have been observed.

Weight Gain during Pregnancy: Combined lifestyle interventions have demonstrated effectiveness in reducing gestational weight gain. Meta-analyses of randomized trials have shown that these interventions result in lower weight gain during pregnancy compared to standard care (mean difference: -1.30 kg, 95% CI -2.26 to -0.35) [72]. In a specific study by Landon et al., the greatest difference in weight gain was observed between the lifestyle intervention group and the control group (2 kg vs. 5 kg, respectively) [73].

Postnatal Depression: Lifestyle interventions have also shown potential benefits in reducing the incidence of postnatal depression among women with GDM. A study by Crowther et al. found that lifestyle interventions were associated with a lower incidence of postnatal depression, as measured by an Edinburgh Postnatal Depression Score greater than 12, when compared to the control group (relative risk: 0.49, 95% CI 0.31 to 0.78) [74].

Future Directions

Future research should focus on personalized approaches to lifestyle interventions, taking into account individual risk factors, behaviors, and socioeconomic status. Early prenatal screening and risk prediction systems can help identify women at high risk for developing GDM and guide intervention strategies accordingly. It is important to conduct extensive, high-quality studies to determine the specific aspects of lifestyle interventions, such as dietary changes and exercise, that are most effective in reducing the risk of or improving outcomes in women with GDM. Harmonizing clinical practice based on robust evidence is crucial to enhance prevention and management strategies for GDM.

Conclusions

The current literature supports the effectiveness of lifestyle interventions, including physical activity, dietary changes, and supplementation, in reducing the risk of GDM and improving outcomes. Early initiation of interventions and focusing on diet quality have shown positive effects. Vitamin D supple-

mentation has demonstrated benefits in improving glucose metabolism, while myo-inositol supplementation has shown promise in enhancing insulin sensitivity and preventing GDM. Combined interventions have positive effects on glycemic control, weight gain during pregnancy, and the incidence of postnatal depression. However, further research is needed to address research gaps, improve the quality of evidence, and guide clinical practice in preventing and managing GDM effectively.

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Chapter 18

THE IMPORTANCE OF THE DENTIST IN THE DIAGNOSIS AND TREATMENT OF PRECANCEROUS ORAL LESIONS AND ORAL CANCER

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Introduction

Head and neck cancer is the seventh most common type of cancer worldwide (1) and refers to a group of cancers that can occur in various parts of the head and neck region, including the oral cavity, throat, voice box, salivary glands, nasal cavity, and sinuses. Oral cancer (OC) is cancer that originates in the oral cavity, which includes the lips, tongue, gums, lining of the cheeks, floor of the mouth, and hard and soft palate (2). OC is a significant global health concern. Oral squamous cell carcinoma (OSCC) is a common type of cancer among head and neck cancers (2). OSCC often metastasizes to lymph nodes in relation to the level of differentiation. Regional metastases are present in at least 30% of OSCC cases. Despite technological advances in surgery, radiation, and chemotherapy, the five-year survival rate for OC is still 50-55% (3). In this review, the features of OC and the importance of the dentist will be discussed.

Epidemiology

The OC is one of the most common types of cancer worldwide. Although oral cancer is usually seen in middle-aged and elderly individuals, it has also been seen in younger adults in recent years (4). Clinically, an OC occurs in three different types: primary oral cavity carcinomas, lip red carcinomas, and carcinomas arising in the oropharynx. Tumors in the mouth and oropharyngeal region are more common in men than in women. Its male-to-female ratio is more than 2:1 (5). However, in recent years, the incidence of breast cancer among women has increased due to their exposure to carcinogens such as tobacco and alcohol (5).

The incidence and prevalence rates vary across different regions and populations. It is more prevalent in certain parts of the world, including South and Southeast Asia, where tobacco and betel nut use are common.

Risk Factors

Several risk factors contribute to the development of OC (4). The most significant ones include:

1. Tobacco Use: There is a strong relationship between tobacco use and OC. Tobacco in various forms, such as smoking cigarettes, cigars, pipes, or smokeless tobacco products, is a leading risk factor for OC (6). It contains carcinogens that can damage oral tissues. There is a strong relationship between tobacco use and OC. Studies have reported that the risk of developing OC in smokers is five to nine times greater than in non-smokers. This risk was shown to be 17 times higher in those who smoked more than 80 cigarettes a day (5).

2. Alcohol Consumption: Alcohol is an important risk factor for upper digestive tract cancers. Chronic and heavy alcohol consumption is associated with an increased risk of OC (7). But studies have reported that moderate to heavy drinkers have a three- to nine fold greater risk of OC. One study showed that binge drinkers (more than 100 grams daily) had a 30 times greater risk of oropharyngeal cancer and OCs (8). The combined use of tobacco and alcohol significantly amplifies the risk.

3. Betel Quid: Areca nut is the main ingredient of betel quid (BQ), which is consumed by many people around the world (9). BQ use is particularly prevalent in India and Southeast Asia. Quid usually consists of a betel leaf wrapped around a mixture of areca nuts and slaked lime, along with tobacco and different seasonings. The slaked lime causes the release of an alkaloid from the areca nut. This creates a feeling of euphoria and well-being in the person who uses it. Chewing BQ leads to a condition called submucous fibrosis in the mouth. In the study in India, it was shown that 7.6% of this lesion turned into a malignancy (10).

4. Human Papillomavirus (HPV) Infection: HPV is a small, double-stranded DNA virus classified as belonging to the Papillomaviridae family (11). The probability of HPV transforming into malignancy depends on the type of virus, the synergistic effect between different physical, chemical, and biological agents, the genetic structure, and the immune mechanism of the host. Certain strains of HPV, primarily HPV-16 and HPV-18, are associated with an increased risk of OC, especially in younger individuals (12).

5. Sun Exposure: It is known that sun exposure is a confirmed risk factor for skin cancer. But prolonged and excessive exposure to sunlight can increase the risk of lip cancer.

6. Poor Oral Hygiene: Neglecting oral hygiene practices and having poor dental health, including chronic irritation or inflammation, may contribute to the development of OC (13). Some studies have shown poor oral hygiene as an additional factor causing OC (14). However, it has been reported that there is a relationship between poor oral hygiene and OC in different studies (14).

7. Genetic Factors: Many studies have addressed genetic pathways related to the cell cycle, immune apoptosis and angiogenesis, adhesion, and matrix degradation in the etiology of OC.

Specific genetic mutations and family histories of OC may also play a role in some cases (6). In studies using developing genetic technologies, mutations with different pathways have been detected in the genomes of OC patients (15).

Etiology of OC

The etiology of OC involves complex interactions between genetic, environmental, and lifestyle factors. The development of OC typically follows a multi-stage process (16). Exposure to carcinogens, such as tobacco smoke or alcohol, and other risk factors can lead to genetic damage in the oral tissues. This genetic damage can initiate the process of cancer development (6). Further exposure to carcinogens and other factors, such as chronic inflammation or viral infections, can promote the growth and progression of initiated cells. This stage involves changes in gene expression and cellular behavior. The progression stage involves the transformation of pre-cancerous cells into malignant cancer cells. It is characterized by uncontrolled growth, invasion into surrounding tissues, and the potential for metastasis to distant sites.

Diagnosis

The diagnosis of OC typically involves a combination of physical examination, imaging tests (such as CT scans or MRIs), and a biopsy, where a small tissue sample is taken for laboratory analysis (17). The most important evaluation in the diagnosis of OC is a tissue biopsy and histological evaluation. This is an invasive procedure performed by a healthcare professional (18). Once diagnosed, the treatment options depend on various factors, including the type, stage, and location of the cancer.

Treatment

It's important to note that the treatment plan for OC is highly individualized, and the specific treatments used may vary from person to person. The medical team, including oncologists, surgeons, radiation oncologists, and other specialists, will work together to develop the most appropriate treatment approach for each patient (19). The treatment of OC typically involves a multidisciplinary approach, which may include surgery, radiation therapy, chemotherapy, targeted therapy, and immunotherapy. The specific treatment plan depends on various factors, such as the stage and location of the cancer, the patient's overall health, and the preferences of the patient and the medical team (20). Some common treatment options for OC include:

Surgery: Surgery is often the primary treatment for localized OC. The goal is to remove the tumor along with a margin of healthy tissue. The extent of the surgery depends on the size and location of the tumor (21). Surgical options may include tumor excision, laser surgery, or more extensive procedures like partial or total removal of the jawbone or tongue.

Radiation Therapy: Radiation therapy uses high-energy beams to kill cancer cells and shrink tumors. It may be used as the primary treatment

or in combination with surgery and/or chemotherapy (22). External beam radiation therapy is the most common type used for oral cancer, where a machine delivers radiation from outside the body. Brachytherapy is another form in which radioactive implants are placed near or inside the tumor.

Chemotherapy: Chemotherapy uses drugs to kill cancer cells throughout the body. It is often used in combination with other treatments (22). In the case of OC, chemotherapy is typically administered in the form of an intravenous infusion, and it may be given before or after surgery or radiation therapy.

Targeted Therapy: Targeted therapy drugs specifically target certain molecules or pathways that are involved in the growth and spread of cancer cells. These drugs can interfere with the cancer's growth while causing fewer side effects than traditional chemotherapy (23). Targeted therapies may be used in cases where specific genetic mutations or biomarkers are present in the cancer cells.

Immunotherapy: Immunotherapy helps boost the body's immune system to recognize and attack cancer cells. Immune checkpoint inhibitors, such as drugs targeting PD-1 or PD-L1, have shown promising results in the treatment of certain types of OC (24).

Premalignant Oral Lesions

Premalignant oral lesions (PMOLs) are known as lesions that can potentially lead to oral malignancies. It is crucial to identify and manage them because they have the potential to progress into OC (25). Detecting and treating these lesions early can significantly improve the prognosis and increase the chances of successful treatment. Here are some examples of PMOLs:

A. **Leukoplakia**

Leukoplakia is a condition characterized by thickened, white patches that develop on the mucous membranes of the mouth, including the tongue, gums, inner cheeks, and the floor of the mouth (26). These patches cannot be scraped off and may be precancerous, meaning they have the potential to develop into OC. However, not all leukoplakia cases progress to cancer. The exact cause of leukoplakia is unclear, but it is often associated with irritants such as tobacco use (smoking or chewing), alcohol consumption, and chronic irritation from rough teeth, dentures, or dental fillings. In some cases, leukoplakia may also be associated with the HPV infection. It's important to note that leukoplakia should not be ignored, as it has the potential to progress to OC. The management of leukoplakia focuses on removing the cause of irritation and monitoring the lesions for any changes. Treatment options for leukoplakia include (27):

Removing the source of irritation: This involves encouraging the patient to quit smoking, stop using tobacco products, reduce alcohol consumption, and improve oral hygiene. Eliminating these irritants can sometimes lead to the regression of leukoplakia.

Regular monitoring and follow-up: If the patches are small and show no signs of dysplasia (abnormal changes in the cells), the doctor may recommend regular follow-up examinations to monitor any changes in the lesions. This allows for early detection of any potential progression to cancer.

Biopsy and further evaluation: If the leukoplakia patches show dysplasia or other concerning features, a biopsy may be performed (28). During a biopsy, a small sample of tissue is taken from the lesion and examined under a microscope to determine the presence and severity of any abnormal changes. This helps in determining the need for further treatment.

Surgical removal: If the leukoplakia shows severe dysplasia, the doctor may recommend surgical removal of the affected tissue (29). This procedure is typically performed under local anesthesia and may involve excision, laser surgery, or cryosurgery (freezing the abnormal tissue). The removed tissue is then sent for pathological examination.

B. **Erythroplakia**

Erythroplakia is characterized by red, velvety patches that occur in the mouth, particularly on the mucous membranes (30). These patches cannot be attributed to any specific cause or irritation, and they are considered to have a higher risk of being precancerous or already cancerous compared to leukoplakia. Erythroplakia is less common than leukoplakia but is more concerning due to its association with OC. The exact cause of erythroplakia is unknown, but similar to leukoplakia, it is often associated with risk factors such as tobacco use (smoking or chewing), alcohol consumption, chronic irritation, and potentially the HPV infection (31). Early detection and prompt treatment are essential for erythroplakia due to its higher risk of malignancy. If you notice any persistent red patches in your mouth or have any concerns, it is important to consult a healthcare professional or a dentist for a thorough evaluation and appropriate management.

Given the higher potential for malignancy, it is crucial to seek medical attention promptly if you notice any red patches in your mouth. The management of erythroplakia typically involves (31):

Biopsy: The first step in diagnosing erythroplakia is to perform a biopsy. During a biopsy, a small sample of tissue is taken from the red patch and examined under a microscope. This helps to determine the presence of any abnormal or cancerous changes.

Surgical Treatment: If the biopsy confirms the presence of dysplasia or cancer, surgical intervention is typically recommended (32). The extent of surgery depends on the size and location of the abnormal tissue. Surgical options may include excision of the affected area, laser surgery, or more extensive procedures if the cancer has spread to nearby structures.

Follow-Up Care: After treatment, regular follow-up examinations are crucial to monitor for any recurrence or the development of new lesions. These examinations may include physical examinations, imaging tests, and additional biopsies if necessary.

Lifestyle Changes: It is important to address any underlying risk factors to reduce the risk of recurrence or the development of new lesions. This may involve quitting smoking, avoiding tobacco and alcohol use, improving oral hygiene, and adopting a healthy lifestyle.

C. **Oral Submucous Fibrosis**

Oral submucous fibrosis (OSMF) is a chronic, progressive condition that affects the oral cavity (33). It is characterized by the formation of fibrous bands in the submucosal layer of the oral tissues, leading to restricted mouth opening and difficulty chewing and speaking. OSMF is primarily associated with the habitual use of betel quid, which is a combination of areca nut, tobacco, slaked lime, and various flavoring agents (34). Other factors, such as nutritional deficiencies, genetic predisposition, and autoimmune responses, may also play a role in its development. The progression of OSMF can vary from person to person, and it can lead to significant functional impairment and an increased risk of developing oral cancer in some cases. The management of OSMF aims to alleviate symptoms, prevent further progression, and improve quality of life. Some common approaches to the treatment of OSMF include: (34):

Cessation of Betel Quid and Tobacco: The most crucial step in managing OSMF is to discontinue the use of betel quid, tobacco, and any other potentially harmful substances. This can help prevent further damage to the oral tissues and reduce the risk of malignant transformation.

Medications: Medications may be prescribed to reduce symptoms and slow down the progression of fibrosis (35). These may include topical corticosteroids, systemic corticosteroids, and other medications that help improve blood flow and reduce inflammation.

Physiotherapy and Mouth Exercises: Physical therapy techniques and mouth exercises can help improve mouth opening and reduce trismus. These may include jaw-stretching exercises, warm compresses, and the use of oral devices to gradually increase mouth opening.

Nutritional Supplements: Nutritional deficiencies are often associated with OSMF. Therefore, supplementation with vitamins, minerals, and antioxidants may be recommended to support overall health and oral tissue healing (36).

Surgical Intervention: In advanced cases of OSMF where severe functional limitations persist, surgical treatment may be considered. Surgical options can include the release of fibrous bands, grafting procedures to replace the affected tissue, or surgical interventions to improve mouth opening.

Regular monitoring and follow-up are important in OSMF to assess disease progression and ensure appropriate management (37). It is also crucial for individuals with OSMF to undergo regular oral cancer screenings due to the increased risk of malignant transformation. If you suspect you have OSMF or are experiencing any symptoms associated with the condition, it is advisable to consult a healthcare professional or a dentist who can provide a proper diagnosis and develop an individualized treatment plan.

D. **Oral Lichen Planus**

Oral lichen planus (OLP) is a chronic inflammatory condition that affects the mucous membranes inside the mouth, including the cheeks, tongue, gums, and palate (38). It is considered an autoimmune disorder, where the body's immune system mistakenly attacks the cells of the oral mucosa. The exact cause of OLP is unknown, but it is believed to involve a combination of genetic predisposition, immune system dysfunction, and environmental factors. It is not contagious and cannot be spread from person to person. The most common form of OLP is characterized by the presence of white, lacy lines or reticular patterns on the inside of the cheeks and other affected areas (39). These lines may be accompanied by redness and inflammation. In some cases, OLP can cause painful ulcers or sores in the mouth. These ulcers may be covered by a white or grayish coating and can make eating and speaking uncomfortable. Many individuals with OLP experience a burning sensation or discomfort in the affected areas, especially when consuming spicy or acidic foods. There are subtypes of lichen planus (40):

Classical or Typical Lichen Planus: This is the most common subtype, characterized by pruritic (itchy) purple-colored flat-topped papules or plaques that often have fine white lines or streaks called Wickham's striae. It typically affects the skin, particularly the wrists, ankles, lower back, and genital area.

Hypertrophic Lichen Planus: In this subtype, there is a thickening or hypertrophy of the lesions, which appear as raised, reddish-brown, or purplish nodules. It most commonly affects the shins, ankles, and genital areas.

Linear Lichen Planus: This variant presents as a linear or band-like distribution of lichen planus lesions along a specific area of the skin. It may

be associated with trauma or the Koebner phenomenon (lesions developing at the site of injury).

Actinic lichen planus: Also known as lichen planus actinicus, this subtype occurs in sun-exposed areas of the skin, such as the face, neck, and forearms. The lesions may resemble classical lichen planus or may appear more like discoid lupus erythematosus, with scaling and crusting.

Lichen Planopilaris: This variant affects the scalp and hair follicles, leading to patchy hair loss and scarring. It may cause permanent hair loss if not treated promptly.

It's important to note that these subtypes are not mutually exclusive, and some individuals may exhibit features of multiple subtypes concurrently. The diagnosis and management of lichen planus should be conducted by a qualified healthcare professional, such as a dermatologist. The management of OLP focuses on relieving symptoms and preventing complications (41). Treatment options may include:

Topical corticosteroids: corticosteroid creams, gels, or mouthwashes may be prescribed to reduce inflammation and relieve symptoms. These medications are typically applied directly to the affected areas in the mouth (42).

Systemic Corticosteroids: In severe or widespread cases, oral corticosteroids may be prescribed to control inflammation (43). These medications are taken in pill or liquid form, but their use is generally limited to short periods due to potential side effects.

Immune-Modulating Medications: In some cases, medications that modify the immune system, such as retinoids, calcineurin inhibitors, or immunosuppressants, may be used to manage OLP.

Symptomatic Relief Measures: To alleviate discomfort, various measures can be taken, including avoiding irritating foods, practicing good oral hygiene, using gentle mouthwashes or rinses, and maintaining proper hydration.

Regular Follow-Up: OLP is a chronic condition, and regular follow-up visits with a dentist are important to monitor the condition, adjust treatment as needed, and screen for any potential complications.

It's important to note that the treatment approach for OLP may vary based on the severity and extent of the condition (44). It is recommended to consult a healthcare professional or a specialist in oral medicine or dermatology for an accurate diagnosis and an appropriate management plan tailored to your specific needs.

E. **Actinic Cheilitis**

Actinic cheilitis, also known as solar cheilitis or farmer's lip, is a condition characterized by chronic inflammation and damage to the lips, particularly the lower lip (45). It is primarily caused by long-term exposure to ultraviolet (UV) radiation from the sun or other sources, such as tanning beds. Actinic cheilitis most commonly affects fair-skinned individuals and is often seen in people who spend significant time outdoors, such as farmers, sailors, and outdoor workers. The main feature of actinic cheilitis is dry, cracked, and scaly lips. Other symptoms may include redness, swelling, ulceration, and the formation of white patches or rough areas on the lips (46).

Actinic cheilitis is considered a PMOL because it can progress to squamous cell carcinoma, a type of skin cancer, in some cases. The management of actinic cheilitis focuses on protecting the lips from further sun damage and monitoring for any signs of progression to skin cancer (47). Here are some common approaches to the treatment of actinic cheilitis:

Sun Protection: The most important step in managing actinic cheilitis is to minimize sun exposure and protect the lips from UV radiation. This can be achieved by wearing wide-brimmed hats, using sunscreen lip balms with a high sun protection factor, and seeking shade during peak sun hours.

Topical Medications: prescription-strength topical creams or ointments containing ingredients such as fluorouracil (5-FU) or imiquimod may be used to treat actinic cheilitis (48). These medications help to remove precancerous cells and promote healing of the affected lips.

Cryotherapy: In some cases, cryotherapy may be performed, which involves freezing the affected area using liquid nitrogen (48). This helps to destroy abnormal cells and stimulate the growth of healthier tissue.

Surgical İntervention: If actinic cheilitis shows severe dysplasia or there is suspicion of skin cancer, a biopsy may be performed to confirm the diagnosis. In such cases, surgical removal of the affected tissue may be necessary. This may involve excision, laser surgery, or other surgical techniques.

Regular follow-up examinations are important for individuals with actinic cheilitis to monitor for any signs of progression to skin cancer (49). It is recommended to consult a healthcare professional or a dermatologist for a proper diagnosis and management plan, especially if you notice persistent lip changes or have a history of significant sun exposure. Early detection and intervention can help prevent the development of skin cancer and ensure optimal outcomes.

The Importance Of PMOLS

Overall, recognizing PMOLs helps healthcare professionals implement appropriate preventive measures, provide timely interventions, and educate patients about OC risks. The goal is to reduce the incidence and impact of OC and improve the course of the disease by proactively addressing these lesions (50). Here are some reasons why PMOLs are important:

Early Detection: PMOLS provide an opportunity for early detection of potential OC. Regular dental check-ups and oral examinations can help identify these lesions at an early stage, allowing for timely intervention and treatment.

Risk Assessment: The presence of PMOLs helps determine an individual's increased risk of developing OC. Healthcare professionals can assess the characteristics of the lesion, evaluate associated risk factors, and implement appropriate monitoring and preventive measures.

Preventive Strategies: Managing PMOLs can involve implementing preventive strategies to reduce the risk of progression to OC. These strategies may include lifestyle modifications, such as quitting tobacco use and reducing alcohol consumption, as well as improving oral hygiene practices.

Treatment Interventions: Treatment options for PMOLs aim to prevent or reverse the progression to cancer. Depending on the specific condition, treatment may involve surgical removal of the lesion, topical medications, laser therapy, or photodynamic therapy. Close monitoring and follow-up care are typically recommended to ensure that any changes or recurrences can be promptly addressed.

***Patient Education and Awareness*:** Identifying and discussing PMOLs with patients creates an opportunity to educate them about the risks associated with certain habits like smoking or excessive alcohol consumption. It also raises awareness about the importance of regular oral health examinations and encourages individuals to seek professional help if they notice any changes or abnormalities in their mouth.

The importance of the dentist in the diagnosis and treatment of PMOL and OC

Dentists have an important role in the diagnosis and treatment of PMOLs and OCs (51). These can be listed as:

***Regular Dental Check-Ups*:** Most people visit their dentist for routine check-ups at least once or twice a year. During these appointments, dentists thoroughly examine the oral cavity, including the lips, gums, tongue, cheeks, and throat, looking for any abnormalities or suspicious lesions.

***Expertise in Oral Anatomy*:** Dentists are highly knowledgeable about the anatomy of the oral cavity, which enables them to identify any unusual changes or deviations from normal tissue appearance. They are trained to differentiate between normal variations and potentially malignant lesions.

***OC Screenings*:** Dentists often perform OC screenings as part of routine dental check-ups. These screenings involve a visual examination of the oral tissues, palpation of the head and neck region to check for enlarged lymph nodes, and the use of specialized tools like OC screening devices or toluidine blue staining to detect suspicious areas.

Referral for Further Evaluation: If a dentist identifies any suspicious lesions or areas of concern, they can promptly refer the patient to an oral and maxillofacial surgeon or an oral pathologist for a detailed examination, biopsy, and histopathological analysis. Early referral for further evaluation is crucial for an accurate diagnosis and appropriate management.

***Patient Education*:** Dentists also play a vital role in educating their patients about the risk factors associated with oral cancer, such as tobacco and alcohol use, sun exposure, and HPV infection. They can provide guidance on lifestyle modifications, oral hygiene practices, and the importance of self-examination for early detection.

It is crucial to maintain regular dental visits and promptly report any changes or concerns related to the oral cavity to your dentist**.**

Conclusion

OC often starts as a small, painless lesion in the mouth, it may go unnoticed by individuals until it progresses to more advanced stages. Early detection and appropriate treatment of these lesions can significantly reduce the risk of developing OC. It is crucial to note that not all PMOLs will progress to cancer, but it is essential to have them evaluated by a dental or medical professional. Dentists are trained to recognize the signs and symptoms of OC during routine dental examinations, allowing for early detection and timely intervention. Dentists' expertise in oral health, regular check-ups, and the ability to identify suspicious lesions make them key healthcare professionals in diagnosing and managing OC.

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Chapter 19

MALE INFERTILITY

Sevilay ERİMŞAH[1]

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1. Sperm maturation

1.1.Spermatogenesis

Spermatogenesis is the process of production and development of sperm from the spermatogonia. Spermatogonium, located on the basal lamina in testicular tissue, is approximately 12 μm sized stem cells. These cells differentiate into primary spermatocytes, which will divide by mitosis and take their place. Primary spermatocytes form the beginning of meiosis phase of spermatogenesis. After formation of primary spermatocytes, DNA replication and crossing over will occur. Each diploid primary spermatocytes reduce the number of chromosomes to half and also differentiates into two haploid secondary spermatocytes (1).

Secondary spermatocyte goes into prophase of second meiotic division without DNA synthesis. Then sister chromatids separate in metaphase. After division, two haploid spermatids are formed with 1n chromosome and 1c amount of DNA.

Spermiogenesis is the final stage of spermatogenesis. Spermatids develop into developing spermatozoa to transfer the paternal genome to the egg cell. Cell division does not occur at this stage, round spermatids undergo morphological changes. They mature into spermiums about 7-8 μ in diameter. In spermatogenesis, chromatin condensation also occurs in sperm and somatic histones in sperm are replaced by sperm-specific protamine proteins (Fig. 1).

Spermatids have a distinctive Golgi complex, mitochondria, free ribosomes, and endoplasmic reticulum organelles. Small PAS+ proacrosomal granules separated from the Golgi complex accumulate in vesicles in the cytoplasm. They form a single acrosomal vesicle as an acrosomal cap that develops in front of the spermatid nucleus.

In the acrosome phase, the condensed nucleus of the spermatid flattens and elongates. Cytoplasmic microtubules from the posterior margin of the acrosome they form the cuff, a cylindrical sheath that extends towards the posterior pole of the spermatid. For flagellum development, centrioles move to the opposite pole of the acrosome to its surface. At the opposite pole of the acrosome, the centrioles develop to form the neck of the sperm in the future. Nine microtubules develop from the centrioles attached to the head structure and form dense outer fibrils towards the inner part of the tail. This region, which consists of the proximal and distal centriole, where the nucleus joins with the flagellum, is called the connecting piece. Mitochondria form a thickened region called the middle part around the flagellum. This part is where the energy required for the sperms to move is located. Acrosome functions as a specialized lysosome that houses hydrolytic enzymes (2).

1.2. Sperm chromatine condensation

Protamines are small, lysine and arginine rich core proteins that are replaced by histones that package DNA during the late haploid phase of spermatogenesis. Condensation occurs in the core chromatin when somatic histones are replaced by protamines. This fixes and protects the genomic DNA of the sperm. After the replacement of somatic histones with protamines, the nucleosomes disappear and straight chromatin fibers line up to condense the core material.

In spermiogenesis, the majority of histones are regularly replaced by protamines. The strong interaction between the positively charged protamines and the negatively charged DNA skeleton facilitates binding and causes chromatin condensation. The nucleus elongates and condenses, the cuff migrates caudally. Maturation is complete when the core condenses, the cuff begins to disintegrate, and the outer dense fibers are fully organized.

At the beginning of spermiogenesis, haploid spermatids show a typical nucleosomal chromatin structure. Abundant nonribosomal RNA transcription at this stage has activities. However, in the later stages of spermiogenesis, the bead-like chromatin structure that exists due to the classical nucleosome structure leaves its place to straight chromatin fibers. This structure assembles side by side in strands and they no longer transcribe (Fig.1).

As a result, very dense, insoluble and stable chromatin series are formed with the addition of protamines. Condensed sperm chromatin ensures that the genetic structure is preserved during the transport of the paternal genome between the reproductive systems (3). For successful fertilization, histone proteins in the sperm cell must be replaced with protamines and highly packaged.

In addition, this event facilitates the formation of nuclei required for the progressive movement of the sperm, while protecting the paternal DNA from degenerative molecules. In addition, the protamines in the structure of the sperm are necessary for the oocyte to complete meiosis and for a successful fertilization. During fertilization, protamines from the mature sperm genome are replaced by histones in the oocyte genome that are suppressed in metaphase II. Thus, the oocyte completes metaphase II and the polar body is excreted. Any abnormality in the protamine condensation can cause damage to sperm DNA and affect fertilization (4).

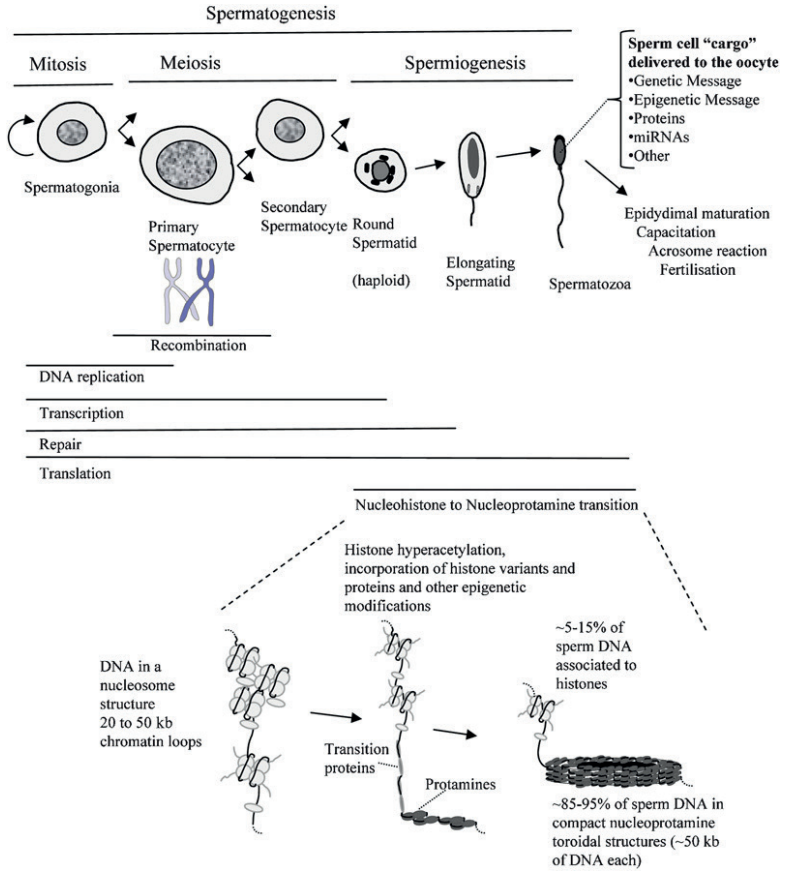

Figure 1. Spermatogenesis and chromatin condensation: *a pattern of chromatin repackaging from the solenoid loop in somatic cells to the ring loop seen in sperm (5).*

1.3. Sperm DNA integrity

Sperm DNA integrity is very important for successful fertilization and healthy embryo development. In the later stages of spermatogenesis, spermatogenesis remodels its nucleus molecularly to preserve condensation and genetic material. Testicular and post-testicular (ROS) transport of spermatozoa play a role in the histopathology of sperm.

Environmental factors, genomic mutations and chromosomal factors may affect all process that can lead to abnormal chromatin related to fertility that occur during spermatogenesis. While the oocyte has the ability to repair a significant amount of damaged sperm DNA, extensive damage may exceed its repair capacity, potentially affecting normal development (6). The molecular mechanisms underlying sperm DNA damages. Today, it is thought to be due to 3 main reasons;

1-Abnormal chromatin packaging

2- Reactive oxygen species

3- Apoptosis

In order to reduce the cyclical stress that occurs in sperm DNA, temporary DNA breaks occur with the loosening and opening of chromatin. DNA fragmentations are repaired by topo 2 before spermatogenesis. However, in cases where the fragments are not repaired, DNA fragmentation can be observed in the ejaculated sperm.

Elevated levels of ROS have been linked to spermatozoa DNA fragmantation. The low ROS parameter has a significant role in sperm maturation. Semen has antioxidants that safeguard paternal genom. However, excessive ROS production exceeding the antioxidant capacity in seminal plasma and the male reproductive system can lead to sperm DNA damage. There is a positive association between DNA fragmentation and the presence of reactive oxygen species. Analysis of semen from infertile men reveals high levels of ROS in approximately 25% of these individuals. The sources of ROS in semen include leukocytes, immature sperm, and sperm with abnormal head morphology (7).

Apoptosis is a programmed cell death process that occurs in various tissues in the body. In the testicles, apoptosis serves to regulate the production of germ cells and eliminate damaged germ cells. Sertoli cells can only support a limited number of germ cells, necessitating the reduction of germ cell population. The initiation of this apoptotic pathway is thought to involve the interaction between Fas ligand (FasL) secreted by Sertoli cells and Fas protein present on the surface of germ cells. However, recent research indicates that apoptosis may not always occur in germ cells of mice with FasL defect. In individuals with poor seminal parameters, high levels of Fas expression have been observed in spermatozoa, often in semen. This has led to the hypothesis that sperm with DNA damage and Fas expression undergo an "unsuccessful apoptosis" process, where apoptosis is initiated but the apoptotic pathway remains incomplete (8).

2.Male infertility

Infertility, as defined by the World Health Organization (WHO, 2013), refers to the inability to conceive a child after two years of regular unprotected sexual intercourse. Male factor infertility has become a significant contributing factor in infertility cases today. The primary causes of male infertility include varicocele (25%), genital tract obstruction (15%), testicular failure (15%), cryptorchidism (14%), idiopathic factors (12%), genetic conditions (8%), infections (3%), ejaculation dysfunction (3%), hormonal dysfunction (2%), immunological conditions (2%), cancer (0.5%), and systemic diseases (0.5%).

While various diagnostic methods are available to assess male reproductive potential, their capabilities are limited. The WHO conventional semen analysis values are commonly used to assess semen parameters, aiding in the clinical evaluation of male fertility potential in vivo. However, it's important to note that although semen parameters provide insights into factors such as sperm morphology, sperm penetration ability, and embryo development, they do not serve as absolute indicators of infertility (9).

Approximately half of infertile couples experience male factor infertility. Routine semen analysis, which includes parameters such as seminal volume, pH, sperm count, motility, and morphology, remains a valuable method for assessing male fertility. However, it is important to note that around 15% of males with infertility exhibit normal semen analysis results, highlighting that a definitive diagnosis of male infertility cannot always be made through routine semen analysis alone. In recent years, there has been an increasing focus on investigating the role of sperm DNA integrity in male factor infertility, with suggestions that it may serve as a better predictor of male infertility compared to routine semen analysis.

Infertile men tend to have higher rates of sperm DNA damage compared to fertile men, and this DNA damage negatively impacts their fertilization potential. High levels of sperm DNA damage are often associated with poor seminal parameters, including decreased sperm count, motility, and normal morphology. Interestingly, it has been reported that 8% of men with normal semen parameters still exhibit sperm DNA damage.

Assisted reproductive techniques (ART) such as intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) are the main options for addressing male subfertility. The choice between IUI, IVF, or ICSI is determined based on semen quality and the clinical presentation of the patient. Each clinic may have its own criteria for selecting quality sperm, as there are no strict rules in place. Sperm quality plays a crucial role in the success of IVF. However, conventional semen parameters alone may not provide comprehensive information about paternal factors that could impact embryo development. Parameters such as volume, sperm count, motility, or morphology cannot accurately predict the blastocyst rate or fertilization outcomes in vitro. Additionally, sperm DNA integrity and chromosomal abnormalities are not routinely assessed through semen analysis, despite their significance in diagnosing and treating male infertility. Paternal factors, including genetic factors like chromatin defects, DNA damage, aneuploidy, microdeletions, and mutations, can have an impact. Moreover, sperm with high levels of DNA damage can still fertilize the oocyte and affect embryo development (10).

3. Semen analysis

Semen analysis serves as the initial step in the laboratory assessment of male infertility. Since semen analyzes can show high variability, semen samples should be taken repeatedly (at least 3 days of abstinence) and evaluated. According to the results of semen analysis, we can comment on the prognosis of male infertility. Semen analyzes contribute greatly in determining and classifying the severity of the male factor (11).

3.1.Conventional analysis

The World Health Organization (WHO) reports a detailed methodology for semen analysis. The semen sample is ideally collected in the laboratory. The procedure should be carried out at room temperature, as the sample must be examined within a short period of time after collection. Morphology evaluation is also performed in sperm samples whose liquefaction period has been completed and analyzed under a microscope (Table 1)(12).

The Reference Limits of a Semen (WHO 2010)	
Ejaculate volume (mL)	≥ 1,5
Total sperm count (million)	≥ 39
Sperm concentration (million/mL)	≥ 15
Total sperm count (%)	≥ 40
Progressive motility (%)	≥ 32
Vitality (live sperms, %)	≥ 58
Sperm Morphology (normal forms, %)	≥ 4
pH	≥ 7,2

Table 1. *The reference limits of a semen analysis (*The World Health Organization *2010)*

3.1.Spesific Tests to Determine Sperm DNA Integrity

A growing number of tests have been developed to assess sperm DNA integrity. These tests used to determine sperm DNA integrity mechanisms differ from each other. Some of these tests measure sperm chromatin anomalies and some directly measure DNA strand breaks.

Hypoosmotic Swelling Test: Live sperm swell when exposed to special solutions, but dead sperm do not. With this test, it is possible to distinguish between live but immobile sperm in assisted reproductive techniques.

Capacitation, Acrosomal Reaction and Sperm Penetration Tests: It is used in cases such as fertilization failure. ICSI and IVF are the preferred treatment methods if there is insufficient evaluation of sperm in any of these tests.

Sperm Viability Staining: This test distinguishes between live but non-motile sperm and non-living sperm. It can help detect the presence of necrospermia (13).

Sperm Chromatin Structure Analysis (SCSA): SCSA was first described 25 years ago. The basis of this assay is based on the fact that sperm chromatins with chromatin anomalies are more prone to acid and temperature denaturation. Using the metachromatic property of Acridine Orange, SCSA measures the sensitivity of acid-induced denaturation of sperm DNA. By treating cells with acid using flow cytometry, the degree of DNA denaturation can be determined with the green to red color transition resulting from the metachromatic property of Acridine Orange. Parameters obtained using SCSA are indicated in studies as an indicator of DNA fragmentation, which is a measure of DNA denaturation (14).

Acridine Orange Test (AO): Metachromatic property of Acridine Orange with color transition from green to red. It is a measure of DNA denaturation susceptibility using with this feature, it is similar to SCSA. However, this method is simpler and cheaper than SCSA. Because it can be observed directly with a fluorescent microscope. There is no need to use flow cytometry like SCSA and special SCSA techniques. However, some negativities such as uncertain colors, fading quickly, and heterogeneous staining may cause difficulties during microscopic examination.

Toluidine Blue (TB): Toluidine blue is one of the main dyes used to determine sperm chromatin integrity. Residual phosphates of sperm with loosely packed chromatin or defective DNA are more prone to staining with basic dyes such as toluidine blue. Therefore, unstained or light-colored sperm seen in light microscope examinations are normal.

While they are evaluated as having chromatin condensation, those with blue and purple staining are evaluated as damaged sperms with chromatin condensation defects.

Aniline Blue (AB): It is one of the acidic dyes used to evaluate sperm DNA integrity. Sperm with damaged DNA often contain remnants of histone proteins. This residues lead to looser DNA packages. Loose chromatin packages increase access to nucleoprotein groups, making DNA prone to staining with acidic dyes such as aniline blue (15).

TUNNEL: The method is used to directly show the amount of DNA breakage in sperm. In this method, deoxyuridine triphosphate (dUTP) is combined into single-stranded and double-stranded DNA fragments with the catalysis of Tdt enzyme. DNA breaks associated with dUTP can be marked and observed and measured with light microscopy, fluorescence microscopy or flow cytometry. Then the sperms are TUNEL positive and TUNEL positive. The percentages are determined according to the total sperm population by grouping them as negative.

In Situ Nick Translation Analysis (NT): NT analysis similar to TUNEL analysis dUTP in DNA breaks calculate their merger. However, unlike the analysis of both double and single-stranded DNA breaks in TUNEL, only single-stranded DNA breaks are detected by DNA polymerase enzyme in NT analysis. Although it is a simple test compared to other analysis methods, its sensitivity is less.

Single Cell Gel Electrophoresis Analysis (COMET): Single cell gel electrophoresis, also known as Comet analysis, is an alternative test that directly assesses DNA breaks in sperm. In this technique, the chromatin structure of sperm is embedded in agarose gel and subjected to electrophoresis. Subsequently, the DNA is stained with a fluorescent dye that binds to DNA molecules. During electrophoresis, low molecular weight DNA fragments, including both single-stranded and double-stranded DNA, migrate away from the head of the comet and form a characteristic "tail" pattern. On the other hand, high molecular weight DNA remains in the head region of the comet. The extent of DNA damage is quantified by measuring parameters such as tail length and fluorescence intensity through imaging. Sperm with a higher number of DNA breaks exhibit longer tail lengths and increased fluorescence intensity. Comet analysis provides a direct evaluation of DNA integrity and can be used as a complementary method alongside other assays to assess sperm DNA damage (16).

4. Sperm selection methods

Semen includes seminal plasma, cellular debris, epithelial cells. Sperm accounts for approximately 5% of the total volüme of semen. The remaining volume consists of seminal plasma, cellular debris, and leukocytes. Seminal plasma serves multiple functions in supporting and protecting spermatozoa. It provides energy sources and nutrients that are essential for sperm motility and survival. Additionally, seminal plasma plays a role in protecting spermatozoa from the acidic environment of the vagina, which can be detrimental to their viability. Furthermore, cellular debris and leukocytes present in semen contribute to the overall composition and function of seminal fluid, potentially influencing fertility outcomes. However, keeping sperm in semen for a long time adversely affects its fertilization capacity. Moreover, semen that has not undergone a washing process can be a significant source of contamination that may jeopardize the entire IVF (in vitro fertilization) program. Therefore, selected and processed washed semen is an essential step for assisted reproductive techniques (ART).

Despite the availability of various methods for selecting sperm, our understanding of the physiological processes of sperm selection within the female reproductive system remains limited. There is ongoing debate as to whether this selection is based on specific characteristics of sperm

or occurs randomly. In a study by Cohen and McNaughton, the process of sperm selection for fertilization in the female genital tract of rabbits was investigated. Sperm collected from the rabbit uterus were inseminated into the uterus for a second time and compared with washed sperm and ejaculated sperm from different genotypes. The findings revealed that sperm obtained from the upper part of the female genital tract exhibited higher fertilization ability. Sperm selection for fertilization is one of the functions performed by the female genital tract. However, due to ethical reasons and technical limitations, similar studies have not been conducted in humans, despite the promising results obtained in the rabbit study (17).

While most sperm isolation techniques focus on selecting motile sperm, relying solely on sperm movement is not sufficient to determine fertilization and pregnancy outcomes. Alternative methods like electrophoretic filtration and density gradient methods (DGC) utilize sperm charge or density but lack physiological relevance. Despite this, pregnancies can still be achieved using sperm obtained through these methods. However, the relationship between charge, density, and fertilization capacity remains unclear. Moreover, comprehensive studies have revealed significant correlations between parameters such as curvilinear and straight-line velocity and the success rates of in vitro fertilization (IVF), which are crucial for achieving successful fertilization and pregnancy. Important sperm parameters including apoptosis, DNA integrity, membrane development, and ultrastructural characteristics, which are often overlooked in routine sperm preparation techniques like DGC and swim-up, exist. However, research indicates that these features can be negatively affected by sperm preparation techniques, ultimately reducing the success of IVF (18).

4.1.Swim up

The swim-up method involves the active movement of sperm from liquefied semen towards a medium spread on the upper surface or a pre-washed cell pellet. It enables the selection of highly motile sperm without the use of specific selection elements. However, a drawback of this method is that sperm come into close contact with each other, as well as cellular debris and leukocytes, which can result in the production of high levels of reactive oxygen species (ROS). Saturated fatty acids, which are present in high concentrations in the sperm plasma membrane, are susceptible to lipid peroxidation caused by ROS in the semen. This can ultimately lead to reduced sperm function and motility.

In this method, ROS can decrease the proportion of spermatozoa with normal chromatin condensation, resulting in reduced success rates in IVF cases where swim-up is applied (17,18).

4.2. Density Gradient Centrifugation (DGC)

Density Gradient Centrifugation (DGC) is a widely used sperm preparation method in assisted reproductive techniques. It involves the use of a saline suspension surrounded by polyvinyl pyrrolidone or colloidal silica particles (15-30 nm). Compared to the swim-up method, DGC is technically more complex as it requires control over different media, centrifugation speed, and time. The method was developed in 1951 and later adapted for human semen.

The principle of DGC relies on the differences in sperm density. Sperm with normal morphology, which have highly compacted chromatin, migrate towards the region of higher gradient density. The process of DGC involves two steps: isolation of sperm using DGC and subsequent washing of the isolated sperm fraction. The semen is gently layered on top of the DGC medium and centrifuged at 300g for 20 minutes. After centrifugation, the supernatant is discarded, and the pellet containing the isolated sperm is resuspended in fresh medium. A second centrifugation is performed to wash the sperm, resulting in the recovery of a purified fraction containing highly motile sperm.

However, DGC does have certain drawbacks. The use of expensive medium and separate equipment for sperm centrifugation can increase costs. Moreover, the centrifugation process itself may induce membrane stress and cell damage. The potential impact of defective spermatozoa used in IVF on DNA damage within the embryo is still a topic of debate, and the centrifugation process has been suggested to potentially affect sperm DNA. In a study conducted by Mahfouz et al. in 2010, levels of H2O2 and O2 in sperm were examined, and it was reported that centrifugation could increase superoxide dismutase activity, which could have a negative effect on sperm quality (17, 20).

4.3. Migration sedimentation

This method was developed in 1984. The method combines the swim-up and sedimentation steps. It requires a plastic or glass tube with a special cone inside. The spermatozoa float towards the supernatant and then sediment towards the inner cone. Compared to methods that involve a centrifugation step, such as DGC (Density Gradient Centrifugation), the swim-up method is considered relatively harmless and gentle (21)

The migration sedimentation method is suitable for obtaining a sufficient number of functional motile spermatozoa. In 1996, it is demonstrated that after 2-3 hours of incubation, an adequate number of motile sperm could be obtained for ICSI in cases of oligo- and asthenozoospermia. These researchers showed that compared to DGC, there was a greater improvement in sperm parameters and a decrease in

the number of apoptotic sperm. However, the efficiency of this method is much lower compared to DGC and swim-up methods. Therefore, this method does not have a wide application in current use. Zavos et al. (2000) proposed a multi-compartment tube for functional sperm recovery in ART, suggesting it as a potential advancement for this method in the future (22).

4.4. Glass wool filtration method

The glass wool filtration method, introduced by Paulson and Polakoski in 1977, is a technique that allows for the separation of motile sperm from semen using densely packed glass wool fibers. This method takes advantage of both the forward movement of spermatozoa and the filtration effect of the glass wool. Here's how the process works:

1. The ejaculate is placed in the upper compartment of a glass wool column.

2. Semen passes through the glass wool column along with a suitable medium.

3. The mixture is then centrifuged at a relatively low speed of 300g for 10 minutes.

4. After centrifugation, the selected sperm, which have passed through the glass wool, can be collected and utilized in assisted reproductive techniques (ART) procedures (23).

One disadvantage of this method is the potential risk of sperm damage due to the presence of glass wool particles in the filtrate. Care must be taken to minimize any potential adverse effects on sperm quality during the filtration process.

Compared to other sperm separation methods, the glass wool filtration technique allows for the utilization of the entire volume of the ejaculate, resulting in a significantly higher total count of motile sperm. Therefore, this method is particularly useful for patients with oligo- and/or asthenospermia, where the concentration and motility of sperm may be low. However, it's worth noting that after the separation of functional sperm from immotile ones, a subsequent centrifugation step is often required to remove seminal plasma and further concentrate the sperm before use in ART procedures.

In addition to separating spermatozoa, this method effectively separates leukocytes. Although a clean sperm fraction can be obtained with this method, it is not widely used due to the lack of standardized protocols and commercial kits (24).

4.5. Electrophoretic filtration

Electrophoretic filtration method, sperm selection based on their negative charge. The highest quality sperm typically carries the most negative charge can be isolated from other cells such as leukocytes and cellular debris. The semen sample is loaded into a chamber and then balanced for 5 minutes with an electrical field consisting of a variable voltage between 18-21 V and a constant current of 7 mA using a specific buffer. The preparation of this method only requires 5 minutes, which can be valuable in minimizing oxidative stress on the sperm.

Studies have compared the effectiveness of sperm isolation methods such as Density Gradient Centrifugation (DGC) and MicroFlow CS-10, and have found no statistically significant difference in fertilization and pregnancy rates between the two methods. However, researchers have noted that the MicroFlow CS-10 method offers the advantage of shorter washing time compared to DGC.

These findings suggest that both DGC and MicroFlow CS-10 can be equally effective in isolating viable sperm for assisted reproductive techniques. The choice between the two methods may depend on other factors such as cost, availability, and specific laboratory protocols. It is important for healthcare professionals and laboratories to evaluate and select the most suitable method based on their specific requirements and resources (25).

5. Novel sperm selection method

Microfluidic chip method is a method developed using microfluidic technology. In this method, cell or particle selection and manipulation is performed using micro-scale channels and fluids.

The microfluidic chip system developed by Schuster et al. consists of two parallel streams: semen stream and ambient stream. In this system, parallel currents can be realized without interference between small channels designed with nano technology. In this way, the recovery of sperm with high motility (at a high rate of 98%) can be achieved (17,26,27).

The microfluidic chip method does not require steps such as centrifugation or pipetting that can damage cells or increase the production of ROS. Therefore, sperm with no cell debris, low sperm DNA fragmentation rate and high motility can be obtained. In microfluidic technologies, sperm selection can be performed using principles such as chemical attractants, fluid flow, and thermotactic forces.

In passive migration-based microfluidic technologies, none of these forces are used, the passage relies only on the sperm's own movement. In passive microfluidic systems, the most motile sperm reach the targeted

exit point in the optimum time, while the less motile or non-motile sperm are left behind. This system has generally been studied on mouse sperm. The optimum time and pore width for the passage of human and mouse sperm through the polycarbonate membrane filter have been investigated, and more comprehensive and new studies are needed on this new method.

Microfluidic technology has emerged as a powerful tool in biological research. It utilizes small fluid volumes and enables the integration, automation, and manipulation of high-throughput analysis. Microfluidics focuses on studying fluid behavior at the submicroliter scale and finds applications in various fields such as cell biology, diagnostics, and analytic medicine.

Microfluidic devices have emerged as a promising tool for sperm sorting and selection in assisted reproductive techniques. These devices mimic in vivo conditions and offer advantages such as precise control, improved efficiency, and reduced DNA fragmentation compared to conventional methods.

One example of a microfluidic device for sperm sorting is the laminar flow-based device developed by Cho et al. This device effectively isolated motile sperm cells from non-motile sperm cells and other cells using the concept of laminar flow. The stable flow of fluid was achieved through a passive pump, resulting in higher purity of motile sperm cells in the outlet chamber.

Microfluidic devices can be classified based on fabrication techniques (emulsion droplet microfluidic method and digital/droplet-based microfluidics) as well as their function. Type 1 devices focus on isolating motile sperm for in vitro fertilization, improving upon the swim-up approach. Type 2 devices selectively trap or sort sperm based on physical characteristics other than motility, aiming to preserve the fertilization capacity of subfertile samples. Type 3 devices enable noninvasive capture and evaluation of individual sperm cells while maintaining their viability.

These microfluidic devices offer advancements in sperm sorting and selection, providing researchers and clinicians with more precise and efficient tools for improving the success rates of assisted reproductive techniques. Continued research and development in this field hold great potential for enhancing fertility treatments.

Overall, microfluidic technology provides innovative solutions for sperm sorting and analysis, offering enhanced efficiency, selectivity, and compatibility with various biomedical applications. Further research and development in this field hold promising potential for improved diagnostics and advancements in assisted reproductive technologies (ART) and IVF (27,28).

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Chapter 20

SPINAL ANESTHESIA COMPLICATIONS AND TREATMENT

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Spinal anesthesia is a type of regional anesthesia that involves the injection of a local anesthetic into the subarachnoid space of the spinal cord to numb a specific area of the body. It is commonly used for surgeries on the lower abdomen, pelvis, and lower extremities. While spinal anesthesia is generally safe and effective, like all medical procedures, it is not without potential complications. In this chapter, we will discuss the most common complications of spinal anesthesia, their causes, and how they can be prevented or treated.

1. Hypotension

One of the most common complications of spinal anesthesia is hypotension, which is a sudden drop in blood pressure that can lead to dizziness, nausea, and in severe cases, loss of consciousness. Hypotension occurs because spinal anesthesia blocks sympathetic nerve fibers that control blood vessel tone and heart rate, resulting in a decrease in blood pressure. Hypotension can be prevented by administering intravenous fluids before and during the procedure, maintaining the patient in a supine position, and by giving vasoconstrictors such as ephedrine, phenylephrine or methoxamine.

2. Bradycardia

Bradycardia is a slow heart rate, which can also occur as a result of spinal anesthesia. It is caused by the same mechanism as hypotension, and is usually associated with hypotension. Bradycardia can be treated by administering intravenous atropine.

3. Headache

Another common complication of spinal anesthesia is a headache. A spinal headache is usually caused by a leak of cerebrospinal fluid (CSF) through the puncture site in the dura mater. The headache can range from mild to severe and may be accompanied by neck pain, nausea, and dizziness. The headache can usually be treated with bed rest, hydration, and analgesics, but if the headache is severe or persistent, a blood patch may be needed to seal the puncture site.

4. Nausea and vomiting

Nausea and vomiting are common side effects of spinal anesthesia, particularly in patients who have a history of motion sickness. It is caused by the anesthetic affecting the vestibular system, which is responsible for maintaining balance and equilibrium. Antiemetic medication such as ondansetron or metoclopramide can be given to prevent or treat nausea and vomiting.

5. Back pain

Some patients may experience back pain after spinal anesthesia. This is usually caused by the positioning of the patient during the procedure, and

can be prevented by ensuring that the patient is positioned correctly and comfortably during the procedure.

6. Urinary retention

Urinary retention, or the inability to empty the bladder, can occur after spinal anesthesia, particularly in male patients. This is caused by the anesthetic affecting the nerves that control the bladder muscles. Urinary retention can be prevented by inserting a urinary catheter prior to the procedure or by closely monitoring the patient's urine output and catheterizing if necessary.

7. Respiratory depression

Spinal anesthesia can cause respiratory depression, which is a decrease in respiratory rate and depth. This is more common in elderly patients and patients with pre-existing respiratory disease. Respiratory depression can be prevented by closely monitoring the patient's oxygen saturation and respiratory rate, and by administering supplemental oxygen if necessary.

8. Infection

Spinal anesthesia can also carry the risk of infection. The risk of infection can be minimized by using sterile techniques during the procedure, and by administering antibiotics prophylactically.

9. Neurological complications

In rare cases, spinal anesthesia can cause neurological complications, such as nerve damage, paralysis, or seizures. These complications are usually associated with traumatic needle insertion or the use of an incorrect needle size

1.Post-spinal hypotension

It is caused by a decrease in blood pressure following the injection of local anesthetic into the spinal fluid. This can lead to symptoms such as dizziness, lightheadedness, nausea, and in severe cases, loss of consciousness.

Treatment for post-spinal hypotension involves conservative measures such as laying the patient down and elevating their legs to increase blood flow to the heart and brain. In addition, intravenous fluids may be administered to help increase blood volume and blood pressure. Medications such as ephedrine or phenylephrine may also be given to help increase blood pressure.

Prevention of post-spinal hypotension involves careful monitoring of the patient's blood pressure before and after the procedure, and adjusting the dose of local anesthetic accordingly. Patients at high risk for post-spinal hypotension, such as those with pre-existing hypertension or on medications that lower blood pressure, may be given prophylactic medications to prevent hypotension.

It's important for patients to report any symptoms of post-spinal hypotension to their healthcare provider, as prompt treatment can help prevent further complications such as cerebral hypoxia or cardiac arrest.

In summary, post-spinal hypotension is a common complication of spinal anesthesia that can be effectively treated with conservative measures and medications. Prevention involves careful monitoring of blood pressure and adjusting the dose of local anesthetic accordingly, as well as prophylactic medications for high-risk patients.

2. Bradycardia

Bradycardia, or a slower than normal heart rate, is a potential complication of spinal anesthesia. The local anesthetic used during the procedure can affect the nerves that regulate heart rate, leading to a decrease in heart rate.

Factors that can contribute to the development of bradycardia include the use of certain medications such as beta-blockers, pre-existing heart disease, or advanced age. Symptoms of bradycardia can include lightheadedness, dizziness, and fainting.

To prevent bradycardia during spinal anesthesia, the patient's heart rate should be closely monitored throughout the procedure, and any underlying medical conditions that may increase the risk of bradycardia should be identified and managed. If bradycardia does occur, treatment options include administering medication such as atropine to increase heart rate or other medications that can improve cardiac output.

In rare cases, severe bradycardia may require the use of a temporary pacemaker to regulate heart rate. Patients with a history of heart disease or those who are taking medications that can affect heart rate should be closely monitored for the development of bradycardia after spinal anesthesia.

Overall, while bradycardia is a potential complication of spinal anesthesia, it is relatively uncommon and can be effectively managed when detected early. Patients should be informed of the potential risks and benefits of the procedure before undergoing spinal anesthesia, and any concerns should be discussed with the anesthesiologist or healthcare provider.

3.Postspinal headache

Also known as post-dural puncture headache, is a common complication following spinal anesthesia or a lumbar puncture. It is caused by leakage of cerebrospinal fluid through the puncture site, leading to low pressure in the brain and spinal cord.

Symptoms of postspinal headache include a headache that worsens when sitting or standing and improves when lying down, neck pain, nausea, and dizziness.

Treatment for postspinal headache typically includes conservative measures such as rest, hydration, and over-the-counter pain relievers such as acetaminophen or ibuprofen. Caffeine may also be helpful in alleviating the headache, as it acts as a vasoconstrictor and can help decrease the size of blood vessels in the brain.

If conservative measures are not effective, a blood patch may be necessary. This involves injecting the patient's own blood into the spinal fluid to help seal the leak that is causing the headache. The blood forms a clot that seals the hole in the dura, allowing the cerebrospinal fluid to remain at a normal pressure.

A blood patch is a safe and effective procedure that can be done as an outpatient procedure. It is typically performed by a trained anesthesiologist or pain specialist.

Prevention of postspinal headache involves using a smaller gauge needle for spinal anesthesia or lumbar puncture, using an atraumatic needle that causes less trauma to the dura, and maintaining strict aseptic technique during the procedure.

In conclusion, postspinal headache is a common complication following spinal anesthesia or lumbar puncture, but can be effectively treated with conservative measures or a blood patch. Patients should report any symptoms of postspinal headache to their healthcare provider to ensure prompt treatment and prevent further complications.

4. Postspinal nausea and vomiting

It is caused by a combination of factors, including the effects of the local anesthetic on the gastrointestinal system, changes in blood pressure and circulation, and psychological stress.

Treatment for postspinal nausea and vomiting typically involves antiemetic medications such as ondansetron or metoclopramide to help alleviate symptoms. Rest and hydration may also be helpful in reducing nausea and vomiting.

Prevention of postspinal nausea and vomiting involves careful monitoring of the patient's blood pressure and hydration status, as well as providing psychological support to help reduce stress and anxiety. Patients at high risk for postspinal nausea and vomiting, such as those with a history of motion sickness or previous episodes of nausea and vomiting following anesthesia, may be given prophylactic medications before the procedure to prevent these symptoms.

It's important for patients to report any symptoms of postspinal nausea and vomiting to their healthcare provider, as prompt treatment can help prevent further complications such as dehydration or electrolyte imbalances.

In summary, postspinal nausea and vomiting is a common complication of spinal anesthesia that can be effectively treated with antiemetic medications, rest, and hydration. Prevention involves careful monitoring of the patient's blood pressure and hydration status, as well as providing psychological support and prophylactic medications for high-risk patients.

5. Postspinal urinary retention

It is caused by the effects of the local anesthetic on the nerves that control the bladder, leading to difficulty in emptying the bladder.

Treatment for postspinal urinary retention typically involves measures such as bladder catheterization, which involves inserting a tube into the bladder to drain urine. If catheterization is not feasible, medications such as bethanechol may be used to help stimulate bladder contraction and promote urine flow.

Prevention of postspinal urinary retention involves careful monitoring of the patient's urinary function and providing prophylactic measures such as bladder catheterization prior to the procedure for high-risk patients.

It's important for patients to report any symptoms of postspinal urinary retention to their healthcare provider, as prompt treatment can help prevent further complications such as urinary tract infection or bladder damage.

In summary, postspinal urinary retention is a common complication of spinal anesthesia that can be effectively treated with bladder catheterization or medications to promote urine flow. Prevention involves careful monitoring of the patient's urinary function and providing prophylactic measures for high-risk patients.

6. Postspinal backache

It is caused by the needle used to administer the anesthetic, which can cause trauma to the surrounding tissues and muscles, resulting in pain and discomfort.

Treatment for postspinal backache typically involves nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or acetaminophen to help alleviate pain and inflammation. In addition, physical therapy and gentle exercises may be helpful in relieving back pain and restoring normal movement.

Prevention of postspinal backache involves careful technique during the procedure, including the use of smaller needles and avoiding repeated punctures. In addition, patients may be advised to rest and avoid strenuous activities following the procedure to help reduce the risk of back pain.

It's important for patients to report any symptoms of postspinal backache

to their healthcare provider, as prompt treatment can help prevent further complications such as chronic pain or mobility issues.

In summary, postspinal backache is a common complication of spinal anesthesia that can be effectively treated with NSAIDs, physical therapy, and rest. Prevention involves careful technique during the procedure and advising patients to avoid strenuous activities following the procedure.

7. Postspinal nerve injury

It can occur due to trauma to the spinal cord or nerve roots during the administration of the local anesthetic, or due to nerve damage caused by inflammation or compression.

Symptoms of postspinal nerve injury may include numbness, tingling, weakness, or paralysis in the affected area. Treatment for postspinal nerve injury typically involves a combination of pain management, physical therapy, and in some cases, surgical intervention.

Prevention of postspinal nerve injury involves careful technique during the procedure, including the use of appropriate equipment and monitoring the patient for any signs of neurological damage. Patients at high risk for nerve injury, such as those with pre-existing neurological conditions or spinal deformities, may require special precautions or alternative methods of anesthesia.

It's important for patients to report any symptoms of postspinal nerve injury to their healthcare provider, as prompt diagnosis and treatment can help prevent further damage and improve outcomes.

In summary, postspinal nerve injury is a rare but serious complication of spinal anesthesia that requires prompt diagnosis and treatment. Prevention involves careful technique during the procedure and monitoring for signs of neurological damage, especially in high-risk patients.

8. **Respiratory depression**

Respiratory depression is a potential complication of spinal anesthesia, which is characterized by a decrease in respiratory rate and depth. This can result in low oxygen levels in the blood, which can be dangerous or even life-threatening.

Respiratory depression occurs because the local anesthetic used in spinal anesthesia can affect the nerves that control breathing, particularly in patients with pre-existing respiratory disease or in elderly patients. Other factors that can contribute to respiratory depression include the use of other medications that can depress breathing, such as opioids or sedatives, and the positioning of the patient during the procedure.

To prevent respiratory depression, the patient's respiratory rate and oxygen saturation should be closely monitored throughout the procedure, and supplemental oxygen should be administered if necessary. In addition, the use of medications that can depress breathing should be minimized or avoided, and the patient's medical history should be carefully evaluated to identify any risk factors for respiratory depression.

If respiratory depression does occur, prompt treatment is essential. The patient may need to be given oxygen or assisted with ventilation using a bag-mask device or a mechanical ventilator. Medications that can reverse the effects of the local anesthetic, such as naloxone or flumazenil, may also be used in certain cases.

Overall, while respiratory depression is a potential complication of spinal anesthesia, it is rare when proper monitoring and precautions are taken. Patients should be informed of the potential risks and benefits of the procedure before undergoing spinal anesthesia, and any concerns should be discussed with the anesthesiologist or healthcare provider.

9. **Infection**

Infection is a potential complication of spinal anesthesia, which can occur when bacteria or other pathogens enter the subarachnoid space during the procedure. The risk of infection can be minimized by using sterile techniques during the procedure and by administering antibiotics prophylactically.

Infection can cause symptoms such as fever, headache, neck stiffness, and back pain. In severe cases, infection can lead to meningitis, which is a serious inflammation of the membranes surrounding the brain and spinal cord.

If an infection is suspected after spinal anesthesia, prompt treatment is essential to prevent further complications. Antibiotics may be administered to treat the infection, and supportive care may be provided to manage symptoms such as fever and pain. In severe cases, hospitalization may be required for close monitoring and intravenous antibiotic therapy.

To prevent infection after spinal anesthesia, the anesthesiologist and healthcare team should adhere to strict sterile techniques, including the use of sterile gloves and drapes, and the proper cleaning and preparation of the injection site. Patients should also be screened for any pre-existing infections or medical conditions that may increase the risk of infection.

If a patient experiences symptoms of infection after spinal anesthesia, they should contact their healthcare provider immediately. Early diagnosis and treatment can help prevent the spread of infection and reduce the risk of serious complications.

Precautions to be taken to avoid complications after spinal anesthesia

To avoid complications after spinal anesthesia, there are several precautions that healthcare providers can take before, during, and after the procedure:

Patient selection: Screening patients for pre-existing medical conditions that could increase their risk of complications is important. For example, patients with a history of chronic pain, allergies, diabetes, or other medical conditions should be assessed and managed accordingly.

Informed consent: Patients should be informed about the risks and benefits of spinal anesthesia, and provide their informed consent before the procedure.

Proper positioning: The patient should be positioned properly during the procedure to avoid any strain on the back or neck. This may include using a headrest, a pillow or cushion, or adjusting the bed position.

Sterile technique: The procedure should be carried out using sterile technique to reduce the risk of infection.

Monitoring: The patient's vital signs, including blood pressure, heart rate, oxygen saturation, and respiratory rate should be closely monitored during the procedure and in the recovery period to detect any signs of complications early.

Hydration: Patients should be encouraged to drink plenty of fluids before and after the procedure to prevent dehydration.

Medication management: Patients should be given appropriate medications before and after the procedure to manage pain, nausea, and other side effects.

Follow-up care: Patients should be monitored closely in the days and weeks after the procedure to detect any signs of complications, and provided with appropriate follow-up care.

In summary, to avoid complications after spinal anesthesia, healthcare providers should take precautions such as patient selection, informed consent, proper positioning, sterile technique, monitoring, hydration, medication management, and follow-up care.

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Chapter 21

THE EFFECT OF INFERTILITY ON SEXUAL HEALTH

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Introduction

Infertility is a reproductive system problem defined as the inability to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. This problem affects millions of people of reproductive age, their families and communities (WHO, 2018). Although its incidence varies throughout the world, it is a very common public health problem (Küçükdurmaz & Taşkıran, 2015).

In the demographic studies conducted since the beginning of the 1950s, it has been determined that there has been a decrease in birth rates, especially in European countries. In addition, in recent years, population-based studies in developing and developed countries have revealed that the timing of the first birth is delayed. This situation has led to an increase in the number of couples who want to have children with assisted reproductive techniques and the number of health institutions providing services in this regard (Demirci & Beji, 2017).

It is estimated that an average of 48.5 million couples worldwide cannot have children (WHO, 2018, Demirci & Beji, 2017). In a population-based study conducted in China, one in seven couples in the UK was infertile (Datta et al., 2016), the prevalence of infertility was 15.5% (Zhou et al., 2018), and 24% in France (Slama et al. , 2012) was determined. It has been determined that infertility affects 30% of women of reproductive age in low-income South and Central Asia, Sub-Saharan, North African and Middle Eastern countries (Petraglia et al., 2013; Inhorn, & Patrizio 2015; Tsevat, et al., 2017). In our country, the rate of infertility varies between 10-20% among married couples (Çağlar & Oskay, 2020).

Every person has the right to enjoy the highest attainable standard of physical and mental health. Couples have the right to decide the number, timing and spacing of their children. Infertility can hinder the realization of these basic human rights (Zegers-Hochschild et al., 2013; WHO, 2018). Defined as an unexpected and sudden life crisis, infertility is an unexpected, perhaps unexplained, condition that has been diagnosed and treated over a long period of time, creates extreme stress and challenges adaptation mechanisms (Uğur, 2014). Psychological problems such as anxiety, stress, depressive symptoms, decreased self-esteem in infertility couples; guilt and shame lead to psycho-social problems such as marital problems, sexual dysfunction and decreased quality of life (Beji & Kaya, 2012; Algül & Aksun, 2014). Social expectations also cause the mourning and grief experienced by the infertile couple to deepen and become permanent (Beji & Kaya, 2012).

Social and familial pressures for the continuation of the family name consume infertile couples. In addition, the physical, psychological and economic strains of assisted reproductive techniques will continue to affect

couples in the future (Güleç et al., 2011). Infertility and its treatment cause different effects and life changes in men and women (Broeck et al., 2010). Infertility negatively affects the emotional states, social lives, marital relations and sexual lives of spouses (Şen et al., 2014). It has been reported that men and women may perceive their bodies and infertility differently, have different ways of coping with the problem, and exhibit different psychiatric symptoms. Regardless of whether the cause of infertility is female, women are more negatively affected by infertility. Because, even if the cause of infertility is in men, it is usually women who are exposed to complex interventional treatment methods (Güleç et al., 2011).

The general expectation from marriage in all societies is to have children. Couples who cannot have children feel responsible for the society and feel emotionally inadequate and under pressure (Durat et al., 2018). Expensive and tiring infertility treatments on the one hand and unsuccessful attempts to have children on the other hand affect sexual life problems in infertile couples (Bokaie et al., 2015). However, infertility, which also causes sexual discomfort, can cause couples to experience sexual dysfunctions by avoiding sexual intercourse, loss of pleasure and desire. The diagnosis and treatment process of infertility can sometimes take months or even years. In this process, the sexual intercourse of the spouses is completely aimed at achieving pregnancy. By determining the time of ovulation, the spouses have sexual intercourse especially during this period. Acts such as sexual desire and initiation of sexual intercourse may become meaningless at times other than the ovulation period, and sexuality may turn into an act performed only for reproduction for the spouses. (Bacanak, 2019; Broeck et al., 2010). The individual may feel sexually inadequate due to his infertile situation, and this feeling of inadequacy may cause a decrease in the interest of the spouses and their satisfaction with sexual life (Valsangkar et al., 2011). It is suggested that the fact that couples undergoing infertility treatment have to be constantly vigilant about ovulation and menstrual cycle and that sexuality cannot get rid of its "reproductive" purpose may cause sexual acts to become "meaningless" especially for female partners, outside of "fertile" times (Güleç et al., 2011).

Due to the aggressive and time-consuming diagnosis and treatment process of infertility, it can negatively affect the sexual lives, marital relations, economic and psychosocial situations of couples and create a life crisis that complicates the couple's adjustment (Koçak & Büyükkayacı Duman, 2016). Depression, anxiety, sexual dysfunction increase in women, emotional well-being and quality of life are negatively affected (Carter et al., 2011). In the treatment of male-induced infertility, men may experience many problems such as low self-esteem, loss of self-confidence, thoughts of incompetence, isolation, loneliness, guilt, fear, anger, shame and disappointment. Emotional stress, crisis and not being a father also change the perspective of being a man.

While men accept the inability to have children more easily, women are more willing and determined about treatment (Beji & Kaya, 2012).

Sexuality is a natural and healthy part of life. According to the World Health Organization, sexual health is defined not only as the absence of disease and infirmity, but also as a state of physical, emotional, mental and social well-being related to sexuality. Sexual dysfunction (SD) is defined as the inability to have sexual intercourse despite the person's desire for various reasons. sexual health; It requires a respectful approach to sexuality and sexual relations, as well as enjoyable and safe sexual experiences that are free of coercion, discrimination and violence (WHO, 2019). Starting from the intrauterine period, sexuality is shaped by the biological, physiological and psychological characteristics of the individual, the religious beliefs, values, attitudes and laws of the society, and life periods such as adolescence, pregnancy and menopause (Bozdemir & Özcan, 2011; Aydın & Beji, 2011). 2013; Smoke, 2018). The person's health status, dyadic adjustment, self-concept and previous experiences affect the sexual development process (Koç & Oskay, 2015; Aydın & Beji, 2013).

Sexuality and sexual health are welcomed in every part of the society in terms of general health, well-being and quality of life (Zeren & Gürsoy, 2018). However, traditional taboos are still influential on sexuality from past to present, as it is perceived as forbidden topics that are not easily talked about (Gürsoy & Gençalp, 2010). Due to these traditional taboos, individuals are exposed to insufficient, incomplete and incorrect information about concepts such as sexuality, sexual health and reproductive health. This conservative view of sexuality, which is a basic instinct, creates obstacles for individuals to express their sexual problems and find solutions (Zeren & Gürsoy, 2018). Individuals who have problems with sexual health are not only affected physically, but also in terms of mental health. In this case, it can negatively affect family and community health in circles (Bozdemir & Özcan, 2011).

When we look at the literature, there are four types of relationships between sexuality and infertility: Men and women with sexual dysfunction may experience infertility problems afterwards. Sexual functions may be affected due to tests and applications for the diagnosis and treatment of infertility. Stress, depression and anxiety can be experienced due to the effects of infertility on feelings such as sexual focus and guilt, inferiority, aggression and passivity. Psychological effects and sexual reactions are associated with each other in infertile individuals (Aydin & Beji, 2013). In this crisis period, where assisted reproductive techniques have a negative effect on the sexual relations of couples, it is important to provide counseling and support to individuals in order to combat the crisis (Akın & Şahin, 2020).

The Effect of Infertility on Women's Sexual Life

Although infertility is actually a problem of the married couple, as individuals, men and women can show different emotional reactions. In comparative studies, it was observed that clinical depression and anxiety were more common in women than in men. Regardless of the source of infertility, women feel more guilty, take more responsibility and are exposed to more medical tests. This explains the emergence of more psychological problems in women (Rockliff et al., 2014).

Sexual dysfunction is a problem that affects both men and women intensely (Çoban & Dinç, 2013). In a study conducted with 150 couples receiving infertility treatment, it was determined that 77% of women and 23% of men had sexual dysfunction problems (Çoban & Dinç, 2013). Turan et al. (2014) found that treatment duration of three years or longer in infertile women is the main risk factor for sexual dysfunction. Arslan et al. (2008) in a study examining the sexual dysfunction and quality of life of infertile couples, it is reported that women have more sexual dysfunction and more significant decreases in their quality of life compared to men. In a study conducted with 200 infertile women in India, it was reported that 105 of the women had one or more sexual problems. The most frequently reported complaints are decreased frequency of intercourse and anorgasmia (Ramezanzadeh et al., 2006). In another study, it was reported that only 7% of 100 infertile Iranian women had normal sexual functionality and arousal disorder was the most common (Nelson et al., 2008).

Facchin et al. (2019) in his study with 269 infertile women, 30% of the women experienced sexual dysfunction, as a result of the research, in fact, infertility may not be associated with sexual dysfunction, and the quality of female sexual function may be more related to certain psychological risk factors such as social, relational and sexual concerns about infertility. is considered. Millheiser et al. (2010) in the study of 119 infertile and 99 fertile women aged 18-45 years, sexual dysfunction was found in 25% of fertile women, while this rate was found to be 40% in infertile women. Women diagnosed with infertility were found to be at higher risk for sexual dysfunction compared to fertile women. Wekker et al. (2018) In a randomized controlled study conducted with 177 infertile women (84 interventions, 93 controls) in the Netherlands, lifestyle changes (physical activity, diet and behavior changes) were recommended for the intervention group for 6 months, while the control group received routine infertility care. At the end of 6 months, it was determined that the intervention group experienced less sexual dysfunction and vaginal lubrication problems compared to the control group.

The Effect of Infertility on Men's Sexual Life

Compared to women, infertile men were found to experience less sexual satisfaction. It is emphasized that this situation may be related to the psychological pressure caused by making the timing of sexual intercourse dependent on the woman's ovulation cycle (Monga et al., 2004). Considering the 3-6 year period in infertile couples caused by the male factor, it was emphasized that the sexual satisfaction levels were low in both partners (Drosdzol & Skrzypulec 2009). Sexual dysfunctions that develop during the infertility process affect the marital relationship by causing deterioration in sexual satisfaction over time (Shindel et al., 2008) Tao et al. (2011) stated in their review that infertile men have lower self-esteem in relation to changes in stress level due to treatment. Dooley et al. (2014) in their study, male partners among infertile couples were evaluated in terms of "ideal male condition", "mental health", "relationship satisfaction" and "self number", and it was reported that male partners were negatively affected in terms of these factors. Boorjian et al. (2007) found in their study with infertile couples that there was an 11% decrease in libido in men and 28% in women.

Lee et al. (2001) found that more than 50% of men with azoospermia in couples struggling with infertility entered the impotence process. Sahebalzamani et al. (2018) In a study conducted in Iran, 46.7% of men were found to have sexual dysfunction. It has been determined that increased health literacy in men is associated with increased sexual function and sexual satisfaction. It has been reported that approximately 20% of male partners in infertile couples have mild to moderate erectile dysfunction, as well as decreased sexual satisfaction after diagnosis of infertility or in the case of female sexual dysfunction (Shindel et al., 2008a). In the study of Drosdzol and Skrzypulec (2008) in Poland, 206 infertile and 190 fertile couples were compared in terms of their sexual lives. Erectile dysfunction was found in 23.9% of infertile men and 13.7% of fertile men. It has been determined that male infertility is the biggest cause that negatively affects male sexual function.

In another study conducted with 100 infertile couples in Tehran, only 2% of male participants were found to have severe erectile dysfunction (Khademi et al., 2008). Shindel et al. (2008a) in a study with 121 infertile men, it was determined that 18% of the men had mild and 4% had moderate erectile dysfunction. In a study conducted with 73 couples in the USA, it was found that half of the men had premature ejaculation problems (Shindel et al., 2008b).

Nursing Approach to Infertile Couples with Sexual Dysfunction

The infertility nurse plays an important role as a counselor to support infertile couples and guide them through the treatment process. Some highlights of the counseling role of the infertility nurse (Aşçı & Kızılkaya Beji 2012; Karakoyunlu & Öncel 2009; Koçak et al., 2016; Güngör & Kızılkaya Beji 2015):

Information and Education: The infertility nurse provides detailed information about infertility to couples and provides education about treatment options, procedures and processes. The nurse is an important resource to inform couples about the treatment process and to help them establish realistic expectations about expected results.

Emotional Support: The infertility nurse provides support for the emotional needs of couples. Infertility can cause stress, sadness, and anxiety among couples. The nurse empathizes with couples, provides emotional support and guides them through the emotionally challenging process.

Communication Skills: The nurse helps them improve communication between couples. The infertility process can cause communication difficulties between couples. The nurse encourages couples to strengthen their communication skills and communicate in an emotionally supportive environment.

Supporting the Decision-Making Process: During infertility treatment, couples may have to make a series of decisions. The nurse helps couples make informed decisions, explains the options, explains the risks and benefits, and supports couples in their treatment decisions.

Resource Referral: The nurse provides couples with information about infertility resources and directs other professionals who can provide the support and services they need. These resources may include various areas such as psychological counselling, support groups or financial counseling.

Infertility nurse provides important support and counseling to infertile couples during the treatment process. This role of nurses allows couples to feel better informed, emotionally supported and safe during the treatment process. In this way, couples can have a stronger foundation to deal with infertility.

Infertility nursing; Evaluation of the couple is a special area that provides care to couples with a sensitive, holistic and evidence-based approach in the diagnosis and treatment processes. Providing services that cover the basic roles of nurses such as the evaluation of individuals with infertility problems (history taking, assistance with examination and application of tests, monitoring of daily records, etc.), planning and implementation of the determined treatment, prevention of complications in the treatment process and counseling, strengthens the communication between the couples and positively affects the treatment success. (Koçak et al., 2016; Güngör & Kızılkaya Beji 2015).

The infertility nurse should be able to define the individual's sexuality in order to ensure the safety of the patients and to provide counseling on issues related to sexuality and sexual health. He should be comfortable with sexual

matters, be a good listener, and avoid judgmental attitudes. Should have knowledge about sexual functions and developmental status of sexuality in life cycle. Likewise, they should develop their counseling and communication skills. Because the evaluation of infertile individuals and taking a detailed psychological history enable to predict the problems that may occur during the treatment process, and the determination of family and social relations from the beginning can be effective in reducing the severity of the psychological effects that may occur (Aşçı & Kızılkaya Beji 2012; Karakoyunlu & Öncel 2009).

Sexual dysfunction is a health problem that significantly affects the quality of sexual life of couples and should be evaluated within the framework of sexual counseling (Bitzer et al., 2007; Lamont, 2012). They stated that they were concerned about whether unresolved sexual problems affected the quality of sexual life of couples, intimacy between spouses and treatment processes, and they wanted to get information about this issue (Southard & Keller 2009; Bal, 2014). It was stated that the patients first shared their concerns and problems regarding sexuality and sexual health with the nurses. However, very few nurses can evaluate sexuality (Akıncı et al., 2010). Because nurses state that they do not have enough knowledge and skills to provide counseling on sexual health and how diseases affect sexual life (Saunamaki et al., 2010). However, nurses can help patients by increasing their sexual knowledge and skills, assessing the sexual needs of infertile couples and choosing the best strategy to meet these needs (Saunamaki et al., 2010). For this, first of all, nurses should have enough information about sexuality and sexual function or dysfunctions, be a good listener, not show a judgmental attitude, develop sexual communication skills, and be able to take a detailed sexual history. Using a model that integrates information about sexual behaviors with general care in this process can also help nurses to learn and evaluate sexual health and thus examine possible concerns and problems faced by this patient group (Saunamaki et al., 2010; Bal, 2014)

Conclusion

It is a fact that men and women may encounter sexual dysfunctions during the infertility treatment process. Therefore, it is important to investigate these issues as part of the treatment process and to take the necessary precautions. Evaluation of the sexual life of infertile women and interventions for sexual problems should be a part of health services.

Before a diagnosis of infertility is made, the nurse's counseling role is of great importance. Nurses should take an effective role in informing and educating individuals or couples. These trainings should be in the form of providing information on issues that directly affect individuals or couples and informing the public in general.

In-service training programs should be organized for health workers in order to ensure that sexual education and counseling services for infertile couples meet the requirements and be of high quality. In this way, healthcare professionals can provide couples with appropriate support and guidance.

Infertility is a condition that directly or indirectly affects couples' lives in many ways. Sexuality is an important component that needs to be addressed in this process. Infertility treatment centers and specialists should provide the necessary medical and psychological support so that couples can better manage this difficult process. This support can help couples cope with problems that affect their sexual functioning and relationships, and can reduce stress during the treatment process.

As a result, it is important to pay attention to sexual dysfunctions, integrate them into treatment and provide appropriate support to couples during the infertility treatment process. Training programs for health workers should be organized and sexual education and counseling services should be provided to couples. In this way, it will be possible to address the problems related to the sexual lives and relationships of the couples and to find solutions.

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Chapter 23

PSYCHOLOGICAL EFFECTS OF COVID-19 ON DENTISTRY STUDENTS

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INTRODUCTION

Covid-19, which was first reported in Wuhan, China in December 2019, spread to many countries in a short time and caused a worldwide epidemic (Lai et al. 2020). After China, most of the countries where Covid-19 has spread have implemented nationwide quarantines to prevent the transmission of the disease (Dubey et al. 2020). While this strategy has been an effective practice in controlling the spread of Covid-19, it has had several negative effects on the economy and people's lives. In a recent study, high levels of income loss and deterioration in quality of life in the general population were reported due to the Covid-19 outbreak (Tran et al., 2020).

In addition, there have been developments that changed the lives of university students such as adapting to new living conditions, leaving universities and adapting to online learning platforms. Faculties with applied laboratory courses involving artistic performance were more negatively affected in this process (Kecojevic et al., 2020).

While the first aim of dentistry education is to specialize in theory, the second and more important aim is to gain clinical competence. Clinical practice is carried out by the 4th and 5th grade students themselves, under the supervision of a professional (Cao et al., 2020). Previous studies have shown that dental students have higher levels of stress than the general population (Odriozola-González et al., 2020; Xiong et al., 2020).

In an already stressful dental education, common challenges such as uncertainty about how lessons work, lack of guidance and fear of exposure to Covid-19 can further increase the stress on dental students. The biggest disadvantage for 4th and 5th year dental students is that clinical education cannot be sustained by direct patient care, which is an important component of the dental curriculum. This situation creates some concerns in terms of professional knowledge and skills in students who are about to graduate, and in terms of performing the profession after graduation. The biological and physical repercussions of an epidemic are the focus; however, mental health problems related to the covid-19 outbreak are also important issues to be addressed.

GENERAL INFORMATION

Coronaviruses

Coronaviruses are enveloped viruses with a positive single-stranded RNA genome. The size of the helical symmetric nucleocapsids is about 26-32 kb. With this feature, it is the virus with the largest genome investigated among RNA viruses (Sexton et al., 2016). Coronaviruses have a fundamental similarity in their organization and genome expression (Su et al., 2016). Previously, coronaviruses were thought to only cause enzootic infections in a

number of animals, including certain birds and mammals, but recent findings show the diversity of this family of viruses with different antigenic groups and that they can also infect humans (Schoeman & Fielding, 2019). These rapidly mutating RNA viruses can cause mild cold symptoms in humans, as well as life-threatening diseases such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), which are on the list of infectious diseases threatening societies published by the World Health Organization. (Sweileh, 2017).

Other Infections Spread by Droplet

Dentists are at risk of transmission of infections caused by various microorganisms such as mycobacterium tuberculosis, hepatitis B and hepatitis C viruses, streptococci, staphylococci, HSV type 1, HIV, mumps, rubella (Smith et al., 2001). It shows that they were infected with the virus as a result of injuries with sharp instruments contaminated with blood or saliva. Since nasopharyngeal secretions carry the virus, there is also the possibility of aerosol transmission of infection (Withers, 1980). Since sharps are frequently used in the dentistry routine, it carries a great risk of exposure to infectious diseases. All healthcare professionals, especially dentists, who use instruments contaminated with mucosa, blood or body fluids, should pay due attention to the precautions to be taken in order to minimize the risk of infection.

Covid-19 and Dentistry

Covid-19 disease is transmitted from person to person directly by droplets, nasal and eye mucosa, respiratory system or indirectly by contacting the mouth, eye and nasal mucosa after contact with infected surfaces (To et al., 2020). Aerosol that occurs during the treatments applied by dentists and working directly in the respiratory tract are among the factors that increase the risk of transmission of Covid-19 disease. During the operation of high-speed and ultrasonic instruments, droplets containing the patient's saliva and blood particles hang in the air and these particles can accumulate on the surfaces and cause contact infection. It has also been shown that coronaviruses can survive on glass, metal and plastic surfaces for several days and maintain virulence levels from 2 hours to 9 days at room temperature (Kampf et al., 2020). A Covid-19 carrier can infect 2.2 to 3.58 people. This means that the number of people with coronavirus could double within 7 days (Velavan & Meyer, 2020). As a matter of fact, the first case in our country was seen in March 2020 and then spread rapidly.

Psychological Effects of Covid-19

After the World Health Organization declared the new coronavirus a pandemic on March 11, 2020, many governments had to impose certain restrictions and take precautions regarding social and working life. As a result, while infection rates continue to increase, the psychological aspects

of this disease have also emerged due to the increased death rates associated with Covid-19, the loss of some people's jobs, and social isolation (Lee et al., 2020). It is known that people tend to experience clinically significant fear and anxiety when infectious diseases turn into epidemics. Thus, the high infection and death rates associated with Covid-19 have caused widespread fear and anxiety (Taylor et al., 2019). With this epidemic, many health workers became infected with the virus and went into quarantine while performing their duties. Studies have shown that stress factors that threaten life and health during the epidemic have a greater impact on young people and those exposed to regular contact with patients (Huang & Zhao, 2021; Temsah et al., 2020).

Economic, social, psychological and educational changes have occurred worldwide since March 2021, when the World Health Organization declared the Covid-19 epidemic (Nelson et al., 2020). However, as this outbreak does not affect different segments of the population equally, several target groups have been studied specifically for the negative psychological impact of Covid-19. One of these groups is university students (Wu et al., 2021). One of the first measures taken to contain the epidemic and stop the spread of the virus has been to close universities with significant associated psychological symptoms (Viner et al., 2020). University education had to become online and students had to adapt quickly to this change. Students had to adapt to disruptions in academic activities, transition to online education, homework management, projects and other ongoing evaluations (Cao et al., 2020). As a result of all this, students began to fear that the epidemic could have a serious impact on their careers (Idoiaga Mondragon et al., 2021; Sahu et al., 2020). They also went through a period of concern about their health, safety and well-being of their families (Zhai & Du, 2020). Social distancing, curfews, and other restrictions and norms to stop the spread of the virus are factors associated with increased rates of depression in the general population and especially among college students (Chang et al., 2020). This increased level of depression appears to be associated with fear of being infected and difficulty adjusting to personal, academic, and professional restrictions (Shigemura et al., 2020). Previous studies have shown that dental students had significant depression before the epidemic (Galan et al., 2014). Pre-existing psychological problems may have resulted from the high emotional burden of dental work due to long working hours, workload, and clinical demands (Puthran et al., 2016). In addition, in clinical practice, dental students must work closely with patients. This proximity has put them at higher risk of viral exposure and being infected with Covid-19 (Kateeb et al., 2021). In fact, droplets and aerosols produced during most dental procedures are a potential danger for transmission of Covid-19 (García et al., 2021).

Precautions to be Taken in Dentistry Regarding Covid-19

According to the statement made by the New York Times, dentistry is one of the professions most exposed to Covid-19 transmission (Gamio, 2020). In order to avoid infections and the spread of the virus, it is necessary to establish a clinical protocol to be applied in the workplace. In daily clinical practice, materials contaminated with the patient's saliva and unit surfaces should be considered as a source of contamination for the dentist, assistant staff and the patient himself. Saliva and blood droplets accumulating on surfaces, inhalation of aerosol created by rotary instruments and ultrasonic hand tools pose a risk to people in this environment. Therefore, the use of disinfectants and personal protective equipment is vital for the dental profession (Luzzi et al., 2020). The sudden spread of Covid-19 has created the need to change both preventive and therapeutic protocols in dental practice.

In various studies, it has been revealed that a questionnaire to obtain as much information as possible about the symptoms of the patients and their relatives in the last 14 days should be done via telephone (Marui et al., 2019; Meng et al., 2020; Peng et al., 2020). Measuring the body temperature of the patients when they come to the dental clinic and postponing the dental treatment in case this value exceeds 37.3 is another recommended measure (Peng et al., 2020). In patients with Covid-19 infection, the American Dental Association (ADA) guidelines recommend rescheduling treatment at least 72 hours after symptoms resolve (American Dental Association Interim Guidance for Management of Emergency and Urgent Dental Care. United States of America, 2020). In another study by Meng et al., the required recovery time before performing dental treatment in infected patients was stated as 30 days (Meng et al., 2020).

The American Dental Association recommends avoiding the overlap of two or more appointments, and if this is not possible, the minimum distance between one patient and the other is at least 2 meters. In addition, asking patients to wait in their vehicles or close to the dental clinic if possible, and informing them by phone or message when it is their turn is stated as another precaution that can be taken (American Dental Association ADA Interim Guidance for Minimizing Risk of COVID-19 Transmission. United States of America, 2020). Regarding the pedodontics clinic, parents of pediatric patients should be informed that they should attend as few appointments as possible, wear protective masks, wait in the waiting room, and not participate in the treatment of the patient in order to avoid the risk of aerosol inhalation (Luzzi et al., 2020).

Various studies have been conducted to show how mouthwashes just prior to dental treatment change the amount of bacteria, viruses and fungi present in the oral biofilm and reduce the risk of cross-contamination due to aerosols; Costa et al. emphasized the importance of using 0.12% and 0.20%

chlorhexidine mouthwash in a study in 2019. (Marui et al., 2019). Because Covid-19 is sensitive to oxidation, Peng et al. recommended gargling with 1% hydrogen peroxide or alternatively 0.2% povidone-iodine (Peng et al., 2020).

Regardless of the type of treatment planned, healthcare professionals, especially dentists and auxiliary personnel, must comply with the protocols regarding personal protective equipment (Marui et al., 2019; Meng et al., 2020; Peng et al., 2020). Hair caps, safety glasses, surgical masks or N95 masks, disposable surgical gowns, special shoes should be used Personal protective equipment should be used as specified in the instructions in the user manual and disposed of as special waste (Lo Giudice et al., 2020)

Literature Review on the Psychological Impact of Covid-19 on Dentistry Students

Studies have revealed several sociodemographic variables that may be associated with higher rates of depression during the Covid-19 outbreak. While higher rates of depression are observed among women in general (Özdin & Özdin, 2020), there are also studies suggesting that there is no difference between men and women (Cénat et al., 2021; Prati & Mancini, 2021). In addition, significant differences were found in the prevalence of depression among university students by country (Ochnik et al., 2021).

Depression has become one of the most important psychological consequences of the Covid-19 pandemic among university students in general and dentistry students in particular.

Among the studies analyzed, a study conducted by Khanagar & Alfadley in Saudi Arabia in 2020 found a relatively lower prevalence of depression among dental students, with a prevalence of 20.9% (Khanagar & Alfadley, 2020). However, Hakami et al. found a prevalence of 60.7% in the same country in 2021 (Hakami et al., 2021). On the other hand, the highest depression rate among dentistry students was found in a study by Keskin et al. in Turkey in 2021 with 75.3% (Keskin et al., 2021). Previous meta-analyses of depression levels in the general population during the pandemic have reported prevalence rates of 22.8% (Pappa et al., 2020) to 33.7% (Salari et al., 2020). As for university students, a large variability has been reported in terms of depressive symptomatology. While previous reviews have determined that the prevalence rate of depression during the epidemic ranged from 12.2% (Z.-H. Wang et al., 2020) to 31.2% (Batra et al., 2021), more recent reviews in 2021 revealed these rates to be 1%. 32 (Deng et al., 2021) to 37% (C. Wang et al., 2021).

It has also been found in pre-epidemic studies that the prevalence of depression among dental students was generally higher than that observed among university students. (Uraz et al., 2013)

In a study, the most important difference in depression was found between geographical regions. Studies in Asia (42%) showed a higher prevalence of depression compared to those in Europe or the Americas (27%). In contrast, a previous meta-analysis study of the general population and university students found that Asia was the region with the lowest prevalence of depression. (Batra et al., 2021).

The Covid-19 pandemic has created a long-term psychological burden for people. Therefore, it is important to establish preventive intervention programs to reduce transmission, especially in groups with a high risk of transmission, such as dental students. It is also important to continue collecting data on the impact of Covid-19 on dental students, especially in countries where this data is still not available.

CONCLUSION AND RECOMMENDATIONS

Considering that university students in general and dentistry students in particular have higher depressive symptoms, various short and long-term psychological consequences may occur in the personal, academic and professional lives of students due to the lack of interventions and policies to improve mental health in universities. For this reason, universities should also carry out various studies to adapt their education policies to this new situation.

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Chapter 23

LYMPHEDEMA

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Introduction

The circulatory system of mammals consists of both cardiovascular and lymphatic systems. The closed circulatory system, known as the cardiovascular system, is necessary for the exchange of waste products resulting from hormones, nutrients, oxygen and metabolism between tissue and blood, and this is provided by blood circulating in the body. In contrast, the lymphatic system, which works to assist the cardiovascular system, is a blind-tipped, one-way system that helps in addition to the cardiovascular system by absorbing the interstitial fluid leaking into extracellular spaces (1).

Lymphedema is a kind of localized tissue expansion that occurs as a result of lymphatic fluid retention and lymphatic drainage in the intercellular space. Lymphomed consists of two components: primary and secondary lymphedema. Primary lymphedema occurs mostly as a result of abnormalities that occur during the development of lymphatic vessels. Secondary lymphedema is mostly hereditary, not acquired, and occurs as a result of a systemic disease, trauma or surgery (2).

Lymphedema cannot be cured. However, if properly diagnosed and treated, the progression, worsening of the disease and potential complications that may occur as a result can be reduced (3).

Unlike the cardiovascular system, not much research has been done for the lymphatic system yet.. Since most studies focus on blood circulation and related disorders, lymphatic circulation is still the part of the circulatory system that is not sufficiently interested and ignored (4).

History of the Lymphatic System

Since ancient times, physicians have observed structures that make up the system known today as the lymphatic system. (5-8) Hippocrates described 'white blood at nodes' in the oldest known medical instrument on the Cloths. Aristotle has described fibers containing colorless fluid between the nerves and blood vessels. In the following times, lymphatic drainage, lymph and lymphatic anatomy began to be explored much more and attracted attention. Although the lymphatic system was first described by Gasparo Aselli in 1627', the blood circulation system was described by William Harvey in the same century. Swammerdam is the first to discover the lids in the collector lymphatic vessels using wax injections with classic works in the mid-1600s. Louis Petit proved that breast cancer spread to axillary lymph nodes in the early 1700s. As a result, the anatomy of the lymphatic system was fully revealed in the early 19th century. While research on blood circulation has continued to increase tremendously in the last century, research on the lymphatic system has progressed at a rather slow pace. It is also controversial today whether lymphatic vessels as the origin of the lymphatic system are

budding through the vessels, whether they are made up of lymphangioblasts or both (9). Although the lymphatic spread of many cancers has been observed for centuries, unfortunately it is still not understood how lymphatic metastasis developed.

Definition

Lymphedema is a long-lasting, progressive and stubborn disease in which the drainage of intercellular fluid into the lymphatic system is impaired and causes rich fluid accumulation from protein in the intercellular area. As a result of this fluid accumulation, an inflammatory process develops that induces lipogenesis and fat accumulation with excessive growth in connective tissue, causing fibrosis. As a result of all this, individuals with this disease develop irreversible enduration and fibrosis in the affected area (10).

Anatomy

The lymphatic circulatory system has five main structures: capillaries, collection vessels, lymph nodes, bodies and channels. Lymph fluid is formed by the intercellular fluid entering the lymphatic capillaries. There are several pressure factors, such as oncotic pressure hydrostatic pressure, which affect the formation of this. The liquid that enters the lymphatic capillaries then travels through the capillaries through at least one set of lymph nodes, but often flows into the numerous collection arteries. Collector vessels enter and leave the lymph nodes where the lymph communicate with the blood through nodal vasculature. These ships then flow into larger bodies that connect to the canals. Finally, the channels insert the lymph into the bloodstream and complete the liquid transport cycle (11).

The lymphatic system is arranged in three parts: soft tissue lymphatics, intestinal lymphatics, and liver lymphatics. Liver and intestinal lymph fluid produces about 80% of body lymph, making up the most important and functional part of the lymphatic system.. However, the normal lymph-producing structures of the liver and intestines and their pathological changes are little known. The lymphatic structure in the extremities consists of two parts: a superficial (epifacial) lymphatic system that collects lymph fluid from the skin and subcutaneous tissue; muscle, a deeper lymphatic system that empties subfaccial structures consisting of bone and deep blood vessels . The lower limb superficial and deep lymphatic systems merge in the pelvis, while the upper limb combines superficial and deep systems in the axillary region. Since both lymphatic drainage structures are interconnected, the lymphatic fluid participates in lymph transport from the surface when there is lymphatic obstruction developing in the deep lymphatic system(12). Superficial and deep systems are emptied in diametrically opposite amounts. Sub-regional transport carries slower than the epifacial transport system and transfers less lymph fluid. All three systems are connected and eventually

merge in the cistern chyli region to form the chest channel (TD) (13). TD usually starts with cistern chyli, which is seen as a fusiform dilation at the level of L1-L2 vertebra. Behind the lower part of the right crumb column, the aorta and lower vena cava are between (IVC). Cisterna chyli is formed by the combination of the left lumbar body, the intestinal body, and in rare cases the right lumbar body, and these connections have significant heterogeneity. As an analogy, the thoracic canal is the trunk of an inverted lymphatic tree, and a simple approach is a thoracic channel reverse tree as the trunk (14) .

Lymphovenous connections drain the lymphatic fluid into the venous system. The combination of the thoracic canal and left subclavian and internal jugular veins forms the main lympho-venous connection. It should be emphasized that although the body has several additional normal lympho-venous connections, functional or physical obstruction of the lymphatic arteries flowing downwards can cause the formation of the novel, pathological lympho-venous connections that can become clinically significant. (15,16)

Physiopathology

The lymphatic system consists of lymph nodes, tonsils, lymphatic organs such as thymus and spleen. These are all linked by a lymphatic vessel network that extends with venous blood circulation. The lymphatic circulatory system has three main functions: interstitial fluid drainage, oil absorption and immunological surveillance. Liquid leaking from the blood capillaries into tissue cavities is called interstitial fluid. The majority of interstitial fluid (90%) is reabsorbed and returns to the vascular system by venous microcirculation. The protein ratio of the remaining interstitial fluid (10%) is very high and is discharged through non-end lymphatic capillaries. This interstitial fluid with a high amount of protein becomes lymph when it reaches lymphatic capillaries. The resulting lymph fluid is carried by the collection of lymphatic arteries, filtered by its nodes and eventually returns to the circulatory system at the point where peripheral venous circulation enters the right heart (17). The average lymphatic flow rate per day is two or three lists (18). Unlike lymphatic capillaries, the collector lymphatic vessels have smooth muscle walls, so they can contract and move the lymphatic fluid.

It is characterized by interstitial fluid buildup caused by a decrease in drainage capacity due to lymphedema obstruction or lymphatic hypoplasia, and consequently tissue swelling. Chronic inflammation and the resulting reactive tissue fibrosis are caused by the decrease in the amount of oxygen in that area over time. The lymphatic system plays an important role in the functioning of the immune system. To regulate the response of the immense against antigens, the circulating lymph transmits various antigens and cells that offer active antigens to the lymph nodes (19). Lymphatic capillaries are abundant on the skin. Due to the accumulation of antigens in peripheral

tissues, lymphedema patients are prone to recurrent skin infections. Continuous inflammation development as a result of lymphedema and fibrosis in soft tissue are attributed to a TH2 immune response triggered by lymphatic stagnation (20).

Incidence and Prevelance

Lymphomidem is said to affect 200 million people globally. Women are more likely to be affected by lymphedema than men. Primary lymphedema is very rare and the frequency of estimation is 1 in 100,000 people. It usually occurs in infancy, but it can develop at any age (21,22). Secondary disease affects almost 99% of lymphedema patients. Secondary lymphedema affects one in every 1000 people, and the average age of the affected people is between 50 and 58 years (23-25). Secondary lymphedema is most common in the United States and Western countries, especially in individuals who have been lymphadenectomy or received radiation therapy for breast cancer treatment (26). However, it can also occur after any cancer that changes lymphatic flow. The most detected cause of my secondary lymphedema in the world is flariasis disease.

Lymphedema with lower limb is much more common than lymphedema with upper limb and is often associated with obesity, chronic venous insufficiency, infection, treatment of rapamycin in patients with kidney failure, uterine cancer, prostate cancer, lymphoma and melanoma (27) . The most common cause of lymphedema in the upper limb is breast cancer. Lymphedema affects about 30% of patients receiving irradiation therapy after mastectomy (28,29). Systematic review and metaanalysis of secondary lymphedema caused by cancer, the overall incidence is 15.5% (, the ratio varies according to malignancy) and the overall incidence 16% (upper limb; lower limb, melanoma) 28% (, 5% after upper limb therapy; lower limb, 28%). Patients receiving radiation therapy had the highest risk of lymphedema (31%)(30).

Primary Lymphedema

Primary lymphedema is rare and is caused by genetic abnormalities that cause lymphatic channel underdevelopment and dysfunction of the lymphatic flow system. Primary lymphedema can occur as an independent condition or as part of a larger syndrome. Most of the examples are autosomal dominant with variable expression and incomplete penetration.. Approximately 30% of primary lymphedema patients have detectable genetic abnormalities, often found in the VEGFR C signal path (31). Primary lymphedema has been associated with more than 20 gene lymphatic abnormalities (32). However, significant genetic heterogeneity has been detected(32). Primary lymphedema most often affects the lower extremities, but in rare cases it can also affect the genital organs and upper extremities(33). The incidence of lymphedema is twice as high as in girls(33).

Primary lymphedema is classified into three types according to the age of onset:

1.Congenital lymphedema (at birth or shortly after birth),

2.Lymphedema praecox (during adolescence or shortly)

3.Lymphedema tarda (advanced age).

As a result of mutations in the VEGFR3 gene, lymphatic hypoplasia is induced in Milroy's disease, which creates a defective valve structure and causes early lymphatic fluid absorption (34). Milroy's disease is a hereditary disease, other types of primary lymphedema often occur later. Primary lymphedema is more common in non-familial situations than in familial conditions. Since individuals with genetic abnormalities can also create lymphedema during adolescence or in the more advanced stages of life, we think it is simple and deceptive to categorize patients only by age, because it does not lead to certain phenotypic properties or a common pathobiological condition is not detected . A strict phenotype-genotype relationship should support optimum categorization.

Secondary Lymphedema

Primary lymphedema is significantly less common than secondary lymphedema. Disease processes are formed by obstruction of lymphatics as a result of obesity, trauma, recurrent infection, surgery, radiation therapy, cancer, malignancy treatment (35). Posturgical lymphedema, caused by lymph node dissection and postflebitic syndrome, are the most known causes of regional lymphedema in secondary countries (36). Rosacea can cause secondary periorbital lymphedema (37). Lymphatic dysfunction can be attributed to both chronic venous hypertension and venous ulcers (38,39). Secondary lymphatic injury from fluid overload affects 20% of individuals with chronic venous disease. Lymphedema caused by permanent venous disease is called phlebolimpedema. Genetic predisposition may also occur in secondary lymphedema (32,40).

Infection

The most common cause of secondary lymphedema worldwide is lymphatic filarya. A mosquito-derived nematode called Wucheria bancrofti causes (round wolf). The disease affects people living or traveling in endemic countries, primarily in India and Sub-Saharan Africa. Mosquitoes leave the larvae of adult roundworms into the skin. The larvae move from here to lymphatics, preventing lymphatic drainage. The information form of the World Health Organization, updated in March 2017, states that more than 120 million people were affected and the disease disrupted and neutralized about 40 million people (41).

Lymphedema can in rare cases be caused by herpesvirus infection. In a case study, an acquired lymphedema developed in a patient with herpetic whiteness (42). Recurrent cellulite and erysipelas cause cutaneous lymphatic injury and can cause lymphedema holding the single limb (43). Lymphogranuloma venerum is a sexually transmitted disease caused by chlamidia trachomatis and can cause external genital lymphedema (44). Scrofula is a disease caused by the retention of lymph nodes in the neck, especially as a)result of tuberculosis, and is a very rare cause of infection-induced lymphedema (45).

Therapeutic interventions associated with malignancy

Removing lymph nodes after mastectomy for breast cancer treatment and after surgical dissection for melanoma treatment disrupts lymphatic flow. Subsequent radiation therapy induces cutaneous lymphatic channel loss and nodal fibrosis, making lymphatic regeneration impossible (46). Lymphedema caused by cancer-related treatments often occurs as swelling of permanent unilateral extremities. However, surgery on the cervix and prostate can result in edema of both lower extremities (47,48).

Podoconiosis

Podoconiosis is an unpleasant variety of filariasis caused by barefoot walking to irritating clay soil with silica microparticles. It is the second most common cause of endemic lymphedema in the world (49). Lymphatic inflammation occurs by absorbing microparticles through the skin, subendothelial lymphatic edema develops, and eventually the lymphatic channel is blocked. Podoconiosis is endemic in the plateaus of tropical Africa, in the north of India and in Central America (50).

Morbid Obesity

Morbid obesity is an independent risk factor for lymphedema development. A BMI of more than 60 kg / m2 causes decreased lymphatic flow (25). The increase in adipose tissue in dependent areas increases lymphatic vascular obstruction (35). Decreasing physical activity of obese patients is another factor contributing to lymphatic obstruction. A large umbilical pannus can restrict lymphatic flow to the lower extremities. Large localized lymphedema is a non-malignant growth of lymphoproliferative tissue, which occurs as a large pedunculated lump in morbid obese people (51,52).

Clinical Classification

To recognize and classify lymphedema, the International Association of Lymphology (ISL) combines two criteria: softness and firmness. As the stage rises, the prognosis worsens. The subclinical or secret state where edema does not appear despite the decrease in lymphatic transport is called stage

0 lymphedema. Volume increase in stage I lymphedema is considered as 20% and mild lymphedema. The volume increase is stage stage II, which is between 20% and 40%, and is a moderate severe condition. The increase of over 40 percent is also called stage III and is the stage that is severe (53).

Lymph transport in lymphedema with stage 0 (aka stage Ia) decreases, minor changes in tissue fluid and composition develop, and changes in subjective symptoms occur. With stage 0 lymphedema subclinical and a generally unsymptomatic secret condition, some patients feel that their limb is getting heavier. Stage 0 may not be permanent or remain silent for a long time before obvious lymphedema progresses.

Stage I lymphedema is usually the edema that develops within 24 hours and improves with the raising of the limb. It has a relatively higher protein content compared to venous edema. Pitting is possible. In addition, a proliferation may occur in various types of proliferating cells of soft tissue. This is known as reversible edema. The stage I described above is known as the lightest lymphedema stage.

Stage II lymphedema is characterized by larger changes in solid structures. The rise of the limb in this stage is generally not sufficient to pass tissue swelling and pitting can be seen. As the subcutaneous fat and fibrosis tissue increases in stage II, pitting in the limb decreases and may not even be seen. This stage is known as self-recycling lymphedema and is classified as moderate lymphedema.

In stage III lymphedema, pitting is no longer seen. This stage is a lymphostatic filtiazis that contains trophic skin changes such as acanthosis, changes in skin character and thickness, more fat accumulation, fibrosis tissue development and excessive growth of the wart. Stage III is related to the most severe degree of lymphedema. It should be noted that a limb can contain all of the simultaneous various lymphedema phases.

Euphoria of the limb environment: The American Physical Treatment Association (APTA) uses the measurement of the limb circumference, an anthropometric measurement of the limb when classifying lymphedema (54). It measures the affected and unaffected part of the limb difference. This method is used for general upper limb.

Classification according to this measurement is as follows (54)**:**

Lightweight lymphedema - maximum 3 cm environmental difference

Medium lymphedema - maximum 3 to 5 cm environmental difference

Severe lymphedema - Difference more than 5 cm

Clinical staging: National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) makes clinical classification taking

into account the presence of lymphedema examination findings and limb dysfunction (55). According to this classification, the following phases occur:

Class 1 - the amount of thickness or color change in the limb is small

Class 2 - Significant color change in limb, leathery skin texture, skin papillary development, limiting daily life activities (ADL) is seen

In most cases before determining the form of treatment, the Campisi staging approach is used to determine the lymphedema stage (56).

Campisi staging:

Stage IA: is the stage where there is no edema and lymphatic dysfunction. For example, the stage where the diameter difference does not develop between the upper extremities after mastectomy or axillary lymph node dissection

Stage IB: temporary edema that can be eased by limb elimination and night rest

Stage 2: permanent edema, which regresses with limb elimination and night rest

Stage 3: A permanent iridescent edema (acute erysipeloid lymphangites)

Stage 4: The fibrotic lymphedema stage, where lymphatic verrucoses are seen for the first time. A colon-shaped limb

Stage 5: Severe limb deformation, scleroindurative pachidermites and elephantiasis with pronounced and common lymphatic verrucoses (56)

Skin changes

Lymphedema is classified as one-sided or two-sided. People often complain about the weight and pain in the affected leg, especially developing towards the end of the day (57). In freshly formed lymphedema, temporary nontender pitting edema develops. The affected skin pits over time or becomes a dimpled tissue (peau d'orange). Due to the developing fibrosis and thickening of the skin, the skin becomes saturated and leathery as the condition improves. Incompatible edema suggests that lymphedema is progressing to an irreversible level. Chronic lymphedema is characterized by the tightening of the skin fold at the base of the second toe (Kaposi-Stemmer mark) . Elephantiasis nostras verrucosa develops over time. The skin of the affected area shows a wart hyperkeratotic "paving stone" or ''mossy'' appearance. Fissures, ulceration and recurrent cellulite are common in chronic lymphedema skin. Group A Streptococcus is the most common cause of cellulite. Each bout of cellulitis worsens the patient's condition by progressively damaging the lymphatic system (58,59). Tinea pedis is a very frequent condition caused by prolonged interdigital maceration. Lenforre is characterized by the dripping and leakage of light yellow and clear liquid. Impetigo is also common.

The chance of developing angiosarcoma was 10% in patients with permanent lymphedema for 10 years. This aggressive course tumor often manifests itself as red-like purple spots or nodules to grow, form ulcer tissue and produce satellite lesions. Stewart-Treves syndrome is a kind of cutaneous angiosarcoma that develops in people with lymphedema after postmastectomy operation (60). Angiosarcoma is a very fast-moving malignant tumor with a 10% 5-year survival rate (61). Other cancers associated with lymphedema include basal cell carcinoma (BCC), squamous cell carcinoma (SCC) (62), malignant melanoma (63), cutaneous lymphomas (64), and Kaposi's sarcoma (65). There is no obvious link between lymphedema and tumor growth, but a decrease in the function of the local immune system in the limb as a result of lymphedema may explain this somewhat(66).

Imaging Techniques

Imaging techniques like computed tomography (CT), lymphoscintigraphy, magnetic resonance imaging/MR lymphography, and indocyanine green (ICG) lymphangiography have been used to diagnose lymphedema. Unfortunately, none of these imaging studies use the same methods, so the results are not always the same. Protocols can be very different for lymphoscintigraphy in particular. Still, methods that use different techniques can show the unique lymphatic problems in places that specialize in lymphatic diseases.

Venous duplex ultrasound: Ultrasound is used to rule out venous clotting, venous weakness, and/or valve failure in patients who are going to have a physiological treatment (67).

Treatment of Lymphedema

Lymphedema is treated and managed with a combination of conservative therapy and surgery, such as lymphatic venous anastomosis. Complete Decongestive Therapy (CDT) is a conservative treatment that includes skin care, medical manual lymphatic drainage , compression therapy utilizing elastic bandages and compression clothing, exercise with compression, and advice on everyday living (68) . Lymphedema has no known cure, therefore it's critical to take care of the sufferer as soon as it manifests and stop their illness from becoming worse.

Conservative, multimodal therapy consists of and can be applied at all stages of lymphedema (69)**:**

-General personal care methods: self-monitoring, proper skin care, weight loss

-Changing levels of compression therapy: compression bandage, compression clothing, intermittent pneumatic compression

-Physiotherapy: manual lymphatic drainage, such as full decongestive

therapy. In physiotherapy methods, specific intervention method is selected depending on the disease stage.

It is crucial to emphasize that the evidence to support any of the treatment choices listed here is limited and largely of poor quality.

General self-care interventions are aimed at reducing the degree of edema and delaying the pace of development. there is no scientific evidence to support the efficacy of any of these measures. The International Society of Lymphology (ISL) guidelines recommend self-monitoring, limb elevation, maintaining ideal body weight, avoiding infection/injury, and avoiding constricting garments/ extremity cuffs for all patients with lymphedema, regardless of severity, and for those at risk for lymphedema following surgery (69,70).

Self-monitoring: Patients should be taught how to monitor their lymphedema, including serial limb circumference measurements. They should be advised to report any changes in size, color, temperature, feeling or skin condition as soon as possible.

Limb elevation: Elevating a lymphedematous limb may help decrease swelling, especially in the initial stages of lymphedema (71) . However, elevation alone is not a long-term successful treatment. Patients should avoid putting their limbs in gravity-dependent positions for extended periods of time, such as standing, sitting, or crossing their legs.

Diet and exercise: It's important to help people stay at their target weight. In addition to making lymphedema more likely to happen, obesity may also make it harder for compression pumps or bands to work (72) . Most people can exercise and lift weights safely, and they should be allowed to do so as long as they wear a compression gear that fits them well. Exercise is safe after axillary or groin lymph node dissection, and it is typically suggested to recover complete range of motion in the afflicted extremity (73).

Sports like rowing, tennis, or golf that require repeated motions against resistance are typically not recommended for those with developed lymphedema. However, randomized studies are gathering evidence of the safety of exercise and weight training in the damaged limb, as well as additional advantages associated with exercise, such as increases in cardiovascular fitness and quality of life.

Avoid skin infection/injury: Recommend detailed skin cleansing and nail care to avoid the portal to infection that can lead to soft tissue infection. After minor skin breaches caused by a paper cut or abrasions, pinpricks, insect bites, or pet scratches, patients should be advised to apply skin moisturizers and topical antibiotic treatments. Exposed skin should be protected by using sunscreen and wearing gloves while engaging in activities that might cause skin harm. Similarly, patients should avoid medical operations in the afflicted

limb that may introduce infection, such as immunization, acupuncture, phlebotomy, intravenous lines, and venography, if feasible. The patient should also avoid severe temperatures, which may increase the risk of tissue harm in the lymphedematous limb. Furthermore, individuals who utilize saunas, steam baths, or hot tubs may have their lymphedema aggravated.

Cellulitis should be addressed at all times. Cellulitis should be treated with antibiotics that are effective against gram-positive cocci. Intravenous antibiotics are required for severe cellulitis, lymphangitis, or bacteremia. If a patient has three or more bouts of cellulitis in a year, long-term antibiotic therapy is recommended (74).

Avoid limb restriction: Although there is widespread support for avoiding limb constriction (e.g., tight-fitting clothes, blood pressure devices), scientific data is sparse (75). Those who support limb constriction as a risk factor for lymphedema think that restriction (however temporary) might raise pressure in the limb, increasing lymph production and possibly leading to lymphatic vessel stenosis and fibrosis.

Conservative therapy based on severity: Our approach to lymphedema treatment is severity-based.

At risk for lymphedema: Physiotherapy is recommend to enhance mobility for all patients at risk for lymphedema after surgery (ISL stage 0). Other indicators are often considered significant, but there is insufficient data to establish effectiveness or to compare the benefit of one modality to another.

•**Mild lymphedema:** Physiotherapy (simple lymphatic drainage, a frequently taught self-help movement) and compression garments for individuals with mild lymphedema (ISL stage I) are recommend rather than more intense treatment (Grade 2B).

•**Moderate-to-severe lymphedema:** Extensive physiotherapy is recommend generally in the form of comprehensive decongestive treatment, rather than less intense therapy (Grade 2B) For patient that disease severity is moderate to severe lymphedema (ISL stage II to III) and no contraindications.

•**Severe lymphedema:** In addition to extensive decongestive therapy, intermittent pneumatic compression (IPC) therapy may be useful in patients with severe lymphedema (ISL stage III) (74).

Other therapies: Pharmacologic or non-pharmacologic treatments are not often employed. There has been no evidence that any medicine is useful. Diuretics, in particular, should be avoided since edema fluid cannot be readily recruited into the vascular area. Any patient with lymphedema should be referred for surgery, especially if conservative therapy has failed or if the patient is willing to explore other therapies (74).

Indications for Surgery

The first method for lymphedema treatment is nonoperative measures such as compression garments and decongestive therapy. The aims of surgical lymphedema care are to relieve pain and suffering, maintain or restore function, minimize infection risk, avoid disease progression, enhance cosmesis, and restrict deformity (75) .

The following are the indications for surgical treatment of primary and secondary lymphedema (76-82):

Failed nonoperative management of localized primary lesions (including microcystic and macroscopic lymphatic malformations)

Recurring soft tissue infection

Leaking of lymph into body cavities, organs or outside the body

Limiting function

deformity or deformity

Pain

Decreasing quality of life, including emotional disorders and psychological and social problems

There is no agreement on the best time for surgery or the best surgical intervention (82). An operational method to treat lymphedema should be decided on a case-by-case basis.

The following preoperative assessments are performed:

- Assess the degree of lymphedema
- Lymphedema stage
- Venous duplex ultrasound

Management of Operations

Lymphedema surgical therapy is divided into two categories: physiologic procedures and reductive techniques. To get the best outcomes, some surgeons mix lymph node transplantation with lymphovenous bypass.Lymph node transplantation, in which healthy lymph nodes are harvested from a donor area and microsurgically transplanted to the lymphedematous limb (arterial and venous circulation are reattached), or lymphovenous bypass, lymphatic fluid is discharged into the venous circulation or lymphatic collectors in the more proximal of the lymphatic obstruction area, are the two most common physiologic treatments. Physiological treatments are performed for individuals with early-stage lymphedema (ie Campisi stage I, II or early-stage III) before extra fat accumulation in tissues and significant irreversible fibrosis in tissue

develops. The goal of lymphedema reduction procedures is to eliminate the accumulated fibrofatty tissue. Liposuction using tiny cannulas placed into the subcutaneous space or, less usually, radical removal of the extra tissues are used in these treatments. Reductive treatments are best employed in individuals who does not benefit from conservative therapy or who have more advanced lymphedema due to fat accumulation and tissue fibrosis (Campisi stage III or IV). (82)

Physiologic procedures: Physiologic techniques develop new channels in the lymphatic system to boost its ability to transmit lymph fluid (83). In general, surgical intervention is viable as long as tissue alterations in the afflicted limb are not severe. Physiologic approaches have been used to treat individuals with more advanced lymphedema; however, the outcomes have been inconsistent, and only a small number of patients have been studied.

Lymphatic bypass operations, flap transposition procedures, and vascularized lymph node transfers are some of the surgical treatments used to achieve this aim. The lymphatic bypass methods are the most often employed physiologic approaches. These treatments need a high degree of technical competence, and they should only be performed by surgeons with experience in microvascular surgery.

Lymphatic bypass methods are used in the following cases (84)**:**

Failure in non-operative treatment methods

Recurrent soft tissue infectious or lymphangitis

Failure to adapt to compression clothing or deterioration in quality of life

The following are contraindications to lymphatic bypass surgery (84)**:**

Comprehensive tissue fibrosis

Changes in late stages of lymphedema

Venous hypertension

Repeating cancer in the ipsilateral limb or metastatic disease

Incompatibility with compression therapy or failure to comply with postoperative care methods

This method may be used to treat either primary or secondary lymphedema, while the effectiveness of these operations in primary lymphedema is debatable (84). Lymphatic bypass techniques are divided into two types: lymphatic-lymphatic bypass and lymphovenous bypass. Lymphovenular bypass method is included in the lymphovenous method.

Lymphatic-lymphatic bypass — Lympholymphatic bypass transfers the soft tissue resected from an unaffected area to a region close to the region

affected by lymphedema, followed by direct anastomosis of lymphatic vessels (85).

Lymphovenous bypass technique is a second option to lymphatic-lymphatic technique (86). A vascular interposition graft is used to connect lymphatic vessels in the distal of obstruction to the vessels in the proximal of obstruction. In this technique, lymphatic vessels are used as proximal vessels, the vessels next to it, or the lower part and larger vessels. Numerous lymphatic vessels can be connected simultaneously to the vessel transplant through anastomosis.

In the lymphaticovenular anastomosis method, the distal subdermal lymphatic vessels and adjacent venules with a diameter of less than 0.8 mm anastomose (87). This method is a supermicrosurgery technique . It does not develop much from lymphedema in deeper lymphatic channels and is used less than distal subdermal lymphatics for a bypass procedure.

Vascularized lymph node transplant — Lymph node is removed from the area where healthy lymph nodes are located as a whole (88). The recipient region may be the regions where the previous lymph node excision was made, or a different non-anatomical region may also be selected. Some doctors working on this topic have assumed that a "lymphatic pump" can produce if the lymph nodes are transplanted non-anatomically; however, they could not fully explain the mechanisms governing this lymphatic repair (89). In most cases, when transplanting lymph nodes, arterial and venous anastomosis is performed in the receiving region by microcerrrahi methods, but no lymphatic anastomosis is performed. Lymphedema may develop in the donor limb, which is a limitation of this technique. Given this possibility, mesenteric lymph nodes, the region where lymphedema does not develop after lymph node excision, are used as another alternative (90). However, these concerns must be weighed against the risks of intra-abdominal procedures. There is a need for prospective studies to identify the clinical settings in which patients are most likely to benefit from this procedure.

Responses to lymphatic bypass procedures are extremely variable, ranging from a complete response to none. Differences in the evaluation of volume change or limb diameter in the affected area, follow-up time of patients, lack of a standard of use of compression clothing and/or physical therapy modalities in the postoperative period, and the use of unstandardized or unverified surveys for patient analysis results in different results between studies (91).

Approximately 1800 patients undergoing microsurgical lymphatic-venous bypass were included in a large retrospective study. 87 percent reported subjective improvement, 83 percent objective improvement, and 67 percent average volume reduction. Following lymphatic bypass procedures,

the incidence of cellulitis was decreased by 87 percent. Several additional case reports and small series indicate that lymphatic bypass procedures may be effective in reducing primary and secondary genital lymphedema. In order to evaluate the efficacy of lymphatic bypass procedures in this setting, prospective studies are required. (92)

Reductive procedures (also known as ablative techniques) are used to eliminate fibrofatty tissue that has formed as a result of prolonged lymphatic fluid stasis. The reductive operations are intended to minimize lymphedema mass and are palliative rather than curative in individuals with secondary lymphedema , but may be curative in patients with isolated initial lymphatic abnormalities. Direct excision and liposuction are examples of operations in this category. These methods' aims are the same as those of physiologic treatments: to relieve pain, restore function, minimize swelling, and restrict deformity.

Direct excision: A number of direct excision methods for the treatment of extremity and genital lymphedema have been reported (93) . Lymphedematous tissues, including the skin and soft tissues, are removed in one piece. Tissue flaps (Homans, Sistrunk, Thompson procedures) or skin grafts (Charles technique) are used to cover the ensuing deformities.

Skin and subcutaneous tissue excision is often reserved for individuals with severe illness who have failed all other treatment options. These treatments are especially effective in cases when liposuction is not an option such as scrotal edema. These treatments are much more intrusive than liposuction and may cause significant morbidity (94).

Liposuction: Liposuction has been used in a small number of individuals to treat lower limb lymphedema (95). Liposuction is quite successful in the upper extremities. Success rate in the lower limb is less. This technique is quite simple. If the compression garments are always worn regularly after the operation, the patients treated in this way may have a long-term decrease in limb volume and an increase in quality of life (96). However, liposuction does not improve the underlying condition, and neglecting the use of compression clothing often causes rapid re-accumulation of fibrofatty tissues within three months.

Liposuction is an alternative treatment in the treatment of upper limb lymphedema after breast cancer surgery. To treat lyphedema of the upper extremities, liposuction should be handled with compression clothing and physiological methods (97).

Indications for upper limb lymphedema are (98)**:**

Non-pitting edema that failed in surgery without surgery for more than three months

- Arm volume inequalities of at least 600 cc

Symptomatic symptoms such as weight, shoulder and / or neck strain or discomfort

Recurring infections

Liposuction for upper limb lymphedema has the following contraindications (98)**:**

-Lymphangiosarcoma of the relevant upper limb

-Lenfematous upper limb with open wounds

The perception that the surgical procedure was beneficial for the reduction of lymphedematous tissue of the extremities and/or genitalia was significantly higher among prospectively evaluated patients (83 versus 56 percent) compared to retrospectively evaluated patients. The difference in perception may be the result of weak memory or a decline in life quality over time. Patients undergoing genitalia reduction had a substantially higher quality of life than those undergoing surgery for lymphedematous extremities (71% versus 34%).

After surgery, patients are kept in the hospital for one to five days to take care of their wounds and manage postoperative discomfort . For shorter treatments, the limbs are raised for 24 hours; for longer procedures, they may be elevated for up to one week (99).

Following physiologic and reductive therapies, compression garments are used postoperatively to avoid the recurrence of lymphedema.. Following surgery, antibiotics are normally taken for one week. Water-based, mild moisturizing lotions are used to treat the skin to stop it from drying out or breaking.(100)

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Chapter 24

CAN SEX-BASED REGULATION OF IMMUNE SYSTEM AND GENE EXPRESSION AFFECT COVID-19 DISEASE SEVERITY?

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Introduction

Coronaviruses (CoV) are a family of single-stranded RNA viruses that are transmitted to humans from animals and cause respiratory system-related diseases. Severe Acute Respiratory Syndrome (SARS-CoV), Middle East Respiratory Syndrome (MERS-CoV) (Chhikara, B.S., et al., 2020) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) were identified as a member of the family of coronaviruses. The symptoms of SARS-CoV-2 infection, which became a pandemic in early 2020, are fever, cough and fatigue after an incubation period of about 5 days. In addition to these symptoms, sputum, hemoptysis, headache, diarrhea and lymphopenia can be observed as other symptoms (Rothan, H. A., & Byrareddy, S. N., 2020). SARS-COV-2 virus which is factor of Coronavirus Disease 2019 (COVID-19) enters the cell expressing angiotensin converting enzyme 2 (ACE2) and does DNA, amino acid and protein cycles to produce and assemble all the parts necessary for a new virus. Its genome consists of nucleotide sequences ranging from 29.8 kilobases to 29.9 kilobases corresponding to 1-16 open reading window (ORF), Envelope (E) protein, Membrane (M) protein, Nucleocapsid (N) protein and Spike (S) protein (Khailany, R. A., 2020). The SARS-CoV-2 spike (S) glycoprotein binds ACE2, allowing the virus to enter the host cell. It uses host transmembrane serine protease 2 (TMPRSS2) or cysteine proteases cathepsin B or L (CTSB/L) to allow the fusion of virus and host cell membranes via in irreversible conformational changes of the S protein (Wang, X., et al., 2020).

Findings that differ in the severity of SARS-CoV-2 disease in men and women

Information from prostate cancer research has revealed the androgen sensitive regulation of TMPRSS2. TMPRSS2 mRNA levels increase with the activated androgen receptor in the presence of androgen, while androgen deprivation therapy decreases TMPRSS2 mRNA levels. Furthermore, administration of exogenous testosterone in vitro increases TMPRSS2 expression (Ory, J., et al., 2020). In a study investigating the duration of SARS CoV-2 positivity with quantitative real-time polymerase chain reaction (qRT-PCR) from nasopharyngeal/oropharyngeal swab samples; it has been observed that women have an average of 2 days earlier viral clearance than men. It was determined that ACE2 was highly expressed in testicular cells at both gene and protein levels, while it was found at lower expression levels in ovarian tissue (Shastri, A., et al., 2020). Researchers suggested that ACE2 is overexpressed in testes, even than in lung tissue which is the main target of SARS-CoV-2 infection and it has been stated that ACE2 contributes to the development of the cells of Sertoli and Leydig. Sperm DNA fragmentation caused by the attack of the coronavirus on the male reproductive system, changes in hormone levels and the formation of anti-sperm antibodies are among the important causes of male infertility. It has also been reported that high fever, which is

among the symptoms of COVID-19, may indirectly impair spermatogonesis, negatively affect sperm motility, and impair sperm DNA integrity (Dolanlı Gelenbay, E., et al., 2022). Although it was previously determined that there was no significant difference between the sexes in terms of the conversion of individuals with COVID-19 into positive cases, Ceylan et al. determined that the rate of SARS-CoV-2 positive cases among individuals who came into contact with a COVID-19 patient was higher in women. It was suggested that the reason for this difference is that Turkish women are more in contact with other family members in the household and the assumption that the ACE2 gene on the X chromosome can express higher levels of ACE2 receptors in women (Ceylan, Ş., et al., 2021).

In a study conducted in China, advanced age and multiple comorbidities were related to higher severity and mortality in both COVID-19 and SARS patients and men tended to have a more severe symptoms than women. While the range of men who died from COVID-19 was 2.4 fold higher that of women. Researchers suggested that men with COVID-19 were determined to be at risk of worse outcomes and death, regardless of age (Jin, J. M., et al., 2020). Abate et al. found that the prevalence of symptomatic COVID-19 was lower in women than in men. It has been determined that high rates of alcohol consumption and smoking contribute to the high prevalence of COVID-19 in men (Abate, B. B., et al., 2020). In another study, hospitalization, intensive care unit (ICU), endotracheal intubation and vasopressor support were found higher in men (Gomez, J. M. D., et al., 2021). Bienvenu et al. determined that men were 50% more likely to be admitted to the ICU than women, and the male:female case fatality ratio ranged from 1.7 to 1.8. The male mortality rate was similar in each age groups, with data from 227219 confirmed COVID-19 cases shows the highest mortality rate was in middle-aged men (Bienvenu, L. A., et al., 2020). In Turkey, a study involving 576 COVID-19 patients; the age of smoking patients was found lower than non-smokers for the ICU hospitalization. Furthermore, smokers had higher rate of the presence of chronic obstructive pulmonary disease, coronary artery disease and malignancy. Atar et al. found no relationship between ICU and hospital stay and mortality in smokers. However, neutrophil and white blood cell count were found higher in smokers and monitoring of these parameters was recommended (Atar, F., et al., 2022). However, in another study conducted with 3137 patients in Turkey; 51.2% of the patients were male, and the male gender of the patients transferred to ICU was statistically higher and mortality rate of men was found elevated in all patients, and it was suggested that male gender increases the mortality rate 1.5 times (Topal, S., et al., 2023).

It was reported that the expression of ACE2 was similar in both sexes in a number of tissues, while plasma ACE2 concentrations were reported to be higher in men compared to women. Since the transcription of TMPRSS2 is

expressed at a higher rate in men since an androgen receptor binding element and androgenic ligands in its promoter and it was thought that this gene may contribute to the increase in the severity of COVID-19 in men (Bienvenu, L. A., et al., 2020). Upregulation of both ACE2 and TMPRSS2 was found in males and in polyciliated cells with age, and it was emphasized that co-expression of these two genes would contribute to explain the SARS-CoV-2 nasal viral reservoir (Muus, C., et al., 2020). In experiments with transgenic rats harboring the human renin and angiotensinogen genes, CTSL was found to differ depending on sex. It has been reported that urinary CTSL in the kidneys of 7-week-old male rats is approximately 4 fold higher than that of females, and 40% of male rats die at the end of the 8th week (Bauer, Y., et al., 2011). Female CTSB knockout mice showed a 31% reduction in cholesterol absorption and approximately 50% reduction in CTSB expression following reduced cholesterol absorption, suggesting that specific genetic elements mediate the reduction of CTSB expression. Although CTSB has previously been shown to participate in the effects induced by angiotensin II, β-adrenaline and estrogens, Wang et al. found that gender differences in serum CTSB level in women (Wang, N., et al., 2016).

Differential immune responses to SARS-CoV-2 infection in men and women

Both host and environmental factors such as smoking, stress, genetic factors, microbiota, age, gender, nutritional status, vaccination status, hygiene status, breast milk intake, body mass index (BMI) and physical activity status affect the development of immune system (Acar Tek, N. & Koçak, T., 2020). The Y chromosome in males is much smaller and encodes mainly sex-specific genes. However, while many genes on the X chromosome that play a key role in both innate and adaptive immune responses, particularly against viral infections. Considering that men have a single X chromosome; if men has an X-related gene mutation, it will show its effects, while women can be protected from these mutations thanks to their two X chromosomes (Bienvenu, L. A., et al., 2020). Transcriptional analysis demonstrated that genes associated with the immune response to SARS-CoV-2 in female and young subjects have sex-specific transcriptional modulation. It has been stated that the mRNA levels of key pro-inflammatory/neutrophil-related genes are lower in women compared to men, both in nasopharyngeal cells and peripheral blood of COVID-19 patients, and this correlates with a protective effect against inflammatory damage (Paccielli Freire, P., et al., 2020).

In infectious diseases, it has been found that women produce a stronger immune response compared to men, respond more strongly to pathogens and produce higher amounts of interferon and antibodies. It is thought that this protective effect is derived from estrogen and that the effects of testosterone on immune responses are not as strong as estrogen. It also affects viral load

as it changes the expression of sex hormones ACE2 and TMPRSS2 receptors. Sex hormones can also shape the clinical manifestations, complications, and prognosis of COVID-19. Moreover, sex hormones that decrease with age may lead to increased proinflammatory response and exacerbation of COVID-19 infection in elderly individuals (Yılmaz, A., et al., 2021). Moreover, women have a higher activity of macrophages and neutrophils. ACE2 in-vivo studies suggest that male patients have higher levels of ACE2 expression in their kidneys than female patients, and this may affect COVID-19 susceptibility and differences in the course of the disease (Kopel, J., et al., 2020). Since the level of E2, which is the most produced form of estrogens and has the highest receptor affinity, it causes a stronger immune response in viral infections such as coronavirus and vaccination in women than men (Klein, S. L., et al., 2015). In addition, E2 at high concentrations suppresses the production of proinflammatory cytokines such as IL-6, IL1β, TNF-alpha and reduces macrophage proliferation (Mauvais-Jarvis, F., et al., 2020). When SARS-CoV-2 virus binds to its receptors on epithelial cells, it activates the innate and adaptive immune system, causing the secretion of many cytokines, including IL-6. IL-6 is a cytokine with pro-inflammatory and anti-inflammatory effects and synthesized by T-cells, B-cells, monocytes, fibroblasts, endothelial cells, and various tumor cells, and plays a key role in cytokine storm. The cytokine storm in COVID-19 has been associated with poor prognosis, lung injury and multi-organ failure. In a study conducted in Turkey, IL-6 and ferritin levels were found significantly higher in male patients at admission to the ICU than in female patients (Polat, Ö., et al., 2020). Wang et al. detected IL-6 levels 10 fold higher in bronchoalveolar fluid sample compared to blood which is found dangerous in terms of local cytokine storm and organ damage (Wang, C., et al., 2020). In COVID-19, male patients have higher plasma innate immune cytokine levels such as IL-8 and IL-18 and while non-classical monocytes were found to have stronger induction, female patients were observed to have stronger T cell activation (Takahashi, T., et al., 2020). Another finding that may help explain the difference in COVID-19 disease severity between men and women is that the testicles may harbor coronavirus, and accordingly, men may show delayed viral clearance (Pradhan, A., & Olsson, P. E. 2020). When the effect degrees of the five criteria effective in COVID-19 related death with DEMATEL method and their relations in the system are evaluated; It was determined that the comorbidity factor was the most effective factor on mortality. While the factors affecting after comorbidity were age and immune system, it was seen that smoking and gender had a relatively lower effect on the mortality rate due to COVID-19. It was stated that the criterion with the least effect among these five criteria was gender. However, the presence of one or more of the disease groups such as cardiovascular, diabetes, chronic respiratory, high blood pressure and cancer in people was evaluated as comorbidity in the study. It has been reported that men are more prone to COVID-19 disease because there are relationships with

hypertension, cardiovascular and some chronic lung diseases in individuals with COVID-19 disease, and these diseases are more common in men than women (Şen, G., et al., 2021).

Result

As a result, it is understood that male and female gender may indirectly affect the course of the disease in COVID-19. Although women are thought to be more advantageous than men against infectious diseases due to the fact that they contain two X chromosomes, it can be deduced that many chronic diseases are more common in men and that these diseases accompany COVID-19 may affect the poor prognosis and mortality of the disease in men. Moreover, thanks to the immunomodulatory effects of the estrogen hormone, women's immune systems work more actively than men, which can also affect the response to infection, resulting in milder COVID-19 symptoms. Accompanying a disease such as hypertension in men with SARS-CoV-2 infection and deficiencies in immune system responses may cause these patients to experience more severe COVID-19 symptoms. Moreover, other factors such as age and smoking may contribute to the prolongation of the duration of infection, and the higher incidence of hospitalization and ICU transfer in men in comorbidities such as chronic obstructive pulmonary disease. In addition, as mentioned, there are findings in the literature that testes in males act as a reservoir for SARS-CoV-2, delaying viral clearance, and that ACE2, TMPRSS2 and CTSB/L, which are genes that are effective in the entry of SARS-CoV-2 virus into the cell, can be affected by sex hormones. Although it is not wrong to conclude that all of these factors may contribute to prognosis, length of hospital stay, ICU transfer, and intubation, it would not be a correct approach to say that the male gender factor is one of the main causes contributing to mortality in COVID-19.

Keywords: Gender, Hormone, Immune response, COVID-19

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