Current Researches in Health Sciences-III

Editor: Prof. Dr. Ali Bilgili

ÖZGÜR YAYINLARI

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15 Temmuz Mah. 148136. Sk. No: 9 Şehitkamil/Gaziantep
+90.850 260 09 97
+90.532 289 82 15
www.ozguryayınlari.com
info@ozguryayinlari.com

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ÖZGÜR YAYINLARI

Preface

This editorial scientific book brings together academic articles from various disciplines in the field of health sciences. It strives to address contemporary and significant topics, offering a rich compilation of the latest scientific research in the realm of health. Authors provide in-depth analyses and new perspectives, presenting readers with the most recent developments in the field of health sciences. This book serves as a comprehensive resource for healthcare professionals, researchers, and anyone working in related disciplines, emerging as a prominent reference work that contributes to the field of health sciences.

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Chapter 1

Cartilage Oligomeric Matrix Protein (COMP) As a Candidate Biomarker in Osteoarthritis

Fatih Baygutalp[1]

Lale Duysak[2]

Abstract

Biomarker research aims to provide better diagnostic and prognostic tools, potentially leading to improved treatments for the condition. Biomarker research in osteoarthritis is a field of study focused on identifying and understanding specific molecules, genes, or other measurable indicators that can help diagnose, predict, and monitor the progression of osteoarthritis. These biomarkers can be found in blood, synovial fluid or urine. Osteoarthritis is a degenerative joint condition that causes pain, stiffness, and limited movement due to the breakdown of cartilage in the joints. There are a significant number of studies investigating possible biomarkers which may be useful in the diagnosis and prognosis of osteoarthritis. These biomarkers are included in the groups of synthesis and degradation of bone/cargilage/synovium, inflammation, metabolic parameters and microRNAs. In this book chapter, we aim to focus the current status of studies about cartilage oligomeric matrix protein, which is an indicator of cartilage degradation. When we evaluate the current literature findings, we can say that cartilage oligomeric matrix protein may be a promosing biomarker for the diagnosis of osteoarthritis, but there is not sufficient data for the role of cartilage oligomeric matrix protein in the prognosis of osteoarthritis.

1. Osteoarthritis

Osteoarthritis (OA) is a very common type of age-related, injury-related joint degenerative disorder [1]. The most prevalent type of arthritis worldwide is OA, frequently seen in the elderly population, leading to erosion

1 Department of Physical Therapy and Rehabilitation, Faculty of Medicine, Ataturk University, Erzurum

2 Department of Biochemistry, Faculty of Pharmacy, Ataturk University, Erzurum

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in the joint cartilage, osteophyte formations, and subchondral sclerosis [2]. Epidemiological studies conducted in various parts of the world have reported that symptomatic knee OA is seen in 10-30% of people who are over 65 years old [2].The prevalence of symptomatic knee OA in adults over the age of 55 was found to be 13% [3]. Data from the Framingham OA study shows that the prevalence is 11% in women and 7% in men. In a prevalence study conducted in Turkey, the prevalence of symptomatic knee OA in the population aged 50 and over was 14.8%, and it was reported as 22.5% in women and 8% in men [3].

OA may be associated with obesity, type 2 diabetes, insulin resistance, dyslipidemia, hypertension and metabolic syndrome, either together or separately [4]. Today, we understand that osteoarthritis is more than just a condition where the illness is brought on by mechanical stress-induced cartilage loss, but also a pathological process that affects all tissues in the joints and causes discernible alterations in tissue structure, metabolism, and function. All these changes are mediated by the complex and as yet incompletely investigated interplay of pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and adipokines; all of these can be measured in serum, synovial fluid, and histological samples and potentially evaluated as biomarker candidates for disease staging and prognosis [5-11].

Obesity refers to the condition of body weight being higher than normal. Excessive weight on the joint surfaces can put excessive stress on the joints, which can cause wear of the joint cartilage. The risk of osteoarthritis increases significantly in obese individuals, especially in large joints such as the knee and hip joints. Additionally, obesity can lead to arthritis, which can trigger osteoarthritis. On the other hand, patients with osteoarthritis may limit their physical activities due to movement restrictions. This can promote weight gain and increase the risk of obesity. Additionally, patients who have difficulty maintaining an active lifestyle due to pain and stiffness engage in less physical activity, which may promote obesity [12].

Traditionally, radiological imaging techniques that corroborate clinical symptoms are used to diagnose OA. However, primary OA, which develops without any macro trauma attack, begins years before radiological findings become apparent, and pathology often cannot be detected early. The course of the disease is usually slow and spreads over many years [13].

2. The Role of Biochemical Markers in Osteoarthritis

Early diagnosis of osteoarthritis (OA) remains a diagnostic challenge due to the limited signs and symptoms presented in the early stage of the disease. The use of magnetic resonance imaging (MRI) for diagnosis is limited due to cost and accessibility issues. The joint is a complex structure composed of bone, cartilage and synovial tissue. Therefore, it would be useful to use markers of these three structures when assessing how much the joint has degenerated [13]. No single biomarker has been recognized that is sufficiently validated for systematic use that can be used to aid early diagnosis. Standard laboratory procedures, such as the extremely sensitive CRP test, are unable to conclusively determine the level of disease activity in OA. Despite the fact that the quantitative CRP values may show slight increases in a patient with synovitis showing signs of inflammation, normal values are usually found. Similarly, serum levels of anti-nuclear antibodies, rheumatoid factor and complement components are normal [13].

We now understand that osteoarthritis is a condition marked not just by cartilage loss due to mechanical loading, but also a pathological process that affects all tissues in the joint and causes detectable changes in tissue architecture, metabolism and function. All these changes are mediated by the complex and as yet incompletely investigated interplay of pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and adipokines; all of these can be measured in serum, synovial fluid, and histological samples and potentially evaluated as biomarker candidates for disease staging and prognosis [14].

A "biomarker" is a characteristic that may be tested and assessed objectively to determine if it represents diseased or normal biological processes or the pharmacological reactions to therapeutic intervention and may be represented by radiographic, histological, physiological, or molecular features. [15]. An ideal biomarker should have following features: It shouldn't be impacted by illnesses that aren't related to the system it belongs to; analysis shouldn't require pricey equipment, and interpreting the biomarker's results should be simple, its quantity shouldn't vary significantly among the broader population. Unfortunately, there is currently no osteoarthritis biomarker that has all of these features.

The Osteoarthritis Biomarkers Network developed a five-point classification scheme called BIPED (burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic) to help provide a common framework for communication amongst researchers in the field of osteoarthritis biomarkers [5]. As a result, an individual with and without

osteoarthritis can be distinguished using a "diagnostic" biomarker that has good positive and negative likelihood ratios (sensitivity and specificity) as well as an area under the curve in the receiving operator curve. Whereas a "prognostic" biomarker forecasts the development of osteoarthritis in those who already have the condition or its advancement in those who do not, a "burden of disease" biomarker evaluates the severity of the disease in people who already have osteoarthritis. In order to assess risk in people without obvious illness, predictive biomarkers may be utilized.

Currently investigated biomarkers of osteoarthritis can be summarized in the following groups [16]:

- Biomarkers of synthesis and degradation of bone
- Biomarkers of synthesis and degradation of cartilage
- Biomarkers of synthesis and degradation of synovium
- Biomarkers of systemic inflammation
- Biomarkers related with obesity and metabolism
- microRNAs related to osteoarthritis

In this book chapter we will focus on one of the degradation biomarkers of cartilage, namely Cartilage Oligomeric Matrix Protein (COMP).

3. Cartilage Oligomeric Matrix Protein (COMP)

Cartilage oligomeric matrix protein (COMP) is a non-collagenous extracellular matrix glycoprotein that is mostly present in the human skeleton system, including articular cartilage, meniscus, ligaments, tendons, and synovium [17, 18]. It is a member of the thrombospondins (TSPs) family, commonly referred to as TSP-5. Additionally, vascular smooth muscle cells, the heart, and the eye's vitreous all express COMP [19]. Moreover, bodily fluids may include COMP and its particles. In the typical population, serum and synovial COMP levels are roughly 5.93 ± 1.95 μg/mL [20] and 33 ± 10 μg/mL [21], respectively.

Apart from osteoarthritis, the role of COMP in other diseases including skeleton diseases, rheumatoid arthritis, malignancies, cardiovascular diseases, fibrosis and some other diseases is a current research topic and there are promising results [22].

COMP is the most well-known cartilage degradation marker and the closest to being a biomarker in osteoarthritis. Although the main source of COMP is cartilage, COMP can also be synthesized by synovial cells, tendon

fibroblasts and osteoblasts. Therefore, an increase in COMP levels may indicate cartilage degradation as well as synovial inflammation. Increased COMP levels are associated with osteoarthritis, as both conditions have a role in the etiopathogenesis of osteoarthritis. Here, we will summarize the recent literature findings of COMP related to osteoarthritis. We can say that although most of the studies have reported higher levels of COMP in osteoarthritis patients, serum COMP measurement was not a reliable indicator of the prognosis for osteoarthritis, according to some research.

3.1. Studies reporting a relationship between COMP levels and osteoarthritis

The clinical usefulness of COMP has been suggested and evaluated in a few studies because of its roles in inflammation and cartilage deterioration. In a study conducted on 56 patients with primary osteoarthritis and 31 healthy controls it was reported that COMP levels were higher in patients with osteoarthritis and the researchers have concluded that COMP levels may reflect structural damage of the knee [23].

Measuring serum COMP levels has the potential to be useful in both OA diagnosis and disease progression prediction [24].

A study by Sharif et al. included 115 participants who had osteoarthritis (OA) of the knee. At the conclusion of the study, serum COMP levels were assessed at baseline and every six months, along with radiographic cartilage loss at 0, 24, 36, and 60 months. It was discovered that the COMP level was linked to progressive joint degradation [25].

Individuals suffering from primary knee OA symptoms (KL stages I–III) and met ACR criteria were included in a study and blood COMP levels were tested at the onset of the trial and three years afterwards. It was discovered that the COMP assessment might function as a possible predictor of the course of the disease [26].

Investigating COMP levels related with exercise in osteoarthritis patients is a current research topic. One study has reported that walking for thirty minutes can raise serum COMP levels in both knee OA patients and healthy age-matched controls [27].

Regardless of age or BMI, high serum COMP values were linked to an increased risk of incident knee OA [28]. Radiographic knee OA development has been linked to high concentration of COMP [29].

Due to the conflicting results of some studies and the concern about whether COMP is a valid biochemical measure, the first meta-analysis

(2019) was conducted thoroughly on 35 human research [30]. According to this meta-analysis, COMP performed moderately well in predicting the course of OA and differentiating between patients with knee or hip OA and control subjects. The study's size and diagnostic criteria had no discernible impact on the performance. Additionally, subgroup analysis revealed that serum COMP was more effective in males than in females, and that synovial fluid performed better for COMP than serum [30]. Obtaining synovial fluid in early OA may be challenging, despite the meta-analysis's conclusion that COMP performs best in synovial fluid.

Another meta-analysis was conducted to investigate the usage of serum COMP as a biomarker for knee OA and its correlation with the severity of the disease. Meta-analysis was performed on selected 9 controlled studies which had been used Kellgren-Lawrence (K-L) classification for knee OA and included data of serum COMP in OA patients and healthy controls. Nine studies were pooled, and the results showed that patients with knee OA had significantly higher serum COMP levels than controls (SMD 0.81, [95% CI, 0.36, 1.25], P = 0.0004). All three categories, with the exception of K-L grade 1 versus control, showed noticeably increased serum COMP when compared to K-L 1-4 and controls. Patients with more critical illness stages had considerably greater serum COMP levels than those with less serious disease stages, according to comparisons between K-L grades 1-4. When compared to K-L grade 1 patients, the elevation in patients with K-L grade 3 did not, however, reach statistical significance. Overall analysis revealed that knee OA patients had considerably greater blood COMP than controls, suggesting that serum COMP may be useful in distinguishing knee OA patients from healthy individuals. The statistic of the meta-analysis demonstrated that serum COMP levels were useful in differentiating between patients with K-L $\geq$ 2 [31].

For early OA, COMP might be a promising biomarker. Results have shown that there exists a possibility for COMP to function as a diagnostic and prognostic indicator, as well as an indicator for the effectiveness of interventions in knee OA [32].

3.2. Studies reporting no relationship between COMP levels and osteoarthritis

In a study conducted by Yıldız et al. the relationship of COMP levels with radiographic and clinical findings of knee osteoarthritis was investigated. Researchers have found that serum COMP and MMP-3 (levels did not significantly change across groups with varying radiographic stages of knee

osteoarthritis, nor did they differ in clinical criteria such as pain intensity and functional status in daily activities [33]. However, we have to mention that this study has a limitation that the lack of a control group.

A specific group of biochemical biomarkers (18 biomarkers) was studied by Kraus et al. as potential indicators of the clinically significant development of osteoarthritis (OA). According to the findings, researchers have reported that measuring serum COMP does not serve as a reliable predictor of deteriorating knee OA [34].

Teirlinck et al. conducted a literature review in order to determine the variables which may be predictive of hip OA patients' eventual progression. Researchers have found that there was no evidence of a relationship between the marker COMP and radiographic advancement [35].

4. Conclusion

Currently available biomarkers do not perform consistently enough to be used in clinical studies as a supplementary or supporting endpoint, as a surrogate result, nor do they discriminate well enough to help diagnose osteoarthritis or predict the prognosis of individuals with or without the disease. There is growing evidence that the most effective approach would involve combining tissue biomarkers with other measures (such radiography or MRI) into a single diagnostic test, or alternatively, a panel of biomarkers that cover the spectrum of physiological effects [36].

When evaluating different results in the literature, it is necessary to consider the strengths and limitations of the studies such as sample sizes of groups, standardization of analyse method, existence of a control group, evaluation of radiographic data. Again, it should be considered that BMI levels, sampling time, exercise status, ethnicity and gender may affect results. We have to mention that COMP levels may decrease in advanced stages of OA, most likely as a result of its depletion in severely deteriorated tissue.

Among the candidate biomarkers, COMP is one of the promising biomarkers for OA. COMP is a cartilage-resident glycoprotein and connective tissues that has been associated with osteoarthritis. Elevated levels of COMP in synovial fluid and serum can be indicative of cartilage degradation and may serve as a potential biomarker for this degenerative joint condition.

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Chapter 2

Bacillus cereus Isolates Obtained from Food Used in Infant and Child Nutrition[1]

Edip Urhan[2]

H. Yeşim Can[3]

Abstract

In the present study, a total of 80 sample materials were used (30 powdered baby formulas, 25 jarred baby foods, and 25 cereal-based products). The isolation of *Bacillus cereus* from the samples was performed with the classical culture technique. The isolates were confirmed as *B. cereus* with PCR targeting the hemolysin gene. Emetic (*ces*) and diarrheal toxin genes (*nhe, hbl, cytK*) were detected by multiplex PCR in confirmed isolates.

As a result of the study, 62.5% (50/80) of the samples were found to be contaminated with *B. cereus*. *B. cereus* could not be detected in jarred baby foods. *B. cereus* was detected at 83.3% (25/30) and 100% (25/25) in powdered baby foods and cereal-based products, respectively. Thirteen (26%) of 50 isolates confirmed by PCR carried at least one gene responsible for toxin synthesis. Only *nhe* gene was found in three (6%) isolates, and *nhe* and *cytK* genes were found together in 10 (20%) isolates. It was found that none of the isolates had *ces* and *hbl* genes and all isolates were able to form hemolysis on blood agar and two isolates were psychrotrophic. In conclusion, the presence of enterotoxigenic and psychrotrophic *B. cereus* strains in powdered baby formulas and cereal-based products may be risky for infants and young children whose immune systems and intestinal microbiota are not fully developed.

1 It was summarized from the master's thesis. This study was financially supported by Hatay Mustafa Kemal University with project number 21.YL.024.

2 Directorate of Mersin Veterinary Border Control Point, Republic of Türkiye Ministry of Agriculture and Forestry, edip.urhan@gmail.com, Orcid ID: 0000-0002-3765-8784

3 Assoc. Prof., Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, yesimcan@mku.edu.tr, Orcid ID: 0000-0002-5191-6268

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1. Introduction

Although babies need to receive breast milk for their healthy growth and development, ready-made formulas can also be used as an alternative source when breast milk is insufficient or is interrupted (WHO/FAO, 2004; Rahimi et al., 2013; WHO, 2021). Infant formulas are prepared in liquid or powder form to meet the nutritional needs of normal infants, can be used instead of breast milk when necessary, and with a composition close to breast milk (Seyrekbasan, 2000).

Although the composition of baby foods varies according to the age of use, it generally includes skimmed milk, skimmed milk powder, whey powder, lactose, vegetable oils, fish oil, starch, vitamins, minerals, emulsifiers, and stabilizers (Gökçay et al., 2012). Powdered infant formulas, dried dairy products, and other medicinal foods are produced by using similar techniques. In production, the first step is mixing the content, and after mixing the raw materials and additives, the mixture is pasteurized. After pasteurization, homogenization and standardization processes are performed in terms of oil, vitamin, and mineral content. Then, it is packaged with the appropriate packaging method and heat treatment (sterilization) is applied. The final stage is spray drying (Becker et al.,1994; WHO/FAO, 2004; Dalkılıç Kaya, 2011; Gökçay et al., 2012).

Previous studies show that the heat treatment applied during the production of baby foods and supplementary foods used in infant and child nutrition can destroy the vegetative forms of pathogenic bacteria that have the potential to be present in the raw material, but the spore forms are not inactivated and can maintain their viability. Milk and dried dairy products and starch contained in baby foods are suitable media for microorganisms (Shadlia-Matug et al., 2008; Bahçeci et al., 2018; Genç and Vural, 2021). Microorganisms likely to be found in foods used in infant and young children's nutrition were examined in three different categories by the World Health Organization and the Food and Agriculture Organization as *Cronobacter sakazakii*, and *Salmonella enterica*, which are known to cause infection in babies in category A, coliform group bacteria in category B, *B. cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Staphylococcus aureus,* and *Listeria monocytogenes* are in C category (WHO/FAO, 2004). It was stated that *B. cereus* can be used as an indicator in determining the microbiological safety and contamination level of infant formulas (Pei et al., 2018).

The *B. cereus* microorganism can be found in various environmental sources in the form of spores, it is resistant to adverse environmental

conditions and can maintain its vitality for a long time (Wong et al., 1988; Reyes et al., 2007; Stenfors Arnesen et al., 2008; Tallent et al., 2012; Gdoura-Ben Amor et al., 2019). By synthesizing two different toxins of diarrheal and emetic types, it causes two different foodborne infections (emetic syndrome and diarrheal syndrome) (Kotiranta et al., 2000; Stoeckel et al., 2013). Diarrheal enterotoxins are responsible for the diarrheal syndrome and are heat-sensitive and are produced by bacteria while growing in the intestinal tract. The diarrheal form is characterized by diarrhea and abdominal pain. The proteins responsible for the diarrheal syndrome are hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K (Park et al., 2009; Logan, 2012). The emetic syndrome is characterized by nausea, vomiting, and abdominal cramps, similar to food intoxication caused by *Staphylococcus aureus*, due to the incubation period and observed symptoms (Tewari and Abdullah, 2015; Abraha et al., 2017). Emetic toxin is heat-stable and synthesized in foods. The emetic toxin is designated as "cereulide" and is encoded by the *ces* gene (Sánchez-Chica et al., 2021).

B. cereus is a natural contaminant of raw milk and can often be found in raw milk in spore form, although at low levels (Wong et al., 1988). Dried dairy products may contain *B. cereus* spores (Becker et al., 1994; Pei et al., 2018) and it has been reported that *B. cereus* is the main contaminant in infant formulas (Rahimi et al., 2013).

The purpose of this study was as follows: i) to determine the presence of *B. cereus* in foods used in infant and child nutrition (powdered baby formulas, jarred baby foods, and cereal-based products), ii) to determine the hemolysis-forming ability and psychrotrophic characteristics of isolates, iii) to detect emetic and diarrheal toxin genes (*ces*, *nhe*, *hbl*, *cytK*) in isolates by multiplex PCR method.

2. Material and Method

2.1. Collection of Samples

In this study, 30 powdered baby formulas from different brands (20 imported, 10 domestic formulas), 25 jarred baby foods, and 25 cereal-based products (packaged or unpackaged) that consisted of flour, starch, rice flour, and semolina were investigated. A total of 80 samples were used as the study material. The samples were collected and analyzed under aseptic conditions from the provinces of Hatay and Mersin in March-April-May 2021.

2.2. Isolation and Identification of *B. cereus*

B. cereus isolation was performed in the samples with the classical culture technique (Tallent et al., 2012). Ten grams of each sample were weighed homogeneously into sterile polyethylene bags under aseptic conditions and 90 ml of Tryptic Soy Broth containing 10 mg/ml concentration of polymyxin B was added to the samples. Then, the pre-enrichment broths were incubated at 30°C for 24 hours under aerobic conditions. A loopful of samples was taken from the pre-enriched samples and inoculated on Brilliance *Bacillus cereus* agar. The plates were incubated at 30°C under aerobic conditions for 24-48 hours. Colonies that formed turquoise green pigmentation on the agar at the end of the incubation were considered suspicious for *B. cereus*. Suspicious colonies were confirmed by PCR by targeting the hemolysin gene for *B. cereus* (Wang et al., 1997).

2.3. DNA Extraction

DNA extraction was performed according to the protocol recommended by the commercial nucleic acid extraction kit (Vivantis, Malaysia). Extracted DNA samples were stored at -20°C until PCR.

2.4. Determination of Hemolysis Ability

The obtained isolates were activated in Brain Hearth Infusion Broth, inoculated on blood agar by the streak plating method, and incubated at 37°C under aerobic conditions for 24-48 hours. At the end of the incubation, the development of a greenish or clear zone of hemolysis around the colonies was considered positive.

2.5. Determination of Psychrotrophic Characteristic

The confirmed isolates were activated in Brain Hearth Infusion Broth and then plated onto Tryptone Soy Agar by the streak plating method and incubated under aerobic conditions at 7°C in a refrigerated incubator. The development of growth in the plates was checked at 24, 48, and 72 hours and finally on the 7th day.

2.6. Investigation of Toxin Genes with Multiplex PCR Method

The presence of diarrheal toxin genes (*nhe, hbl, cytK*) and emetic toxin gene (*ces)* in isolates confirmed as *B. cereus* were found with multiplex PCR method. For this purpose, the amplification conditions and primer pairs recommended by Ehling-Schulz et al. (2005, 2006) were used (Table 1).

Table 1. Primer sequences and amplification conditions used in the study.

Genes	**Primer sequences (5'- 3')**	**Product (bp)**	**Reference**
Hemolysin gene	F; CTGTAGCGAATCGTACGTATC R; TACTGCTCCAGCCACATTAC	185	Wang et al. (1997)
Hbl	F; GTA AAT TAI GAT GAI CAA TTTC R; AGA ATA GGC ATT CAT AGA TT	1091	Ehling-Schulz et al. (2006)
Nhe	F; AAG CIG CTC TTC GIA TTC R; ITI GTT GAA ATA AGC TGT GG	766	
cytK	F; ACA GAT ATC GGI CAA AAT GC R; CAA GTI ACT TGA CCI GTT GC	421	
Ces	F; GGTGACACATTATCATATAAGGTG R; GTAAGCGAACCTGTCTGTAACAACA	1271	Ehling-Schulz et al. (2005)

bp: base pair, F: forward, R: reverse

3. Results

As a result of the study, 62.5% (50/80) of the samples were found to be contaminated with *B. cereus*. *B. cereus* was detected at levels of 83.3% (25/30) and 100% (25/25) in powdered baby formulas and cereal-based products, respectively. *B. cereus* was found as 80% (16/20) in imported powdered baby formulas, and 90% in domestic baby powdered formulas (9/10). However, *B. cereus* could not be detected in jarred baby foods.

A total of 50 isolates were confirmed as *B. cereus* with PCR by targeting hemolysin gene (Figure 1). It was observed that all isolates were able to produce hemolysis on blood agar. Also, two isolates (4%, 2/50) were found to be psychrotrophic. In 13 isolates (26%), at least one gene responsible for enterotoxin synthesis was detected and the isolates were found to have the highest number of *nhe* gene. It was also found that three isolates carried only the *nhe* gene (6%, 3/50), and 10 isolates carried the *nhe and cytK* genes (20%, 10/50) together (Figure 2). It was found that the isolates did not carry the *ces* and *hbl* genes.

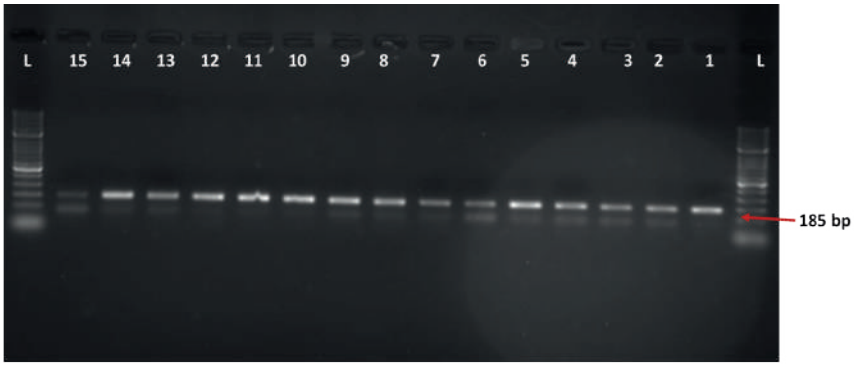

Figure 1. Agarose gel electrophoresis image of the obtained *B. cereus* strains [L; DNA ladder (100 bp), 1: Positive control, 2-15: *B. cereus* positive isolates (185 bp)].

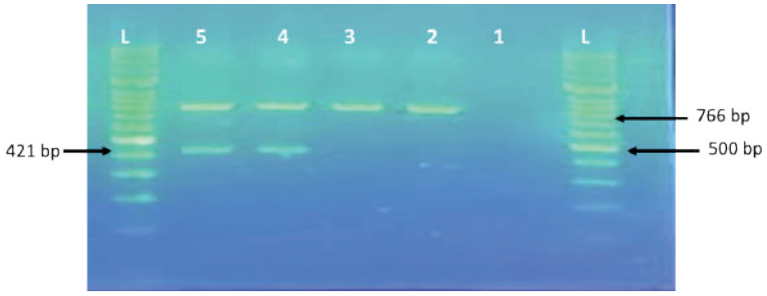

Figure 2. Presence of toxin genes in *B. cereus* positive isolates [L; DNA ladder (100 bp), 1: Negative control, 2-3: Positive for only *nhe* gene (766 bp), 4-5: Positive for both *cytK* and *nhe* genes (421 bp, 766 bp)].

4. Discussion

When the studies performed in our country and abroad were reviewed, Özdemir (2003) reported that *B. cereus* was present in 46.6% of the pasteurized milk offered for sale in Ankara. Can et al. (2022) reported that they detected this bacterium in 12 (34.2%) of 35 raw milk samples. Yıbar et al. (2017) found *B. cereus* at the level of 3.8% (2/53) and 26% (13/26) in raw milk and pasteurized milk samples, respectively.

In the study conducted by Te Giffel et al. (1997) in the Netherlands, *B. cereus* was isolated from 133 (40%) of 334 pasteurized milk samples. In the study conducted by Ahmed et al. (1983) in the United States, 400 milk and its products collected from different retail outlets for 5 months were analyzed for the presence of *B. cereus*. Although *B. cereus* was detected in a total of 103 samples (25.75%), it was found that raw milk and pasteurized milk were contaminated with *B. cereus* at 9% and 35% levels, respectively.

It was reported that the higher presence of *B. cereus* in pasteurized milk compared to raw milk may be due to the germination of spores due to the heat treatment applied in pasteurization. These studies show that pasteurized milk can often be contaminated with *B. cereus*. Competitive microflora is suppressed by the heat treatment applied in pasteurized milk production and due to the absence of competitive microflora in the product, *B. cereus* spores can easily develop as germs (Ahmed et al., 1983; Wong et al., 1988).

In a total of 30 powdered baby formulas analyzed, *B. cereus* was detected at a rate of 83.3%. In this context, when the level of *B. cereus* was examined in baby foods, contrary to the present study findings and at lower levels, Seyrekbasan (2000) stated that *B. cereus* was detected in 1 (2.5%) of a total of 40 imported powdered infant formulas belonging to various companies, Ergün et al. (2002) in 2 (4%) of 50 powdered baby foods, Bahçeci et al. (2018) reported that *B. cereus* was detected in 2 (10%) of a total of 20 imported or local baby foods. Unlike our study findings, Vural and Genç (2022) reported that *B. cereus* could not be detected in a total of 30 samples (15 of infant formula, 15 of follow-on formula) collected from the markets in Diyarbakır.

B. cereus could not be detected in a total of 25 jarred baby foods in our study. Similar to our study findings, Ergün et al. (2002) reported that *B. cereus* could not be detected in a total of 10 jarred baby foods. Unlike our study findings, Vural and Genç (2022) stated that *B. cereus* was was detected in 13.3% (2/15) of fruit-based jarred baby foods.

At a higher level than our study findings, Pei et al. (2018) found that 92.4% of 6656 powdered infant milk and follow-on formula samples collected from markets in China were contaminated with *B. cereus*. At lower levels than our study, in Colombia, Sánchez-Chica et al. (2021) detected *B. cereus* in infant milk by 11% (8/75), Becker et al. (1994) detected *B. cereus* to be 54% of 194 infant milk and follow-up formulas, Rahimi et al. (2013) isolated *B. cereus* from 84 (42%) of 200 infant formulas sold in Iran, Reyes et al. (2007) reported that *B. cereus* was isolated in 175 (45.9%) of a total of 381 dried milk products (milk-rice, milk-semolina, milk-rice-cereal samples, milk powder, milk pudding).

As a result, in studies that were conducted in our country and abroad, the presence of *B. cereus* in baby foods was determined at lower (Becker et al., 1994; Seyrekbasan, 2000; Ergün et al., 2002; Rahimi et al., 2013; Bahçeci et al., 2018; Sánchez-Chica et al., 2021; Vural and Genç, 2022) or higher levels (Pei et al., 2018) than in our study. This may be because of the number of samples was different, the characteristics of the samples (composition,

production technique, packaging condition) collection of samples from different geographical places, the different methods used in the isolation and identification of the bacterium.

In the present study, all cereal-based products were found to be contaminated with *B. cereus* (100%, 25/25). Unlike our study, Vural and Genç (2022) could not detect *B. cereus* in a total of 15 cereal-based supplementary foods. Bahçeci et al. (2018) determined it in 1 (5%) of a total of 20 grain-based foods, and the sample found positive was unpackaged semolina. *B. cereus* was found at the level of 11% in 155 grain-based samples (wheat flour, corn starch) analyzed in Colombia (Sánchez-Chica et al., 2021), and in 25% of the total 293 grain samples (rice, barley) analyzed in Korea (Park et al., 2009), these results are much lower than our study findings.

In this study, it was determined that 4% of 50 isolates identified as *B. cereus* were psychrotrophic. Higher than our study findings, Te Giffel et al. (1997) reported that 53% of 106 isolates were obtained from pasteurized milk samples, and Can et al. (2022) reported that 66.6% of 12 *B. cereus* isolates isolated from raw milk were psychrotrophic. However, contrary to our study findings, Reyes et al. (2007) stated that none of the 28 enterotoxigenic *B. cereus* strains isolated from dried dairy products were psychrotrophic.

It was found in our study that 13 (26%) of 50 isolates confirmed as *B. cereus* carried at least one gene responsible for toxin synthesis. Although it was determined that three isolates carried only *nhe* gene (6%), and 10 isolates carried the *nhe* and *cytK* genes (20%), the *ces* and *hbl* genes were not detected in any isolates. Similar to our study findings, Reyes et al. (2007) reported that 28 (29.8%) of 94 isolates obtained from dried dairy products were enterotoxigenic.

Gdoura-Ben Amor et al. (2019), Park et al. (2009), and Sánchez-Chica et al. (2021) identified enterotoxin genes (*nhe*, *hbl*, *cytK*) at higher levels than our study. Sánchez-Chica et al. (2021) reported that 100%, 63.4%, and 62% of 52 isolates confirmed as *B. cereus* isolated from various food samples in powder form (baby milk, milk powder, wheat flour, corn starch) had *nhe, hbl,* and *cytK* genes, respectively. Gdoura-Ben Amor et al. (2019) reported the highest number of *nhe* (100%), *bceT* (60.9%), *hbl* (52.2%), and *cytK* (39.1%) genes in 23 isolates in cereal products, respectively. Park et al. (2009) reported that 73 *B. cereus* isolates obtained from various cereal-based samples had the most *nhe* (99%), *hbl* (84%), and *cytK* (55%) genes, respectively.

Contrary to our study findings and at a higher level, Te Giffel et al. (1997) found that 76% of 37 isolates isolated from pasteurized milk samples carried the *hbl* gene. Unlike our study, Rahimi et al. (2013) found the most *entFM* gene (61.9%) in 84 isolates obtained from infant formula.

Similar to our study findings, the *ces* gene responsible for emetic syndrome could not be detected in *B. cereus* isolates in the studies of Gdoura-Ben Amor et al. (2019) and Sánchez-Chica et al. (2021). Also, consistent with our study findings, the detection of the highest number of *nhe* genes in different studies (Park et al., 2009; Gdoura-Ben Amor et al., 2019; Sánchez-Chica et al., 2021) supports that nhe is the enterotoxin most responsible for diarrheal syndrome (Stenfors Arnesen et al., 2008).

5. Conclusion

As a result, it can be speculated that the presence of enterotoxigenic and psychrotrophic *B. cereus* in the powdered baby formulas and cereal-based products examined may be dangerous in the nutrition of infants and young children whose immune systems and intestinal microflora are not fully developed. Maximum attention must be paid to the implementation of national and international food quality safety systems at all stages from production to consumption of the foods used in the nutrition of this age group.

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Chapter 3

Psychiatric Effects of Brucellosis: A Little-Known Aspect of an Ancient Disease

Sevil Alkan[1]

Ali Emre Şevik[2]

Abstract

Brucellosis is a zoonotic disease that can lead to a variety of clinical manifestations. This disease, which is endemic in Mediterranean countries including Turkey, is of interest to many specialties due to its multisystem involvement. In this article, we aimed to investigate the lesser-known aspects of brucellosis by examining the psychiatric symptoms associated with this old and persistent disease, to review the published literature on a global level, and to reveal the contribution from our country. Beyond the physical burden of brucellosis, it is crucial to recognize and address the psychiatric effects that this disease can have on individuals. By raising awareness about the psychiatric aspects of brucellosis, healthcare providers can implement comprehensive care strategies that encompass both physical and mental health support. Integrating psychiatric assessment, counseling and appropriate pharmacological interventions is essential to reduce the negative psychiatric impact of brucellosis and promote holistic recovery. Once understood primarily as a physical disorder, the potential impact of brucellosis on mental health is now recognized. The psychiatric effects of brucellosis can range from mood disorders and cognitive impairment to psychotic symptoms and sleep disturbances. Understanding the mechanisms and risk factors underlying these psychiatric symptoms is vital for developing effective interventions and providing holistic care. By shedding light on this often-overlooked aspect of brucellosis, medical professionals can improve patient outcomes and enhance the quality of life of those affected by this infectious disease.

1 Doç. Dr, Çanakkale Onsekiz Mart Üniversitesi Tıp Fakültesi, Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı, mail adresi: s-ewil@hotmail.com, Orcid: 0000-0003-1944-2477

2 Dr. Öğretim Üyesi, Çanakkale Onsekiz Mart Üniversitesi Tıp Fakültesi, Psikiyatri Ana Bilim Dalı, mail adresi:aliemresevik@gmail.com, ORCID ID: 0000-0002-4651-2859

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1. Introduction

Brucellosis is a zoonotic infectious disease caused by the bacteria of the *Brucella* genus. This genus is Gram-negative, facultative intracellular bacteria (Zheng et al.,2018; Arslan et al.,2023; Hayoun et al.,2023).

Remitting fever, undulant fever, Mediterranean fever, Maltese fever, Gibraltar fever, Crimean fever, goat fever, and Bang disease are just a few of its other names (Hayoun et al.,2023). Brucellosis is still endemic in most of the world, but after extensive and expensive control programs, it has been virtually eliminated from livestock in much of Northern Europe, Australia, the United States, and Canada. All of the major agricultural animal species are vulnerable, and the presence of wildlife reservoirs in some areas makes complete control difficult (Thorne et al.,1978). According to the World Health Organization (WHO), brucellosis is a serious illness that affects people all over the world, but it is particularly prevalent in the Mediterranean region of Europe, North and East Africa, the Middle East, South, and Central Asia, and Central and South America (Corbel, 2006). Brucellosis is prevalent in Turkey, making it one of the countries where the disease is endemic (Guven et al.,2013). According to the WHO, there are around 500,000 brucellosis new cases annually worldwide and 10,000–20,000 cases per year in Europe (World Health Organization,2014). Therefore, the disease is primarily either occupational, being associated with farmers, veterinarians, abattoir workers, and laboratory personnel, or is associated with travelers returning from endemic areas in more developed parts of the world where stricter food hygiene measures may be practiced (Young, 2010; Gul&Erdem,2015). Brucellosis primarily affects livestock, such as cattle, goats, and sheep, but can be transmitted to humans through direct contact with infected animals, consumption of contaminated dairy products, or inhalation of airborne particles. Once inside the body, the bacteria primarily target the reproductive organs, causing symptoms such as fever, fatigue, joint pain, and muscle aches (Zheng et al.,2018; Hayoun et al.,2023). However, recent research has shed light on the psychiatric effects toll brucellosis can take on infected individuals (Eren et al.,2006; Gulet al.,2009; de Figueiredo et al.,2015).

Due to *Brucella*'s ability to adapt to intracellular conditions and outwit the host's natural immune defenses, they are facultative intracellular parasites that can survive inside host cells after phagocytosis. This chronic infection is a result of this ability (Shehata et al.,2010). Brucellosis can last for several days or even years, but because it is frequently misdiagnosed, it can result in ineffective treatment and a protracted illness (Zheng et al.,2018).

In previously healthy patients, brucellosis is typically not fatal, but if untreated, it can result in a chronic infection with incapacitating recurrent clinical episodes (Young, 2010; Gul&Erdem,2015). Five of the 10 species that make up the genus *Brucella* have been found to be pathogenic in humans (Bouferraa et al.,2021). *Brucella abortus, Brucella melitensis, Brucella suis*, and *Brucella canis* infections frequently lead to human cases of brucellosis (Young, 2010; Gul&Erdem,2015).

The diagnosis of brucellosis relied on the presence of consistent clinical findings and either a serum agglutinin titer of ≥1:160 in serum tube agglutination (STA) or a positive blood culture (Gul&Erdem,2015).

A wide variety of clinical manifestations may occur as a result of the multisystemic disease brucellosis (Arslan et al.,2023). The most common signs of human infections include fever, malaise, sweats, and lymphadenopathy. However, they can also cause serious complications like endocarditis, meningoencephalitis, arthritis, spondylitis, orchitis, and psychological distress (Young, 2010; Gul&Erdem,2015). While the physical symptoms of brucellosis are well-documented (Zheng et al.,2018; Hayoun et al.,2023), its psychiatric effects often remain overlooked and understudied. However, in recent years, medical experts have begun to recognize that this bacterial infection can also exert significant effects on mental health. The psychiatric implications of brucellosis have become an emerging area of research, shedding light on the hidden impact this disease can have on individuals' psychological well-being (Eren et al.,2006; Gulet al.,2009; de Figueiredo et al.,2015). This article delves into the psychiatric effects of brucellosis, exploring the potential mechanisms, risk factors, and implications for both patients and healthcare professionals.

1.1. Understanding Neurobrucellosis

Neurobrucellosis represents a significant complication that can arise from systemic brucellosis infection (McLean et al.,1992). It was reported that, Florence Nightingale's chronic and severe headaches might have been attributed to a historically significant disease, possibly brucellosis (Young,1995).

The central nervous system may be affected by *Brucella spp.* either directly through invasion of neural tissue or indirectly through endotoxins or immune-inflammatory responses triggered by the bacteria's presence in the body (Bouferraa et al.,2021). Soares et al reported that the incidence of neurobrucellosis is about 4%, and it is virtually always diverse (Soares et al.,2023). In the literature, the occurrence of neuro brucellosis has been

reported with a wide range, varying from 0.5% to 25% (Shakir et al.,1987; Lubani et al.,1989).

An increased risk of nervous system involvement in brucellosis exists in older patients (Bouferraa et al.,2021).

In the majority of cases, the diagnosis of neurobrucellosis is typically established between 2 to 12 months after the initial onset of symptoms (Sanchez-Sousa et al., 1990; Akdeniz et al.,1998). Since it lacks normal clinical signs, other infections frequently mistakenly diagnose it (Soares et al.,2023). The patients may also experience general nonspecific neurological and psychiatric symptoms (Bouferraa et al.,2021).

Meningitis, meningoencephalitis, encephalitis, cranial neuropathies, intracranial hypertension, sinus thrombosis, hemorrhages' radiculitis, peripheral neuropathy, myelitis, and psychiatric symptoms are all included in the neuro brucellosis presentation (Soares et al.,2023).

The diagnosis ought to be made on the basis of neuro brucellosis-specific symptoms and indications that cannot be explained by another neurological condition (Soares et al.,2023). But, the diagnostic criteria for neurobrucellosis pose challenges in the literature. Some authors argue that the diagnosis should rely on clinical neurological symptoms, while others suggest that microbiological and/or biochemical evidence from cerebrospinal fluid should be the basis for diagnosis (Sanchez-Sousa et al., 1990; Bodur et al.,2003; Eren et al.,2006; Yetkin et al.,2006; Karsen et al.,2007).

There are four diagnostic criteria for the diagnosis of neurobrucellosis, which include signs and symptoms suggestive of neurobrucellosis, a positive finding of *Brucella spp*. in the cerebrospinal fluid (CSF), and/or a positive titer of antibodies targeting *Brucella* in the CSF, lymphocytosis with high protein levels and low glucose levels in CSF, and imaging findings (either cranial magnetic resonance imaging or computed tomography) peculiar to neurobrucellosis (Bouferraa et al.,2021).

According to a study from Turkey, agitation (25%), abnormal behavioral patterns (25%), muscle weakness (23%), disorientation (21%), and neck rigidity (17%) were the most prevalent neurological findings, among 128 laboratory-confirmed brucellosis cases who had neurological symptoms, and 48 (37.5%) had neurobrucellosis diagnosis (Guven et al.,2013).

1.2. Physical Symptoms vs. Psychiatric Manifestations

While brucellosis is commonly known for its physical symptoms, such as fever, sweating, fatigue, and joint pain (Hayoun et al.,2023), there is an

emerging body of evidence suggesting that the disease can also impact the central nervous system and mental health (Eren et al.,2006; Gulet al.,2009; de Figueiredo et al.,2015). Psychiatric symptoms associated with brucellosis may vary widely, and the severity and presentation can depend on factors like the strain of *Brucella*, individual susceptibility, and the stage of infection (Bourne et al.,1964; McDevitt,1973; Eren et al.,2006; Gulet al.,2009; de Figueiredo et al.,2015;Esmael et al.,2021).

According to a study, 2 out of 500 nonpsychiatric patients and 3 out of 500 newly diagnosed psychiatric patients with no suspicious brucellosis findings had positive serologic tests. Even though this complication is uncommon, the higher community prevalence of brucellosis leads to the consideration of brucellosis as a significant option in the differential diagnosis of psychiatric symptoms. This study emphasizes that brucellosis should be considered in the differential diagnosis for any psychiatric patient who exhibits psychiatric symptoms, particularly in endemic brucellosis areas (Shoaei&Bidi,2012). According to a study conducted on 400 brucellosis patients in Kuwait, 6% of those individuals experienced psychiatric complications, primarily depression and anxiety during the chronic stage of the illness. According to one study, patients with emotional disorders who did not have acute or chronic brucellosis were found to have *Brucella* deoxyribonucleic acid (DNA) in their blood. Out of 20 patients with emotional disorders, 3 (15%) had *Brucella* DNA found; however, all the controls tested negative (Kechagia et al.,2011).

According to the study conducted by Zou et al, it was observed that the patient's emotional changes, including mood disorder, feelings of sadness, and loss of interest, exhibited slight improvement after undergoing anti-depression therapy. However, these emotional changes were further alleviated through antibiotic therapy. Based on the patient's epidemiological history and the psychiatric manifestations observed after intermittent fever, it was hypothesized that the patient's depression could be attributed to brucellosis. Despite negative cranial magnetic resonance imaging scans and absence of neurological signs, the connection between brucellosis and the psychiatric symptoms was considered (Zhou et al.,2023).

In Eren et al's study (Eren et al.,2006), a total of 34 cases of neurobrucellosis and 30 patients with brucellosis but without neurological involvement were examined. Two psychiatrists conducted interviews with the patients and administered the Hamilton Depression Rating Scale (HDRS) and Mini-Mental State Examination (MMSE) tests. Among the neurobrucellosis cases, the mean MMSE score before antibiotic therapy was 21.6. One week

after therapy, it increased to 22.7, and after two weeks of therapy, it further improved to 24.3 ($p=0.024$ and $p<0.001$, respectively). The mean HDRS score at the time of admission before therapy was 9.9, which decreased to 7.8 after one week of therapy, and reached 5 after two weeks of therapy ($p=0.014$ and $p<0.001$, respectively). The study reported that cognitive and emotional disturbances in neurobrucellosis patients were assessed using the MMSE and HDRS tests. These disturbances showed improvement with antibiotic therapy alone, without the need for additional antidepressant or antipsychotic therapy (Eren et al.,2006).

1.3. The mechanisms underlying the neuropsychological manifestations of brucellosis

Increasing levels of cytokines and microbial-derived antigens in the blood may change the body's neuro-immune balance and, consequently, the behavior, even though the mechanisms underlying the neuropsychological manifestations of brucellosis are unclear (Dantzer,2009; Miller et al.,2013; Pavlov&Tracey,2017; Salvador et al.,2021).

However, the observation that functional sequela occurs in patients with chronic brucellosis supports the possibility that this is the case (Shehata et al.,2010).

The majority of patients advance to a chronic stage of the disease where neuropsychiatric symptoms worsen. It is still unknown what biological mechanisms underlie the development of these symptoms. Localized inflammation brought on by *Brucella* may cause neurochemical changes and, as a result, unrestrained neuropsychiatric disorders. According to the findings of an experimental mouse study, motor impairments, muscular weakness, and decreased motivation in *Brucella* -infected mice were correlated with neurochemical and peripheral immunological disturbances and tended to diminish after 21 days of infection. The current data in this report supported the hypothesis that mood disorders in infected mice may result from disturbed peripheral inflammation and associated neurochemical disruption (Maldonado-García et al.,2021).

1.4. Psychiatric Effects of Brucellosis

1.4.1. Depression and Anxiety

Depression and cognitive impairment are commonly observed symptoms in cases of brucellosis. However, depression stands out as the most prevalent psychiatric disorder and often appears to be disproportionate to the severity

of other symptoms experienced (Shakir et al.,1987; McLean et al.,1992; Akdeniz et al.,1998; Bodur et al.,2003; Young,2010; Shoaei&Bidi,2012; Elzein et al.,2018; Zhou et al.,2023). Brucellosis can significantly impact an individual's mental health. Studies have shown that the chronic nature of the infection, coupled with the prolonged physical symptoms, can lead to the development of depressive symptoms. Feelings of sadness, hopelessness, and anhedonia (inability to experience pleasure) may be present. Additionally, anxiety disorders, such as generalized anxiety disorder and panic disorder, can emerge due to the uncertain and protracted nature of the illness (Bourne et al.,1964; McDevitt,1973; Esmael et al.,2021).

In one study, 50 healthy matched controls and 27 brucellosis patients were compared. 14 (51.9%) of the patients with brucellosis had overt or apparent neurological manifestations, while the remaining 13 (48.2%) did not appear to have any neuropsychiatric involvement. A total of 7 patients (29.2%) had depression (Shehata, et al.,2010).

In a different study, two psychiatrists conducted interviews with the neurobrucellosis patients and administered the MMSE and HDRS tests (Hamilton Depression Rating Scale). There was no antidepressant or antipsychotic therapy used to treat the documented cognitive and emotional disturbances that were improved by antibiotic therapy (Eren et al.,2006).

According to a recent study, patients diagnosed with neurobrucellosis exhibited elevated levels of psychiatric symptoms, including behavioral changes, anxiety, and depression ($p< 0.001$, $p< 0.001$, and p: 0.01, respectively), and considerably poorer cognition than non-neurobrucellosis patients (Esmael et al.,2021).

1.4.2. Cognitive Impairment

Brucellosis has been associated with cognitive impairment, including difficulties with concentration, memory loss, and decreased processing speed. These cognitive deficits can impact daily functioning, work performance, and overall quality of life. The precise mechanisms behind these effects are not fully understood but may be related to the inflammatory response and neurotoxicity caused by the bacteria (Shehata, et al.,2010; Liapina, et al.,2010; Smagina&Shul'diakov,2011).

In a recent study, indices of the quality of life and psycho-functional status parameters of patients with chronic active brucellosis have been identified, and for a group of 40 patients, the therapeutic effectiveness of the immunomodulator cycloferon in the complex treatment of the disease was evaluated. Twenty of the individuals received cycloferon, whereas the other

twenty patients in the control group solely received conventional treatment. It is known that the dimensions of one's quality of life significantly decline when one has chronic active brucellosis. In comparison to results obtained with conventional therapeutic techniques, the administration of cycloferon against the backdrop of a base therapy improves the quality of life and psychoemotional status markers (Smagina&Shul'diakov,2011).

Another study looked at the therapeutic potential of cyclopheron via chronic brucellosis patients as an example. Improvements in quality of life, a decrease in the frequency of exacerbations of the infectious process, and the emergence of concurrent diseases are all positive clinical dynamics during combination therapy with cyclopheron. It has been established that these effects result from cyclopheron's immunomodulating activity and its suppression of lipid peroxidation. Additionally, the medication boosts antioxidant activity while lowering pro-inflammatory (and, to a lesser extent, anti-inflammatory) cytokine levels (Liapina, et al.,2010).

A controlled clinical trial examined the clinical and pathogenetic efficacy of cytoflavin as a component of the combined therapy for chronic brucellosis in 50 patients, who also confirmed the drug's positive impact on quality of life. Based on this study' findings, cytoflavin serves by lessening the severity of endogenous intoxication and systemic inflammation (Shul'diakov et al.,2011).

1.4.3. Sleep Disturbances

Brucellosis can disrupt normal sleep patterns, leading to insomnia or excessive daytime sleepiness. The physical discomfort, pain, and anxiety associated with the infection can interfere with sleep initiation and maintenance. Sleep disturbances, in turn, exacerbate psychiatric symptoms and contribute to an overall decline in mental well-being (Malik et al.,2018; Mansurova&Nazarov,2021; Alimovna,2023). In a study of 70 brucellosis patients, insomnia was found in 12.9% of patients (Malik et al.,2018). Laziness, distractibility, sudden, uncontrollable urges to sleep, and a reversal of the typical sleep-wake cycle are all signs of sleep disturbances. Without treatment, the disease advances to a terminal stage characterized by seizures, extreme somnolence, double stim, cerebral edema, coma, systemic organ failure, and death (Duggan&Hutchington,1966).

1.4.4. Psychosis

Brucellosis can occasionally develop neuro brucellosis, which affects both the central and peripheral nervous systems and can have serious clinical

effects (Eren et al.,2006; Ates et al.,2008; Ghaffarinejad et al.,2008; Shehata et al.,2010).

Although psychiatric findings are uncommon in brucellosis, endemic countries' higher brucellosis prevalence leads to the consideration of brucellosis as a significant alternative in the differential diagnosis of psychiatric symptoms. It is uncommon for neurobrucellosis to present with psychosis. Only a few prior reports of brucellar psychosis were available in the current literature (Ates et al.,2008; Ghaffarinejad et al.,2008).

1.4.5. Post-Traumatic Stress Disorder (PTSD)

Although chronic post-traumatic stress disorders are numerous and diverse, post-traumatic stress disorder (PTSD) continues to be the most well-known. Posttraumatic depression and bereavement can lead to a heightened risk of suicidal crises and self-harming behaviors. When experiencing post-traumatic anxiety disorders, which may include conditions like agoraphobia, specific phobia, obsessive-compulsive disorder, separation anxiety, or social phobia, individuals often exhibit symptoms of re-experiencing, heightened arousal with increased anxious reactivity, and resort to avoidance strategies that intensify anticipatory anxiety. It is not uncommon for post-traumatic symptoms to co-occur with chronic psychotic manifestations, resulting in a clinical picture that is frequently severe. These may include conditions such as post-traumatic schizophrenia, post-traumatic depression with mood-congruent psychotic features, non-schizophrenic post-traumatic psychotic disorder, and bipolar reaction to trauma. Furthermore, if an injury occurs simultaneously with traumatic exposure, the likelihood of later developing post-traumatic stress disorder is heightened (Nohales&Prieto,2018). This, in turn, affects how the body is perceived to be feeling (development of somatoform and psychosomatic disorders, co-morbidity with post-concussion syndrome). Trauma can cause a person's biography to fall apart, as well as their internal physiological processes and social interactions (impacts of instinctive behaviors, personality changes, and adjustment issues on work and personal life) (Auxéméry,2018; Nohales&Prieto,2018).

Recent research indicates that beyond the conventional psychological and behavioral indicators, PTSD diagnosis now takes into account the connection between this condition and changes in immune and inflammatory responses. Studies in epidemiology have revealed a notable correlation between PTSD and heightened prevalence of physical comorbidities related to immune dysregulation, including metabolic syndrome, atherosclerotic cardiovascular disease, and autoimmune disorders (Hori&Kim,2019).

Brucellosis can be a traumatic experience for some individuals, particularly in cases of severe complications or prolonged treatment. In severe cases, brucellosis can lead to the development of PTSD. Individuals who have experienced a prolonged illness, endured invasive medical procedures, or faced significant physical and emotional challenges associated with the infection may be at higher risk. Symptoms of PTSD may include intrusive thoughts, flashbacks, hypervigilance, and avoidance behaviors (Eren et al.,2006, Shehata et al.,2010; Esmael et al.,2021).

1.4.6. Social Isolation and Stigma

The chronic nature of brucellosis and the associated physical and psychiatric symptoms can lead to social isolation. Individuals infected with brucellosis may face stigma and discrimination due to misunderstandings about the disease's transmission or fear of contagion. This social isolation and stigma can further contribute to the development or exacerbation of psychiatric symptoms (Eren et al.,2006,Shehata et al.,2010;Esmael et al.,2021).

2. Conclusion

Brucellosis, a disease primarily known for its physical symptoms, can also have profound psychiatric effects on affected individuals. Depression, anxiety, cognitive impairments, and PTSD can significantly impact the mental health and well-being of those diagnosed with brucellosis. Recognizing and addressing these psychiatric manifestations is vital to ensure comprehensive care for patients and improve their quality of life. Given the potential psychiatric impact of brucellosis, it is crucial to incorporate mental health assessments into the clinical management of affected individuals. A multidisciplinary approach involving psychiatrists, infectious disease specialists, and primary care physicians is necessary to ensure comprehensive care for patients with brucellosis. Early recognition and treatment of psychiatric symptoms can significantly improve the patient's overall well-being and enhance their ability to cope with the challenges associated with the disease. Future research and collaborative efforts are necessary to enhance our understanding of the psychiatric aspects of brucellosis and develop effective interventions to mitigate its impact on mental health. Furthermore, there is a need for further research to elucidate the underlying mechanisms of the psychiatric effects of brucellosis. Longitudinal studies are warranted to investigate the long-term psychiatric outcomes and the risk factors associated with the development of these symptoms. Additionally, developing targeted interventions, such as psychoeducation, cognitive-behavioral therapy, and psychopharmacological approaches, may help alleviate psychiatric symptoms and enhance the recovery process.

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Chapter 4

Understanding Smoking Behaviour: Interpretative Phenomenological Analysis Highlighting Cognitive Dissonance

Buse Keskindağ[1]

Abstract

Literature provides qualitative evidence on smoking behaviour among healthcare professionals. Smoking behaviour of nurses and medical doctors have been examined to understand their attitudes and perceptions regarding their smoking behaviour. Being a healthcare professional and a smoker at the same time may represent some conflict due to the dilemma it creates with a professional position. Therefore it is important to understand how healthcare professionals make sense of their health behaviours. Health psychology professionals aim to promote health related behaviours by conducting various interventions. The aim of the current study is to understand how a health psychology student make sense of their smoking behaviour by focusing on dilemmatic aspects of this position. By using qualitative in-depth interviews, this study aims to understand the perspective of a health psychology student who is a smoker. Interpretative phenomenological analysis was used to conduct analysis on a single case. In total, four super-ordinate themes were developed, one of the themes was reported in detailed. The theme, *justification in terms of escaping from reality*, highlights perceptions relevant to dilemmatic aspects, therefore it was chosen to discuss dilemmatic position of the case. The findings were mainly discussed in the context of cognitive dissonance theory. Analysis showed that the case used rationalisation and denial to justify smoking behaviour. Overall, this study provides interesting narrative that shows how a person on a dilemmatic position make sense of their own behaviour.

1 Assist Prof, Bahçeşehir Cyprus University, buse.keskindag@cyprus.bau.edu.tr, ORCID ID: 0000-0002-0827-0561

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1. Introduction

There is emerging evidence on qualitative evidence to make sense of smoking behaviour particularly among healthcare professionals. Since healthcare professionals are expected to acknowledge harmful effects of smoking, it is not clear why some of them maintain smoking behaviour. There are various interventions in different forms e.g., workshops, psycho-educational programs in order to quit smoking behaviour. Particularly health psychology plays a significant role in terms of helping individuals to adopt positive health behaviours to maintain healthy lifestyle. Adopting this perspective, healthcare professionals including health psychologists are expected not to smoke, otherwise this would create a dilemma in regard to purpose of their position. Health psychologists' health behaviours and perceptions are poorly studied. However, there are some research investigating smoking behaviours and attitudes among nurses, medical doctors' and students studying related fields.

According to Blakey and Seaton (1992), smoking behaviour has been frequently observed among nursing students during their education. This finding shows that many nursing students maintain their smoking behaviour even though they know the harmful effects scientifically. Additionally, it has been reported that 30% to 54% of male doctors and 40% of female doctors were smokers in Europe. In the same study, role of limited knowledge was discussed and it was suggested that smoking behaviour of patients were influenced to some extent from smoking status of patients' healthcare professionals. In other words, quitting behaviour of a patient may be influenced to some extent whether the healthcare professional is a smoker or not (Minh An et al., 2008). Quitting may be very difficult even for healthcare professionals including nurses, medical doctors and students who started to smoke at late adolescence / early adulthood. According to Biraghi and Tortorano, 75.2% of student smokers have at least one parent smoker. In other words, family and peer effect can be seen as important factors among student smokers. Nevertheless, being a healthcare professional and a smoker at the same time may lead some conflict due to dilemma it creates with professional position. Therefore it is important to understand how healthcare professionals make sense of their health behaviours, particularly smoking which is common in healthcare professionals and students studying in relevant academic area.

The aim of the current study is to understand how a health psychology student make sense of their smoking behaviour by focusing on dilemmatic aspects of this position. There is limited evidence on health psychology

professionals' attitudes and perceptions regarding health behaviours. Health psychology practitioners particularly are trained to modify behaviour and design health related interventions to maintain wellbeing. By using qualitative in-depth interview, this study aims to understand perspective of a health psychology student regarding their smoking behaviour. More specifically, the objective of this study is to explore a health psychology student's sense-making of their smoking behaviour by focusing on dilemmatic aspects of their position.

2. Method

2.1. Participant

Previously, John was one of the participants in a small-research project examined attitudes of health psychology students toward smoking behaviour. However, John's narrative seemed rich which led conducting in-depth semi-structured interview with him to highlight idiographic aspects. John was studying health psychology master's degree and he was smoker at the time. John was male and 32 years old. He identified himself as Caucasian.

2.2. Interview Schedule and Procedure

The interview schedule included four parts which investigated participant's history of smoking behaviour, quitting experiences, attitudes about health care advertisements against smoking and dilemmatic relations with health psychology study. History of smoking behaviour questions aimed to identify how participant started to smoke. Quitting experience questions were designed to determine whether the participant attempted to quit. Additionally, questions on health care promotion advertisements were asked to determine whether these had an impact on the participant's behaviour. Finally, last part of the interview questions examined participant's views about his smoking behaviour from health psychology perspective. In total, there were 15 questions on the interview topic guideline. The interview duration was approximately two hours. At the beginning, John was asked to provide informed consent and was informed that his name and interview records will be kept confidential. His original name was changed with a nickname (John) to maintain confidentiality.

2.3. Analytic Procedure

Interpretative Phenomenological Analysis (IPA) was chosen as a methodology for this research. IPA considers understanding of individual`s psychology and their experiences by the individual`s meaning – making

process (Smith & Eatough, 2007). This particular analytic tool assumes the involvement of the researcher with including a double hermeneutic which is unable to be avoided. In addition, researcher tries to understand individual`s perception, while they try to make sense about their own world (Smith & Eatough, 2007). Therefore, IPA was chosen as methodology to use in order to focus on participant's meaning – making about their experience. IPA was appropriate approach for this study as the study aim to understand dilemmatic aspects of a health psychology student related to their smoking behaviour. It was expected that understanding of participant's meaning making about the smoking behaviour would be achieved by letting him "tell the story". Story telling is an appropriate process which helps participant to reveal their views about their smoking behaviour because interview questions included dilemmatic structure.

Firstly, the interview transcript was read several times by the researcher. Throughout this process, before developing emergent themes and superordinate themes, small notes were taken in order to have an initial idea about the potential themes. At second stage, initial notes were promoted to emergent themes that representing psychosocial concepts. This process was revealing participants voice, at the same time some of existing psychological terms were used, therefore analytic process represented both inductive and deductive approaches. Whilst this helped to maintain contextual characteristics, it allowed researcher to use theoretically sensitive interpretation to make sense of the participant experience. At the third stage, emergent themes were examined in terms of how they were connected and how they may form a cluster. In order to deliver idea on the nature of the themes, thematic clusters were labelled (super-ordinate themes). Finally, at four stage, super-ordinate themes, emergent themes and data extracts supporting these themes were reviewed. This process helps researcher to understand and interpret the interview transcript.

The most suitable criteria for assessing this research includes "sensitivity to context" (Yardley, 2000), "coherence" and "grounding in example" (Elliot et al., 1999). Sensitivity to context was chosen as a criteria because if a researcher does not display sensitivity to individual`s language, sociocultural context also previous studies, viewpoints of the researcher would not be grounded data or theoretical context. Additionally "coherence" is another important criteria which plays a significant role while reader tries to understand the researcher`s suggestions related to the data. That is, suggestions should be consistent in order to make it clear for the reader. Moreover, "grounding in examples" is also significant because it is necessary to provide examples in order to make the suggestions to be supported therefore, reader can give

meanings to information that are demonstrated by the researcher. Typically, the most important theme is reported in a research report that use IPA (Lyons & Coyle, 2007).

3. Findings

3.1 Case

Firstly, John's experiences about social influences on his smoking behaviour started in school years when he was 13 years old. He started to smoke at 13 years old with the impact of his peers and school environment. He had a reason for smoking behaviour, he was trying to be "cool" and he was trying to show this with smoking behaviour. One of the social influences was his dad, because his dad used to smoke a lot. Although his attitude toward smoking has changed, he continues to smoke because of psychological dependence. He wish to quit smoking however, he believes that it is early for him to think about quitting. While his attitudes towards smoking changing, he is aware of responsibilities that he has towards his and other's health, particularly, his son's. He feels guilty when he smokes around him. He accepts that his smoking behaviour has an impact on his son's health. He mentioned that he feels different from others while smoking during the breaks between classes at the university. He mentioned that he does not care too much others' opinions. Most of the people expect health psychology students to be more aware of risks and more engaged in positive health behaviours. However, he is aware of responsibilities that he has towards his career or study also his wellbeing. He identified smoking as coping strategy for stressors in life such as; difficulties of work and studying. According to him, smoking is a good excuse in order to have a break. The way he expressed himself provides hints on rebellious personality which was explicitly mentioned during the interview. Somebody else's opinion is not usually important for him. According to him, if someone wants to smoke they should be able to smoke, nobody can judge others' behaviours. Because of this attitude he believes that smoking cessation campaigns are not sufficiently effective. He frequently mentioned that the healthcare advertisements and campaigns against smoking are useless and stupid. This approach may stem from denial such as; not accepting negative consequences of smoking. Therefore, he avoids paying attention to these healthcare advertisements and campaigns against smoking. The analysis included four super-ordinate themes. Since this study focused on dilemmatic position, one of the themes will be reported in this section. This theme was labelled as 'justification in terms of escaping from reality', it highlights perceptions relevant to dilemmatic aspects.

3.2 Justification in Terms of Escaping from Reality

John discussed several ways to justify smoking behaviour. His main excuse was that smoking helped to cope daily stress:

"I wonder, one day, if we wouldn't smoke what would we do something else? And everybody did something else, would there be health psychologist to say "do not do that"? I don't know, like eating. I wouldn't say I prefer smoking but I noticed on myself stress or time out there is always be cost so on , I see thing that make you happy good and you can control and limit to use. I rather being smoker than fat, rather being smoker than alcoholic, rather being smoker than drug addicted, or any other things."

He seemed to find his smoking behaviour as a coping strategy. This can be considered as a way of rationalising smoking behaviour. Actually, he believes that if he would not smoke, he might do something else, e.g., engaging another unhealthy behaviour such as: overeating, drug addiction or alcohol use. In other words, he thinks that there is always a cost of stressful life. Because of this reason, he prefers to be a smoker. Therefore, 'coping strategy' as emergent theme was considered under the title of justification in terms of escaping reality. Additionally, John finds smoking as an opportunity of having a break which helps him to cope with demands of work or study. At the same time, he perceives smoking providing little time for him to relax for a while:

"Generally I find smoking in order to give a break , so I like to smoke to give a break or end of my work, it marks the end of the things and beginning of things it provides me to think also when you are in a group, it gives you something to talk about , or if you are alone it gives you feel that you belonging yourself and think about it something and you do not feel strange because you are standing outside by yourself without doing anything, because you have a reason you are standing outside because having a cigarettes … so it is ok, everybody understand why you are standing outside alone, (laughs) because I also tried a cup of tea for break, it worked for a while, but it is not the same … , it is a small task, easy, and it is not very demanding"

Smoking cigarette was good enough excuse for John to be outside alone for a while. Cigarette was like a tool that helped John take a break from everything. These short breaks were perceived as chance to reflect and think. At the same time, he made sense of smoking as a way out from daily hassles which can represent end of a task. Smoking offered him to have his own time (outside) without feeling of shame or awkward. Smoking cigarette was like a company to him throughout the 'break'. Even if he was

alone outside standing, there was reason, which was smoking cigarette. Therefore, standing alone and having 'break' is no longer weird or strange. At the same time, it provided sense of confidence and comfort. Similarly, being included in a group where most people smokes, John would not feel outsider. Smoking cigarette would provide sense of belongingness, and something that he shares with group members. Hence, smoking cigarette is not only providing an opportunity to relax and have a 'break', it also reduces potential feelings of anxiety and awkwardness in a social environment. For John, smoking cigarette is a coping strategy in multiple ways that has several functions.

Rebellious personality was identified as another emergent theme. This represented how John perceived and recognised himself as rebellious in relation to his smoking behaviour. This was prominent in the interview:

"Fundamentally it sounds terrible but I am buying because I prefer to do in what do you want instead of not doing something because of costing shorter life. Because everybody says that if you smoke it takes time from your life but I believe that there is an end of life so you should live in whatever way you want in. I'm not thinking how many years I am going to live. I'm not worried about that for now".

He expresses himself as independent and as a person who can do whatever he wants in life. He prefers to spend his life according to his beliefs. The most important thing is that he mentioned nobody can stop his smoking behaviour unless he decides to. Although he highlights importance of informed decision, he seems not being sufficiently open for smoking focused healthcare messages. He seemed to reject health information easily. Nevertheless, he relates his own attitudes mainly to his personality. John's reaction towards healthcare messages and campaigns against smoking looked like psychological reactance that led him acting as 'rebellion'. This may be reflection of denial.

"I smoked 12 years. (Laughs) I think at this point advertisement do not work, and it is kind of rebellion that's why I am involved in smoking. And that is possibly why campaigns do not work. Nobody cannot tell me what's right for me or perhaps they could but they need to be very informed and to make me listen."

He can be rebel even to his son. He mentioned that his son always tells him to stop smoking:

"I think it is a part of being rebellion person, for example my son tells me to stop and I am rebel to even him. And I remember when I am smoking,

telling my father to stop smoking. I think it is related when you are being old. We say when it doesn't kill you makes you stronger (Laughs)."

He believes that rebellious personality may contribute to do unhealthy activities in order to maintain independence. Smoking behaviour is one of these unhealthy activities he engages in. It is interesting that he frequently uses his 'rebellious' personality in order to escape from reality. As it was quoted above, he finds a way to rationalise his smoking behaviour as negative impact of smoking on health status is not shown immediately. Moreover, he mentioned that he really does not care about others' views on his smoking behaviour. He clearly showed that he liked being 'independent person' who has freedom to do anything. When he was asked about others' perceptions of him as a health psychology student and being a smoker, he said:

"Many tutors I know they do not see it is a good thing but I do not have any idea about the students (laughs)… who cares people."

In the meantime, he talked about how he perceives health psychology courses in relation to smoking behaviour:

"Reading journals and articles are effective because I decided to read them regarding to my coursework's and it's more effective than advertisements, absolutely it makes me change. The things is that when people try to stop me smoking, it doesn't work I need to give my own decision I think, whereas, reading journals, no one forcing me and I choose article which I will read has a much more impact."

Being smoker and studying health psychology degree seem not to cause any conflict in John. He even seemed powerful to being able to smoke while studying health psychology. Although he recognises importance of evidence as he is occupied with reading articles as part of the course, he also seemed to have strength standing by his choice (of being smoker). This strength may be related to him being 'rebellion' as he frequently refer to. Perhaps this is how he maintains and justifies his smoking behaviour through denial.

4. Discussion

This research aims to provide a health psychology student's rationales about his smoking behaviour and dilemmatic aspects in relation to this position. The analysis highlights ideas and beliefs of a health psychology student in relation to his smoking behaviour. His narrative shows that he is aware of his dilemmatic position, however, he seem to be strongly attached to his justification to maintain smoking. He often made connection between his smoking behaviour and rebellious personality. That is, he uses

his rebellious personality as an excuse for smoking. In the meantime, as a health psychology student, the participant believes that he needs to know more about negative consequences of smoking behaviour. This may be partly related to fact that he finds advertisements useless and not effective. At the same time this might be reflection of denial. There was social influence on his smoking behaviour during his childhood and early adulthood, for instance, his dad was a smoker. One of the good examples for dilemmatic position is that he mentioned that he used to tell his father to stop smoking while he was smoking. Having the knowledge and information do not always lead to behaviour change, which is one of the aspects that health psychologists focus on.

It is common that smokers frequently experience inconsistency between awareness of health outcomes of smoking and maintaining the smoking behaviour itself (Chapman et al., 1993). This inconsistency may be considered in the context of cognitive dissonance theory (Festinger, 1957). Cognitive dissonance theory suggest that person may hold two conflicting cognitions or one's cognition (attitudes, beliefs) may contradict with their behaviour. This tends to lead psychological tension. Coherence is needed in order to reduce this tension, hence a person may change their cognition or behaviour to achieve coherence. For instance, a person who is smoking and who is aware of health outcomes of smoking may experience cognitive dissonance. In this case, they may be expected to reduce or quit smoking, which may reduce inconsistency. On the other hand, they may change smoking related cognitions such as, minimising the importance of evidence showing smoking is harmful, denying the health outcomes of smoking or rationalising the behaviour. These strategies may be even easier to accomplish than behavioural change. Therefore, individuals who smoke are likely to rationalise their smoking behaviour in order to reduce cognitive dissonance. For instance, people who smoke may rationalise smoking and say that it is worth to smoke as they are happy with it. Rationalisation is typical defence mechanism that helps people who smoke to reduce cognitive dissonance hence psychological tension (Orcullo & Teo, 2016). In the current study, the participant attempted to justify his smoking behaviour by suggesting that he has rebellious personality that he owns his freedom to do whatever he wants. Also he used smoking to manage daily stress. In different qualitative studies, people who smoke also hold irrational beliefs to reduce cognitive dissonance even if they were aware of negative health outcomes of smoking (Schmitt et al., 2005; Van Overwalle & Jordens, 2002). For instance, in one of these studies, participants believed that smoking would not be much dangerous

as long as they were moderate smokers. This was their way of rationalising smoking behaviour (Van Overwalle & Jordens, 2002).

Denial, on the other hand, is another strategy people may use as a defence mechanism to reduce cognitive dissonance. It may be sometimes easier for people to deny the facts when they avoid acceptance, this may protect their "ego". Besides, people may deny circumstances unconsciously that are not consistent with their behaviour. In the case of smoking, a person may strongly believe that smoking cigarette is not the only way to harm health status. They may believe that even if they were not smoker, there would be different dangers that may negatively affect their health, therefore, they would be convinced that they there is no need to avoid smoking. In parallel with other qualitative research (Gray et al., 2014; Orcullo & Teo, 2016), this was evident in the current study. The participant strongly believed that there would be other harms to affect his health so he "prefers" smoking. Denial may serve a way to protect self-concept to maintain smoking behaviour.

This research provides interesting narrative that shows how a person on a dilemmatic position make sense of his own behaviour. Although many studies are available in the literature examined healthcare professionals' health behaviours and cognitions, health psychology professionals' health behaviours have been poorly studied. Hence, particularly smoking behaviour should be further investigated in health psychology practitioners. The current study may guide further research addressing similar research questions by using cognitive dissonance theory. Regarding data analysis, conducting IPA was appropriate as it provides a flexible approach in terms of interpreting the transcript by attempting to understand participants' meaning making related to their experiences. Furthermore, it should be mentioned that analysis being conducted on a single case only represents the participant's personal experiences. Although it allows researcher to focus on a deeper level of understanding with idiographic approach, single case study would not be ideal in the context of generalising findings on a broader society. Overall, this study provided a clear illustration of participant's feelings, beliefs, and experiences about smoking and his dilemmatic position regarding his professional aspect.

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Chapter 5

The Importance of Non-vitamin K Antagonists (NOAC) in Their Current Use

Veysel Tosun[1]

Abstract

Non-vitamin K oral anticoagulants are new drugs that are used in the treatment of atrial fibrillation and venous thromboembolism. There are 4 NOACs in use today; dabigatran is a direct thrombin inhibitor, while rivaroxaban, apixaban, and edoxaban are Factor Xa inhibitors. NOACs can be used safely in AF patients, except for patients with moderate to severe rheumatic mitral stenosis, and metallic prosthetic valves. In studies where NOACs were evaluated in terms of effectiveness and safety, similar or better results were obtained with VKAs. With the new two antidotes (idaricuzimab and andexanet alfa) approved for use in NOAC-related bleeding, the potential for use of NOACs in patients with high bleeding risk is expected to increase.

1. Introduction

Protecting patients with atrial fibrillation (AF) from stroke is very important, and vitamin K antagonists (VKA) have long been used for this purpose (1). However, non-vitamin K oral anticoagulants (NOAC) are now considered by AF guidelines worldwide as the preferred choice of anticoagulants to prevent stroke in patients with AF (2-4). NOACs have an efficacy/safety ratio and a predictable anticoagulant effect that does not require routine coagulation monitoring as required with VKAs (5, 6). In recent years, NOACs have begun to be widely used as an alternative to VKA in our country, as well as around the world, to protect against stroke and systemic embolism in AF. There are four preparations used as NOAC today. Of these, dabigatran is a direct thrombin inhibitor, rivaroxaban, apixaban, and edoxaban are factor Xa inhibitors.

1 M.D, Department of Cardiology, T.C. SBU. Van Education and Research Hospital, Van, Turkey, veyseltosun8810@gmail.com, ORCID ID: 0000-0001-7629-2108

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2. NOAC eligibility and dosage

2.1. NOAC eligibility

NOACs are approved especially for stroke prevention in non-valvular AF. In previous guidelines, the term non-valvular AF emphasized AF without a mechanical prosthetic heart valve or with moderate to severe mitral stenosis (especially rheumatic origin) (2, 7, 8). There is no randomized controlled trial (RCT) demonstrating that NOACs are less efficacious in rheumatic mitral stenosis patients. The INVICTUS program investigating the use of VKA, rivaroxaban or acetylsalicylic acid, in patients with rheumatic heart disease is currently ongoing. In patients with mechanical valve replacement, NOACs should not be considered unless there is new evidence reversing existing data that NOACs may be better than VKA in preventing stroke (9, 10). In patients with bioprosthetic valves, in the 'Rivaroxaban for Valve Disease and Atrial Fibrillation' (RIVER) trial, it was non-inferior to warfarin for the median time to the composite endpoint of death, major cardiovascular events or major bleeding (11). Similarly, edoxaban was non-inferior in the 'Efficacy and Safety of Edoxaban in Patients Following Heart Valve Repair or Bioprosthetic Valve Replacement (ENAVLE) study. Observational data showed that early thromboembolic and bleeding events and all-cause mortality were lower with NOACs after TAVI compared with VKA (12, 13).

A summary of the above and other indications and contraindications for NOAC use are listed in Table 1. Additionally, NOACs are contraindicated in pregnancy and women of childbearing age must have reliable contraceptive methods before initiating NOAC therapy (14). Pediatric patients have been excluded from stroke prevention RCTs because AF requiring oral anticoagulation (OAC) is rare in this population (14). It can be considered in fully adult adolescents. Patients with non-valvular AF and antiphospholipid syndrome should be treated with VKAs rather than NOACs as a higher rate of thromboembolic events and major bleeding has been observed with rivaroxaban compared with VKA (15).

Table 1. Selected indications and contraindications for NOAC therapy in AF patients (16).

Condition	Eligibility for NOAC	Comment
-Mechanical prosthetic valve	Contraindicated	-Excluded from pivotal RCTs Data indicating worse outcome
-Moderate to severe mitral stenosis (usually rheumatic)	Contraindicated	-Excluded from pivotal RCTs Little rationale for less efficacy and safety vs. VKA
-Other mild to moderate valvular disease (e.g. degenerative aortic stenosis, mitral regurgitation etc.) -Bioprosthetic valve/valve repair (after >3 months postoperative)	Included in NOAC trials Acceptable	-Data regarding efficacy and safety overall consistent with patients without valvular disease -Some data from NOAC RCTs Single RCT indicating non-inferiority to VKA Patients without AF usually on ASA after 3-6 months post-surgery, hence NOAC therapy acceptable for stroke prevention if diagnosed with AF
-Severe aortic stenosis	Limited data (excluded in RE-LY study)	-No pathophysiological rationale for less efficacy and safety most will undergo intervention
-Trans catheter aortic valve implantation	Acceptable	-Single RCT and observational data
-Percutaneous transluminal aortic valvuloplasty	With caution	-No prospective data
-Hypertrophic cardiomyopathy	Acceptable	-No rational for less efficacy and safety vs. VKA (observational data positive for NOACs)

Abbreviations: NOAC: non-vitamin K antagonist oral anticoagulant; RCT: randomized clinical trials; VKA: vitamin-K antagonist; AF: atrial fibrillation;

2.2. NOAC dosage

Four types of NOACs are used and they have different dosages and different dose reduction criteria for different indications. Therefore, determining the correct dose has become more complicated. Figure 1 provides an overview of available NOACs and their dosages in different indications, including dose reduction criteria (16).

Stroke prevention in atrial fibrillation (SPAF)

	Standard dose	Comments/dose reduction
Apixaban	5 mg BID	2.5 mg BID if two out of three fulfilled: weight ≤60 kg, age ≥80 years, serum creatinine ≥133 µmol/L (1.5 mg/dL) (or single criterion: if CrCl 15–29 mL/min)
Dabigatran	150 mg BID/110 mg BID	No pre-specified dose-reduction criteria in phase III trial[a]
Edoxaban	60 mg QD	30 mg QD if: weight ≤60 kg or CrCl 15–49 mL/min or concomitant therapy with strong P-Gp inhibitor (see 'Pharmacokinetics and drug-drug interactions of NOACs' section)
Rivaroxaban	20 mg QD	15 mg QD if CrCl ≤15–49 mL/min

'SmPc' refers to European SmPc.
BID, twice daily; CrCl, creatinine clearance; GI, gastrointestinal; NOAC, non-vitamin K antagonist oral anticoagulant; QD, once daily.
[a]SmPC: 110 mg BID if age ≥80 years, concomitant verapamil, increased risk of GI bleeding.

NOAC dosing in AF patients post-ACS/PCI (see 'Patients with atrial fibrillation and coronary artery disease' section)

	Standard dose	Comments/dose reduction
Apixaban	5 mg BID	Dose reduction as for SPAF
Dabigatran	150 mg BID or 110 mg BID	110mg as for SPAF
Edoxaban	60 mg QD	Dose reduction as for SPAF
Rivaroxaban	15 mg QD	Dose reduction to 10 mg QD if CrCl 30–49 mL/min

In addition to single/dual antiplatelet therapy, where applicable. See 'Patients with atrial fibrillation and coronary artery disease' section for details.
BID, twice daily; CrCl, creatinine clearance; QD, once daily; SPAF, stroke prevention in atrial fibrillation.

Treatment of DVT/PE

	Initial therapy	Remainder of treatment phase
Apixaban	10 mg BID, 7 days	5 mg BID, no dose reduction
Dabigatran	Heparin/LMWH	150 mg BID, no dose reduction[a]
Edoxaban	Heparin/LMWH	60 mg QD, same dose reduction as for SPAF (see above)
Rivaroxaban	15 mg BID, 21 days	20 mg QD, no dose reduction[b]

BID, twice daily; GI, gastrointestinal; LMWH, low molecular weight heparin; QD, once daily; SPAF, stroke prevention in atrial fibrillation.
[a]Per SmPC: 110 mg BID if age ≥80 years, concomitant verapamil, increased risk of GI bleeding [based on pharmacokinetic/pharmacodynamic (PK/PD) analyses; not studied in this setting].
[b]Per SmPc: 15 mg if risk of bleeding outweighs risk for recurrent DVT and PE (based on PK/PD analyses; not studied in this setting).

Long-term prevention of recurrent DVT/PE

	Standard dose	Comments/dose adjustment
Apixaban	2.5 mg BID	
Dabigatran	150 mg BID	No pre-specified dose-reduction criteria in clinical trial[a]
Edoxaban	60 mg QD[b]	
Rivaroxaban	10 mg QD	[c]

BID, twice daily; QD, once daily.
[a]SmPC: 110 mg BID if age ≥80 years, concomitant verapamil (both based on pharmacokinetics/pharmacodynamics analyses; not studied in this setting).
[b]Not specifically studied, follow-up data available up to 12 months in phase III trial.
[c]SmPc: 20 mg QD in patients at high risk of recurrence.

VTE prevention post-major orthopaedic surgery

	Standard dose	Comments/dose reduction
Apixaban	2.5 mg BID	
Dabigatran	220 mg QD/150 mg QD	[a]
Edoxaban	30 mg QD	Not approved in Europe (only studied in Asia)
Rivaroxaban	10 mg QD	

BID, twice daily; QD, once daily.
[a]SmPC: 1 × 150 mg if CrCl 30–50 mL/min; concomitant verapamil, amiodarone, quinidine; age >75 years.

Secondary prevention of atherothrombotic events post-ACS in patients without AF (i.e. no OAC indication)

	Standard dose	Comments/dose reduction
Rivaroxaban	2.5 mg BID	In addition to aspirin ± P2Y12 inhibitor

BID, twice daily.

Secondary prevention of atherothrombotic events in patients with chronic coronary syndrome and/or symptomatic peripheral artery disease patients without AF (i.e. no OAC indication)

	Standard dose	Comments/dose reduction
Rivaroxaban	2.5 mg BID	In addition to aspirin

AF, atrial fibrillation; BID, twice daily; OAC, oral anticoagulation.

Figure 1. NOACs and approved/studied doses across indications.

3. Pharmacokinetics of NOACs

Treatment with VKAs requires careful consideration of multiple food and drug-drug interactions. These interactions are less in NOACs. Nevertheless, physicians need to consider the pharmacokinetic interactions of concomitant medications and comorbidities when prescribing NOACs. The absorption, distribution, metabolism, and excretion of the different NOACs, are summarized in Figure 2 (17).

	Dabigatran	Apixaban	Edoxaban	Rivaroxaban
Bioavailability	3–7%	50%	62%	15 mg/20 mg: 66% without food, 100% with food
Prodrug	Yes	No	No	No
Clearance non-renal/renal of absorbed dose	20%/80%	73%/27%	50%/50%	65%/35%
Plasma protein binding	35%	87%	55%	95%
Dialysability	50–60% (In part dialysable)	14% (Not dialysable)	NA (Not dialysable)	NA (Not dialysable)
Metabolism	Glucoronic acid conjugation	CYP3A4 (25%), CYP1A2, CYP2J2, CYP2C8, CYP2C9 CYP2C19	CYP3A4 (<4% of elimination)	CYP2A4 (18%)[519], CYP2J2
Absorption with food	No effect	No effect	6–22% more; minimal effect on exposure	+39% more (see above)
Absorption with H2B/PPI	−12% to 30% (not clinically relevant)	No effect	No effect	No effect
Time to peak levels (h)	3	3	2–4	2–4
Elimination half-life (h)	12–17	12	10–14	5–9 (young) 11–13 h (elderly)

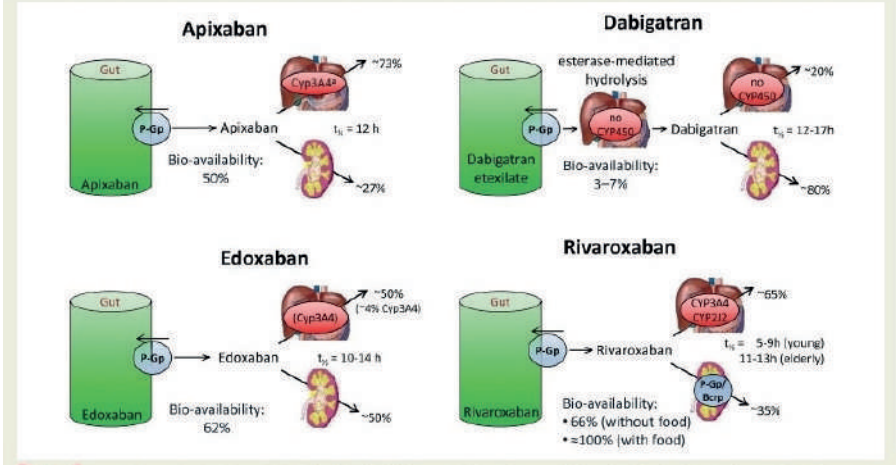

Figure 2. Absorption and metabolism of different NOACs. There are interaction possibilities at the level of absorption or first transformation and at the level of metabolization and excretion. Also via CYP1A2, CYP2J2, CYP2C8, CYP2C9, and CYP2C19.

4. NOACs in patients with chronic kidney disease or advanced liver disease

4.1. Atrial fibrillation and chronic kidney disease

Both bleeding and thrombotic risks are increased in patients with chronic kidney disease (CKD) and AF compared with other AF patients. NOACs all undergo some renal elimination, although at varying rates. 80% of dabigatran, which is eliminated most, and approximately 25% of apixaban, which is the least eliminated, are excreted through the kidneys.

Renal functions should be evaluated at least once a year in patients receiving NOAC therapy, and more frequently in those with renal dysfunction. In patients who develop acute renal failure, NOACs should be discontinued and parenteral anticoagulation should be started (18).

The effectiveness and safety of all four NOAC types in patients with creatinine clearance (CC) above 30 mL/min have been demonstrated in subgroup analyses of phase-III studies of these drugs (19-23). The use of appropriate NOAC doses has great importance in the treatment of patients with CKD. In studies of apixaban, edoxaban, and rivaroxaban, dose reductions were made according to renal functions. In the RELY study, two different patient groups were created, 110 and 150 mg, regardless of renal functions. It is recommended for dabigatran to use 110 mg in patients with CC below 50 mL/min. The dose adjustment of NOACs according to CC is shown in Figure 3 (16).

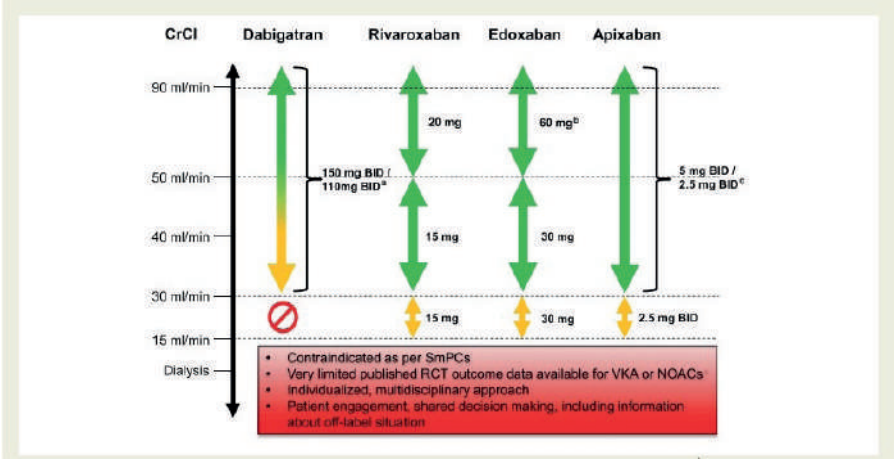

Figure 3. Use of NOACs according to renal functions.

The effectiveness and safety of NOACs in patients with CC below 15 mL/min or in patients undergoing renal replacement treatment are uncertain. In a study, significantly more hospitalizations and deaths due to bleeding occurred with off-label dabigatran and rivaroxaban in patients receiving renal replacement treatment compared to VKAs (24). Although plasma apixaban levels are higher than the therapeutic level in stable dialysis-dependent patients, apixaban treatment of 5 mg twice a day has been approved by the FDA in the USA (25). Besides that, the 2020 ESC guidelines recommend the use of factor Xa inhibitors with caution and at reduced doses for patients with 15-29 mL/min (2).

There are no data regarding the use of NOAC in renal transplant patients. In these patients, dosage adjustments should be made according to renal functions and drug-drug interactions with the immunosuppressive agents used should be taken into consideration.

The use of prophylactic anticoagulants in nephrotic syndrome patients is still a controversial issue today. There is no data in the literature regarding the use of NOAC for thromboprophylaxis in patients with nephrotic syndrome. When deciding which of NOACs or VKAs to prefer in a patient, the pathology causing the nephrotic syndrome, renal functions, serum protein levels, thromboembolism, and bleeding risks should be taken into consideration (26). It should be kept in mind that NOAC may be an alternative for these patients who cannot comply with VKA treatment.

4.2. NOACs in patients with advanced liver disease

Advanced liver disease, like kidney disease, creates a predisposition to both thrombosis and bleeding. In addition, hepatic elimination of drugs, drug metabolism, effectiveness, and drug-induced liver damage differ in liver disease (27). Practical considerations for the use of NOACs in liver disease are presented and summarized in Figure 4 (16).

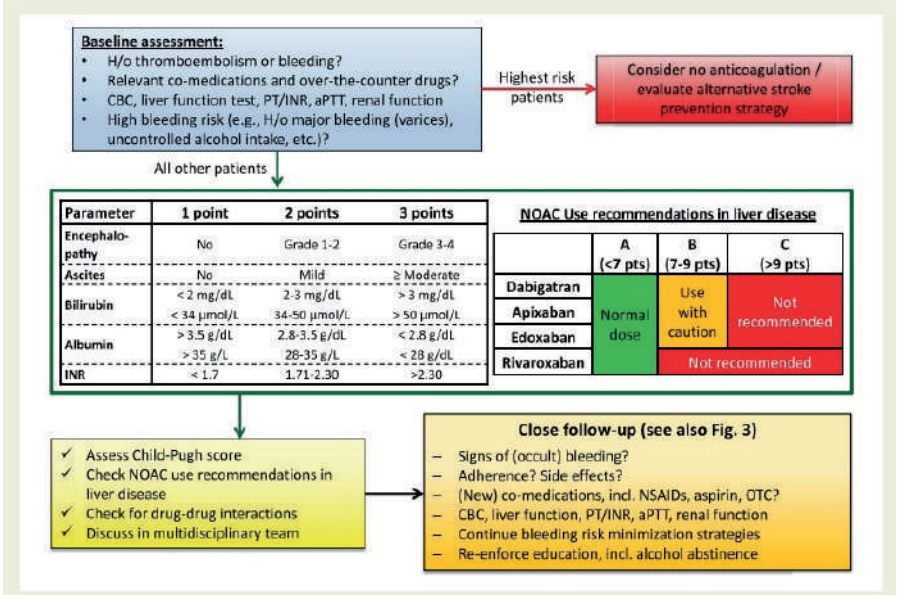

Figure 4. NOACs in patients with liver disease.

4.3. NOAC treatments in cancer patients

The risk of thrombosis is increased in patients with cancer compared with patients without it. There is an increased risk of both arterial and venous thromboembolism in patients with malignancy. At the same time, the risk of bleeding in patients with malignancies raises concerns about the use of anticoagulant drugs. Especially in patients undergoing chemotherapy, it is an approached adopted in current practice to switch to low molecular weight heparins and continue the chemotherapy process with heparin treatment, due to the difficulty in maintaining the therapeutic window and the fact that these patients do not infrequently require diagnostic and treatment interventions during the cancer therapy process. On the other hand, many studies excluded patients with active malignancies, and the remaining studies included small numbers of cancer patients. In the ARISTOTLE study, apixaban was more effective and safer than VKA in patients with active malignancy or a history of malignancy (28).

A published prescription registry analysis showed that bleeding and thrombotic risks were similar in patients with and without malignancy and that NOACs used at standard doses were more effective in both bleeding thromboembolism risks in both groups (29). Another important point to consider is that the interaction of chemotherapeutic drugs and NOACs is not yet fully known, and NOACs should be used more carefully in patients receiving chemotherapy (18). It is important to use proton pump inhibitors along with NOACs in patients with malignancy to reduce the bleeding risk.

5. Cardioversion and NOACs

Current guidelines recommend anticoagulation at least 3 weeks before cardioversion and 4 weeks afterward, regardless of the type of cardioversion (30). Three different studies have been published comparing the use of apixaban, edoxaban, and rivaroxaban with the use of VKAs in patients undergoing cardioversion (31-33). Data regarding dabigatran and cardioversion were presented in a post-hoc analysis of the RELY study (34). As a result of these studies, both thromboembolic events and bleeding rates were observed to be lower with each of the four NOACs compared to warfarin, but none of these studies had the statistical power to evaluate superiority or non-inferiority.

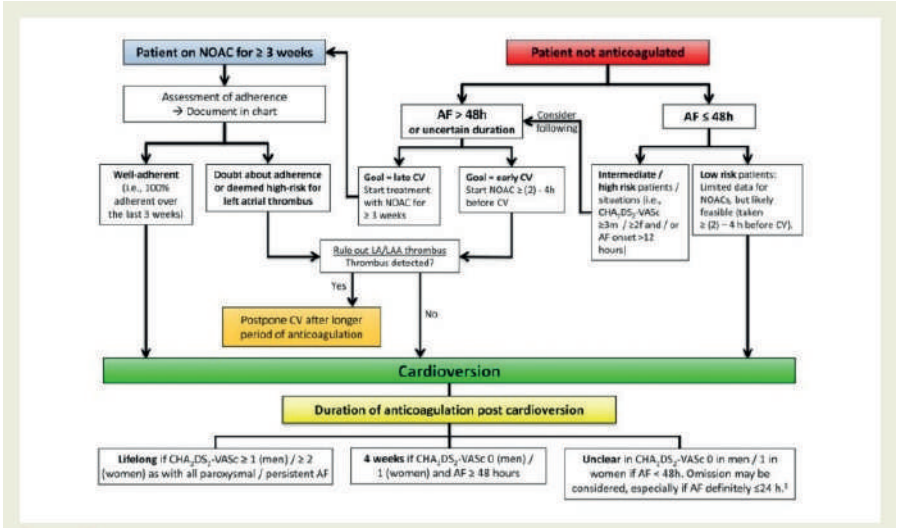

Figure 5. Practical management of patients that cardioverted with or without NOAC therapy (16).

6. NOAC treatment in venous thromboembolism and pulmonary embolism

In RCTs regarding the use of NOACs in the treatment of venous thromboembolism (VTE) and pulmonary embolism (PE), patients using dabigatran and edoxaban received parenteral heparin therapy for at least 5 days before starting oral therapy. Dabigatran 150 mg twice daily and edoxaban 60 mg once daily have been used. In studies of apixaban and rivaroxaban, parenteral anticoagulant treatment was not given beforehand and anticoagulation was started directly with NOAC. The results of these studies showed that NOAC treatment was non-inferiority and safer than standard heparin and VKA treatment (35). As a result of these RCTs and meta-analysis, NOAC treatment was included in the PE guideline with a Class-1 indication (36). In a meta-analysis, 5 RCTs involving a total of 7897 were examined and similar results were obtained with NOAC treatment compared to standard VKA treatment in deep vein thrombosis, PE, recurrent PE, recurrent VTE, all-cause of death and major bleeding (37).

7. AF patients presenting with acute stroke while on NOACs

Ischemic stroke occurs in 1-2% of patients receiving anticoagulant therapy each year. When encountering such patients, medication compliance should be questioned first. If there is an opportunity to optimize treatment

in secondary prevention, drug levels can be measured at admission to the hospital (38). Thrombolytic therapy within 4.5 hours after stroke is an important treatment in suitable patients. Fibrinolytic therapy cannot be used in patients receiving anticoagulant therapy. Thrombolytic therapy should not be administered to patients receiving NOAC until 24 hours after the last dose. Alternatively, anticoagulant therapy with idarucizumab can be rapidly reversed in patients receiving dabigatran. Published case series have reported that intravenous thrombolytic therapy is possible and safe after the reversal of dabigatran effect (39, 40). In addition, fibrinolytic treatment can be applied by measuring the plasma level Factor Xa inhibitors, but the use of rapid tests measuring plasma levels is not yet widespread worldwide. If measurable, fibrinolytic treatment can be safely applied at levels below 30 ng/mL. (41).

There is no RCT on which NOAC treatment should be chosen or drug switching in patients with ischemic stroke under NOAC treatment.

When to restart NOAC treatment after stroke should be determined according to the patient's risk of re-ischemic stroke and hemorrhagic transformation secondary to stroke. In patients who have a transient ischemic attack and are shown to have no bleeding by CT or MRI, anticoagulation should be restarted after 1 day. In patients with mild neurological deficits (National Institutes of Health Stroke Scale score, NIHSS, below 8), it is recommended to restart anticoagulation treatment 3 days after onset of the event. In patients with moderate neurological deficits (NIHSS between 8 and 15) the presence of bleeding should be evaluated with CT or MRI on day 6. If there is no bleeding, restarting anticoagulant treatment should be considered. In patients with the severe neurological deficit (NIHSS over 16), anticoagulant treatment should be started on 12th day and bleeding status should be evaluated with imaging methods (1, 18).

It has been shown that the prognosis of patients receiving NOAC therapy and developing intracranial bleeding is similar to that of patients experiencing bleeding under VKA (42). In these patients, NOACs should be discontinued immediately and the coagulation status should be corrected. Idarucizumab should be used in patients receiving dabigatran therapy. Andexanet alfa can be used in patients who develop bleeding under Factor Xa inhibitors.

The decision and timing of restarting anticoagulant treatment after intracranial bleeding is evaluated together with the degree of regression of intracranial bleeding, the risk of recurrence, and the patient's risk of ischemic stroke. An individualized decision should be made based on the benefit/loss ratio on a patient basis.

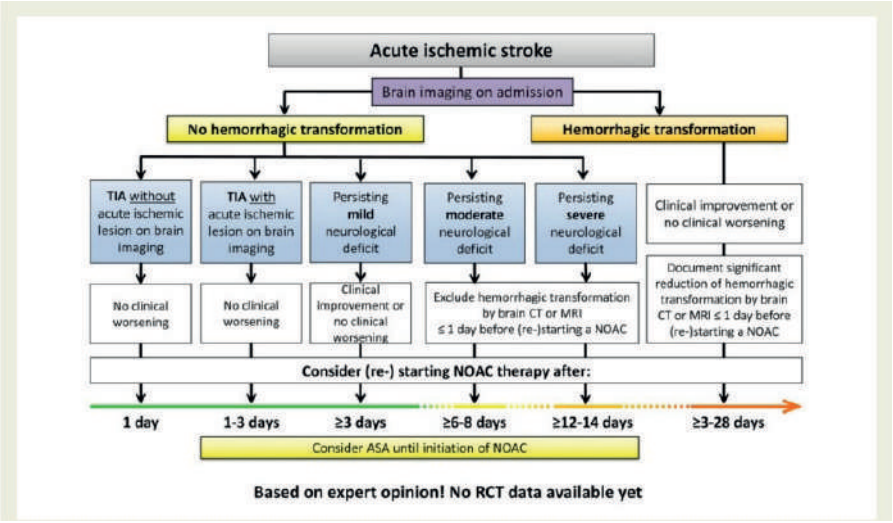

Figure 6. Re-initiation of anticoagulation after TIA/stroke. Without proven evidence/ RCT data available, based on expert opinion (16).

7. AF patients presenting with bleeding on NOACs

Studies have shown that NOACs cause less intracranial and life-threatening bleeding than VKAs. In addition, more positive results were obtained in patients receiving NOAC, especially intracranial bleeding, compared to warfarin (43-45). In patients with bleeding, treatment methods are determined according to the severity of the bleeding. The first thing to do is to increase the diuresis of the drug the wear off. Other options are the use of specific (antidotes) and nonspecific agents (prothrombin complexes). Fresh frozen plasma, protamine and vitamin K are ineffective in bleeding with NOAC (46). Local hemostatic methods should be used in minor bleeding that occurs under NOAC treatment. If recurrent bleeding occurs despite precautions, it is necessary to switch to a NOAC with a different bleeding profile or dose adjustment should be made.

In case of major bleeding that is not life-threatening, adequate diuresis, especially in dabigatran, should be provided. If idarucizumab cannot be reached in case of severe bleeding with dabigatran, dialysis may be considered in patients with renal failure (18). Dialysis is ineffective in bleeding due to the factor Xa inhibitors because they are highly bound to the plasma proteins. Tranexamic acid or desmopressin may be considered, especially in patients with coagulopathy. Studies are showing the benefits of using tranexamic acid, especially in patients with bleeding due to trauma (47).

In case of major bleeding that is life-threatening idarucizumab and andexanet alfa should be used. Idarucizumab is administered as two bolus doses of 2.5 g and its effect begins within minutes. In clinically appropriate patients, dabigatran can be restarted 24 hours after treatment. Andexanet alfa should be used in different doses depending on the NOAC type and the last time the drug was taken. In cases where antidotes are not available, the use of prothrombin or activated prothrombin coagulation complexes should be considered. Which of these two complexes is preferred should be based on the center's experience (18).

8. NOACs for patients undergoing surgical or percutaneous intervention

When to stop NOAC before surgery and when to start again after surgery should be determined according to the characteristics of the patients such as age, bleeding history, kidney functions, and the type of surgical operation. In dental procedures, cataract and glaucoma operations, superficial surgeries, and endoscopies that do not require biopsy, the operation should be performed without discontinuing anticoagulant treatment, even if bleeding can be easily stopped and the risk of bleeding is very low. Such operations can be performed 12-24 hours after the last NOAC dose. The appropriate approach is to start the anticoagulant agent again 6 hours after the procedure. It is recommended to perform the procedure 24 hours after the last NOAC dose in patients with normal kidney functions and low bleeding risk (endoscopic procedure, prostate or bladder biopsy, electrophysiological studies and ablations, pacemaker implantation or non-coronary angiographic interventions).

In patients receiving dabigatran, if the CC is 30-50 mL/min, the last 4 doses should not be given, if it is between 50-80 mL/min, the last 3 doses, and if it is over 80 mL/min, the last 2 doses should not be given and the procedure should be performed. In patients receiving Factor Xa inhibitors and whose CC is between 15-29 mL/min, the last dose should be taken at least 36 hours before the procedure and not continued afterward.

In a big meta-analysis including 9 RCTs, the effectiveness and safety of NOACs (other than edoxaban) and VKA were compared in patients undergoing surgical procedures (48). The majority of patients underwent surgical interventions with a low or very low risk of bleeding. The frequency of embolic events observed in patients receiving NOAC and VKA therapy was similar, but the frequency varied according to the type of surgery performed. Perioperative major bleeding rates were similar in both groups.

Additionally, in the analysis, bleeding occurred more with dabigatran than with warfarin, and with the other two NOACs at rates similar to warfarin. There was no change in bleeding rates according to surgery type.

It is recommended that NOAC should be discontinued 48 hours or earlier in operations such as polypectomy with high-risk bleeding, complex endoscopic procedures, thoracic and abdominal surgeries, epidural, spinal anesthesia, liver, and kidney biopsy. In patients receiving dabigatran, treatment should be stopped gradually according to CC. It should be kept in mind that bridging with LMWH is not recommended before any operations. The bridging therapy causes an increase in the risk of bleeding (49).

If postoperative bleeding control is fully achieved, NOAC can be restarted 6-8 hours after the operation. In types of operations where the risk of bleeding continues for 48-72 hours, thromboprophylaxis should be started 6-8 hours after the operation and NOAC should be postponed during this period. There is no data regarding the use of low-dose NOAC after surgery.

NOACs should be discontinued in patients undergoing emergency surgery. If there is an opportunity for emergency surgeries that need to be performed within minutes, idarucizumab should be used for dabigatran, andexanet alfa should be used for Factor Xa inhibitors (50). If antidotes cannot be obtained, routine coagulation tests should be performed, non-specific bleeding precautions should be taken, and general anesthesia should be preferred for the operation (49). In operations should be performed within hours, the intervention should be postponed at least 12 hours, ideally 24 hours, after the last dose received (18).

9. Conclusion

NOACs are drugs used as an alternative to VKAs in the treatment of AF and venous thromboembolism. In studies, similar or better results were obtained with the use of NOACs than VKAs in terms of effectiveness and reliability. Each NOAC preparation has different metabolic properties. It is expected that the use of NOACs in patients with high bleeding risk will increase with the introduction of two antidotes (idaricuzimab and andexanet alfa) that have recently been approved for use in bleeding. Their use will become safer with ongoing and upcoming new RCTs.

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Chapter 6

The Impact of Genetic Mutational Typing of Endometrial Carcinoma for Adjuvant Oncologic Treatment and Treatment Outcome

Bengü Depboylu[1]

Abstract

The adjuvant treatment of endometrial carcinomas took a different turn when ESGO/ESTRO/ESP announced its prognostic risk group guide in 2020. The Cancer Genome Atlas (TCGA) Research Network Classification and the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) algorithm were integrated into clinical practice. Therefore, by combining genomic traits with molecular subtypes, doctors have enhanced patient care and risk stratification for endometrial cancer. Surgery (hysterectomy and bilateral salphingooopherectomy with or without lymph node dissection) is the primary treatment for early-stage, low-grade, low-risk tumors. Vaginal brachytherapy in an adjuvant setting has secured the treatment success for local control. Intermediate-high-risk cancer patients are scheduled for adjuvant chemoradiation and/or vaginal brachytherapy.

Nevertheless, there is still a 30% of high-risk, high-grade heterogenous endometrial cancer patients whose accurate prognostication needs to be elucidated. Recent and ongoing trials support the superior benefit of chemoradiation combined with targeted therapies for relapse-free and overall survival. This review summarizes the most recent trends in adjuvant oncologic treatments for endometrial cancer according to the validated four subgroups and discusses the results of ongoing trials for adjuvant chemoradiation with targeted therapies.

1 Assistant Prof. Dr, Department of Radiation Oncology, Medical Faculty, Aydın Adnan Menderes University, 09010 Aydın, TURKIYE, bengu.depboylu@adu.edu.tr, ORCID ID: 0000-0002-9777-2917

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1. INTRODUCTION

Endometrial cancer is the most common type of uterine cancer. It arises from the endometrial cells that line the uterus. According to GLOBOCAN 2020 reports, 417000 women were diagnosed, and 917000 women died due to it [1]. The longer the estrogen exposure, the higher the risk of developing endometrial cancer [2]. SEER data reports a 96% survival rate for localized, 72% for regional, and 20% for distant cancer at five years [3]. Surgery (a total abdominal hysterectomy and bilateral salphingooopherectomy) is the primary treatment modality for the early stages. Recently, sentinel lymph node biopsy with indocyanine green is increasingly used with high sensitivity and negative predictive rate for low morbidity (e.g., less lymphedema) [4]. However, higher stages with extensive disease need other adjuvant oncologic treatment modalities of vaginal brachytherapy, pelvic external radiation therapy, and chemotherapy [5]. The cancer stage is not solely enough for proper patient management. Histopathological findings and risk group classifications recommended by international societies determine the cascade of adjuvant oncologic treatments to avoid over or undertreatment [5,6].

Patient's age, cancer stage, tumor grade, histopathologic type, depth of myometrial invasion, and lymphovascular space invasion (LVSI) are essential characteristics for risk group classification [5]. Because histopathological findings might cause conflicts between pathologists in up to 30% of cases, a surrogate system has been developed by The Cancer Genome Atlas (TCGA) Research Network in which four prognostically different groups were identified. The distribution of these prognostically distinct subgroups is DNA polymerase epsilon (POLE) (ultramutated) (7%), Microsatellite instability (MSI) -hypermutated (MMR-D) (28%), Copynumber low (CNL) (39%) and Copy-number high (CNH) (26%) [7,8].

Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) was developed using immunohistochemical analysis rather than molecular analysis to increase the applicability of molecular classification [9,10]. Several studies have confirmed the validity of the proposed molecular classifications over various endometrial cancer patient groups by combining immunohistochemistry (IHC) and mutation analysis for its determination and predicament of disease prognosis [11–13].

The subsequent studies related to TCGA classification have documented that four subgroups contain almost all grades, histologic types, and stages of endometrial cancer. Adjuvant oncologic treatments with new therapeutic targets are being developed in new clinical trials. This review summarizes the

most recent trends in adjuvant oncologic treatments in endometrial cancer according to the validated four subgroups.

2. MANAGEMENT OF ADJUVANT ONCOLOGIC TREATMENTS

2.1. Management Of Adjuvant Oncologic Treatments For Patients With DNA-Polymerase Epsilon (POLE) Ultramutated Tumors

Patients with polymerase epsilon exonuclease domain mutated (POLE EDM) or ultramutated tumors constitute 6 to 12% of all endometrial cancers [8,14–16]. These tumors are often in the endometrioid histological subtype, tend to be of higher grade (grade III), and are rich in lymphocytic infiltrate, but with good prognosis, manifested by early onset of symptoms presented at early stages (stage I-II) in young women that have low body mass index (BMI) [16–19]. In a meta-analysis for pooled patients with POLE EDM tumors, estimated HR for overall survival was 0.90 (95% CI, 0.59 to 1.38), for disease-free survival was 0.41 (95% CI, 0.30 to 0.55), for progression-free survival, was 0.23 (95% CI, 0.08 to 0.64) emphasizing superior survival and favorable prognosis [19].

Previously PORTEC 1 trial showed that external beam radiotherapy was superior to no adjuvant treatment in locoregional disease control at 5 years (4% vs.14%, $p<0.001$) but without overall survival (85% vs. 81%; $p=0.31$)[20]. Furthermore, PORTEC 2 trial showed that neither vaginal brachytherapy nor external pelvic radiotherapy was different from each other in older patients with higher-grade endometrial cancer in locoregional disease control (5% vs. 2%, $p=0.17$) and overall survival (85% vs. 80%; $p=0.57$) at 5 years [21]. Patients with POLE EDM tumors were found to have no recurrence in a further analysis by Stello et al., where they integrated molecular and clinicopathological features into risk assessment for patient cohorts of these trials. The authors stated that the high mutation rate and increased immunogenicity in POLE EDM tumor patients are responsible for this outcome [14]. On the other hand, Van Gool et al. opposed and declared that an increased mutation rate would not be enough to explain the favorable outcome because while none of the POLE EDM patients had a recurrence in the control group of PORTEC 1 trial (0/16), in the POLE wild-type patients 44/229 (19.2%) had a recurrence in the absence of adjuvant oncologic treatment [22]

To support the PORTEC-1 data, van Gool et al. investigated POLE EDM treatment sensitivity in a model system and reported that these mutations exhibited increased sensitivity to nucleoside analogs like cytarabine and

fludarabine. Therefore, the authors concluded that the prognostic benefit of POLE mutations is independent of adjuvant treatment but can be explained by increased immunogenicity [22]. These tumors are platinum-based chemotherapy resistant. However, in vitro comparison to primary POLE wild-type tumors, they are naive to paclitaxel. Bellone et al. have attributed it to higher T-cell infiltration of POLE-ultra mutated endometrium cancers [17]. Among the systemic treatment options, POLE EDM patients are most potentially expected to benefit from immune check-point inhibitors [23].

In their recent metanalysis, McAlpine et al. advocated "de-escalating patient care" for POLE EDM tumor patients as adjuvant oncologic treatment (radiation therapy and chemotherapy) showed no survival benefit in this cohort [24]. Close observation can be advised for them. PORTEC 4a and TAPER trials are ongoing prospective studies to elucidate whether omitting vaginal brachytherapy in cases of favorable molecular profiles is safe and cost-effective [24,25]. The early results of these trials are expected in 2023.

2.2. Management of Adjuvant Oncologic Treatments For Patients With Microsatellite Instability (MSI)- Hypermutated (MMRd) Tumors

In this group of patients, mismatch repair deficiency leads to microsatellite instability because the nuclear expression of several mismatch repair proteins (e.g., MLH-1, MSH2, MSH6, PMS2) is missing. It results in the accumulation of insertions, deletions, and mismatches, predisposing conditions for tumor development [8,9,14]. Repair deficiency in MSH2, MSH6, and PMS2 is associated with hereditary endometrial carcinoma (Lynch Syndrome), whereas MLH1 repair deficiency is a somatic sporadic mutation. MLH1 methylation assays are used to differentiate one another [26].

Approximately 25 to 30% of endometrial cancer patients have MMRd and show diverse heterogeneity in their histology, including cribriform and nonpapillary patterns and mucinous differentiation [27]. Tumor-infiltrating lymphocytes are present in the peritumoral areas. Microsatellite instability assessment is divided into three: high (MSI-H), which means evaluating mutations ≥2 genes; stable (MSS) mutations in zero genes; and low (MSS-L) mutations in 1 gene [28]. Histologically, patients with half of MSI-H tumors are heterogenous and undifferentiated carcinomas; meanwhile, 30% of endometrioid, 16% of serous, and 15% of clear cell carcinomas are MSI-H [29,30].

The microsatellite instability hypermutated/mismatch repair deficiency status is associated with intermediate prognosis due to their high

immunogenicity, and the prognostic value is essential only in early, low-grade, LVSI and/or endometrioid histology [10,14,18,31]. Patients with these tumors have higher BMI and can be of any age but younger than non-MMRd counterparts [18].

Patients with MMR/d MSI hypermutated tumors tended to have lower recurrence with adjuvant oncologic treatment (brachytherapy and pelvic radiotherapy) compared to non-MMR/d patients in the Kim et al. study. However, on multivariate analysis, MMR status was not associated with progression-free and overall survival [31].

MMRd cancers have a high mutational burden, which is essential in systemic treatment with immune check-point inhibitors. Belone et al. reported that the benefit of immune check-point inhibitor treatment is more effective on Lynch Syndrome and Lynch Syndrome-like tumors [32]. Pembrolizumab, a PD-1 inhibitor agent, has recently proven beneficial in MMRd/MSI-H patients by KEYNOTE-158 trial [33]. It is now included in the NCCN treatment guidelines as FDA approved drug for unresectable, advanced, metastatic, or recurrent MMRd patients [34,35]. Dostarlimab and darvalumab are other immune check-point inhibitors that are under study. Interim analysis of the GARNET trial presented a 45% objective response rate (complete response 11%, partial response 34%) with dostarlimab. Mirza et al. recently reported an advantageous progression-free survival with dostarlimab plus carboplatin-paclitaxel in patients with primary advanced or recurrent dMMR-MSI-H endometrial cancer [36]. Durvalumab is also promising, with similar response rates as dostarlimab in a phase II trial by Antill et al. [37]. In advanced or recurrent dMMR-MSI-H endometrial cancer, Avelumab either alone or in combination with either talazoparib (PARP inhibitor) or axitinib (tyrosine kinase) is found 27% objective response rate in a clinical trial which has just completed [38,39].

2.3. Management of Adjuvant Oncologic Treatments For Patients With Copy Number Low (CNL) Tumors

Copy number low patients have no specific mutation profile (NSMP), and they comprise 40-50% of all endometrium cancers. They are also called p53 wild type, MMR proficient, and POLE mut (-) [10]. Prognosis in this group of patients is generally intermediate; however, stage-dependent at a greater extent [8]. Typically, they are of endometrioid histology with squamous differentiation and hormone-positive status. They have a high response rate to hormonal therapy [40,41] Women with copy number low endometrial cancers have the highest BMI [18]. Some mutations like CTNNB1 (beta-catenin 1)

and L1 cell adhesion molecule (L1CAM) for patients are related to poor prognosis and distant recurrence [14,42].

NSMP tumors associated with the PI3K/Akt/mTOR pathway and hormone-positive status are subject to new studies targeting these pathways. A phase II trial on recurrent endometrial cancers evaluated everolimus and letrozol treatment superiority to medroxyprogesterone acetate, and tamoxifen showed 32% ORR [43]. Mirza et al. studied palbociclib (cyclin-dependent kinase inhibitor) and letrozole compared to letrozole alone. Combined treatment was superior to single treatment with a 64% control rate and 5 months of progression-free survival [44].

2.4. Management of Adjuvant Oncologic Treatments For Patients With Copy-Number High (CNH) Tumors

Patients in this group have a high number of somatic copy number alterations and, with their low somatic mutation rate, have high-grade tumors (serous 88%, undifferentiated-clear cell-high grade cancers ranging 30-40%), aggressive resulting in early metastasis and poor prognosis [8,9,14,45]. Almost all these tumors are TP53 mutated, comprising 13-18% of endometrioid tumors[10, 15]. The p53 status is associated with old age and a low BMI [10,46].

Adjuvant oncologic treatment (platinum-based chemotherapy and pelvic radiation) evaluation of p53 abnormal patients in the PORTEC 3 study significantly benefitted at a rate of 22.4% for relapse-free survival and 23.1% for overall survival at five years [45]. However, the diminishing benefit of relapse-free survival at 5 years when chemoradiotherapy and radiotherapy-alone comparison (59% vs. 39%; p=0.019) moves the benefit the patients get from irradiation into question [46].

Recent studies point out a new therapeutic target for p53 mutated endometrial cancers: overexpression of HER 2 protein. HER2++ or HER+++ was present in 31.4% of p53 mutated endometrial cancers. Amplification was prominent in serous, clear cell carcinomas and carcinosarcomas, emphasizing the potential benefits of HER2-targeted therapies for these aggressive forms [47–49]. First update results of an ongoing phase II trial have shown that adding trastuzumab to carboplatin/paclitaxel chemotherapy significantly improved progression-free and overall survival in advanced stages of p53 mutated endometrial carcinoma [50].

Another new therapeutic target regarding p53 mutated high-grade endometrial cancers is reported as homologous recombination deficiency (HRD). In their study, de Jonge et al. reported that HRD is strongly related

to non-endometrioid histology, and patients with p53 mutated HRD tumors may benefit from poly (ADP-ribose) polymerase (PARP) inhibitors added to the carboplatin and paclitaxel chemotherapy, targeting this deficiency [51]. Trials designed to evaluate the combined treatment of PARP inhibitors with chemotherapy are still in progress with promising preliminary results [52].

3. CONCLUSION

Identifying the endometrial tumors on a genomic level would potentially provide crucial clinical benefit because this data would help oncologists increase awareness and clinical point of view to design superior management and obtain therapeutic outcomes in their medical practice and future clinical trials**.** As a result, by focusing on these patients with accurate genomic characterization regarding their typing/grouping, the oncologists may have the comfort to direct the results towards a more appropriate clinical endpoint for the patient, avoiding undertreatment/overtreatment problems of endometrial cancer in which multiple risk factors alter its clinical manifestation and clinical aggressiveness pattern.

By the end of 2022, the RAINBO Research Consortium has announced its new program for refining the adjuvant treatment in endometrial cancer based on molecular features [53]. An overarching research program consisted of four international studies: RED Trial, a phase III trial of p53 abnormal endometrial cancer cases that compares adjuvant chemoradiation followed by two years of olaparib immunotherapy versus radiotherapy alone. The GREEN Trial, a phase III trial of stage II (LVSI positive patients) or stage III MMRd patients, compares adjuvant radiotherapy alone with radiotherapy plus concurrent darvolumab followed by one year of adjuvant darvolumab. The ORANGE Trial, a phase III trial of stage II (estrogen receptor and LVSI positive) or stage III NSMP patients comparing adjuvant chemoradiation to radiation followed by two years of progestin. The BLUE Trial, a phase II trial of stage I-III POLE-mut patients, compared no adjuvant therapy for the low-risk group and no adjuvant therapy or radiotherapy for the high-risk group.

The main results of the RAINBO clinical program are expected to be announced by 2028. The shareholders aim to fill the void of whether molecular-directed adjuvant treatment is the more effective, less toxic, better quality of life provider than the current patient management principles for patients with endometrial cancer[53].

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Chapter 7

Advances in Lung Cancer Diagnosis

Ege Rıza Karagür[1]

Abstract

The most prevalent kind of cancer worldwide and the leading cause of cancer death is lung cancer. Lung cancer is discovered at an advanced stage in 70–80% of patients. Currently used diagnostic tools do not make it possible to diagnose the disease at an early stage. The preferred methods in the treatment of lung cancer are now shifting to targeted drugs supported by molecular diagnosis. Early diagnosis of lung cancer and treatment regimen may be possible by identifying distinctive genetic markers. With advancing technology, next-generation sequencing and liquid biopsy can increase the success rates of molecular testing in clinical settings by simultaneously detecting many targets and multiple types of changes, even with small amounts of sample. This approach allows us to eliminate the disadvantages that we have experienced before, such as investigation of a limited number of targets, insufficient tumor tissue, small amounts of nucleic acid production and tumor heterogeneity, which were the reasons for failure. This chapter's purpose is to provide a summary of the most recent techniques used to analyze genetic and epigenetic changes in lung cancer.

1. Introduction

Lung cancer is an important health problem globally. In 2020, more than 2.2 million individuals have received a lung cancer diagnosis, and there have been around 1.8 million lung cancer-related fatalities globally, according to the most recent GLOBOCAN statistics. As a result, lung cancer is currently the largest cause of cancer-related deaths worldwide. (Sung et al., 2021). The leading cause of cancer deaths worldwide is still lung cancer (18.4% of all cancer deaths), which places a heavy cost on society and has a negative impact on the economy (Siegel et al., 2022). Smoking is responsible for

1 Asst. Prof. Dr. Ege Rıza KARAGÜR, Pamukkale University, Faculty of Medicine, Department of Medical Genetics, Denizli/Turkey, ekaragur@pau.edu.tr, ORCID ID: 0000-0003-2189-8553

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almost 80% of lung cancer fatalities. Additional risk factors for lung cancer include radon, asbestos, extended and cumulative exposure to air pollution, particularly emissions of polycyclic aromatic hydrocarbons (PAH), and a personal or family history of the disease. (Kanwal et al., 2017). Lung cancer is subdivided two main subtypes as non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) based on histopathology. 85% and 15%, respectively, of all lung cancer cases are NSCLC and SCLC. (Sher et al., 2008). Squamous-cell carcinoma, adenocarcinoma and large-cell carcinoma are the other three subtypes of non-small-cell lung cancer. Squamous-cell carcinoma develops from early forms of squamous cells in the bronchial tubes in the middle of the lungs' airways. It is strongly correlated with tobacco use (Kenfield et al., 2008). Adenocarcinoma, which comprises around 40% of all cases of lung cancer, is the most prevalent subtype of non-small-cell lung cancer. Adenocarcinoma develops from type II alveolar cells that line the small airways and release mucus and other substances (Noguchi et al., 1995). Regardless of age, men and women with and without a history of smoking develop adenocarcinoma, the most prevalent type of lung cancer (Couraud et al., 2012). Additionally, adenocarcinoma has a non-aggressive attitude compared to other subtypes. Large cell (undifferentiated) carcinomas make up 10–17% of all the non-small cell lung cancers. Large carcinomas generally are shown up the central part of the lungs, sometimes into nearby lymph nodes and into the chest wall as well as distant organs (Rodriguez-Canales et al., 2016). SCLC is classified as a limited disease SCLC, when it is confined to a hemithorax, where curative treatment with radiochemotherapy is feasible; and an extensive disease SCLC, defined as the presence of metastatic disease outside the hemithorax at first diagnosis (Micke et al., 2002).

Numerous researches have been conducted over the past two decades' years to explain the biology process of oncogenesis in lung cancer. The term "oncogene addiction" describes the reliance of tumor cells on a particular oncogene activity that is active or overexpressed.

The main oncogenic factors in thoracic oncology are mutations in the EGFR, KRAS, and ALK genes. Some of the most recent molecular targets to be discovered are ROS1 and RET new translocations, HER2 and PIK3CA mutations, BRAF mutations, and HER2 and PIK3CA. The strategy of chemotherapy drugs used today, such as monoclonal antibodies and tyrosine kinase inhibitors, is to block the oncogenic pathway or molecule that plays a key role in the signaling pathways.

Today, imaging and follow-up methods used for both diagnosis and treatment of lung cancer have some limitations in use; such as high false positive rate, overdiagnosis, and increased radiation exposure. Additionally, detection of early-stage lung malignancies still requires tissue diagnosis.

As technology continues to advance in the field of interventional pulmonology, tissue acquisition for the diagnosis of lung lesions has become faster, safer, and more accurate. However, to ensure patient comfort and reduce invasive indications, a molecular approach with the use of minimally invasive liquid biopsy or blood sample has gained importance.

This area of study is evolving quickly and is not only becoming more involved in lung cancer diagnosis but also in lung cancer staging and treatment. A less invasive and more convenient method that could be used in addition to or instead of both imaging and minimally invasive tissue collection would certainly be more attractive. It would have the advantage of providing sufficient information for "individualized" cancer treatment (molecular analysis). In this context, liquid biopsy or blood sample analysis combined with the evaluation of various circulating tumor biomarkers has emerged as a practical alternative in diagnosis and is currently the subject of intense study worldwide (He et al., 2009).

2. Advances in Lung Cancer

2.1. Next Generation Sequencing (NGS)

Nowadays, automated Sanger sequencing is referred to as "first-generation" DNA sequencing technology. Advances in sequencing technology have led to reasonably affordable clinical testing platforms that can reliably produce results with anywhere between a few and several hundred nanograms of DNA. These platforms allow for multiplexing of gene targets spanning several orders of magnitude (Metzker, 2010). The Sanger sequencing constraint was addressed by the NGS technology, which later developed to be employed in all aspects of genomic research, starting with DNA, RNA, miRNA, ChIP, and methylation sequencing (Slatko et al., 2018). Cost, anticipated testing volume, necessary sensitivity and planned scope of genomic targets, requirement for highly effective bioinformatics tools and trained employees for both experimental and data processing are some of its drawbacks (Levy and Myers, 2016; Rizzo and Buck, 2012). Table 1 lists some of the NGS's advantages and disadvantages (Cainap et al., 2021).

Table 1. Advantages and disadvantages of NGS

Criteria	Advantages	Disadvantages
Price	*	-
Need for specialized software and computers for data analysis	-	*
Short time from library preparation to results	-	*
No standardization or availability of standardized material for clinical application	-	*
Variety of applications	*	-
Still expensive in some developing countries	-	*
Useful both in research and clinic	*	-
High number of commercially available NGS platforms and specialized kits	*	-

2.2. Liquid Biopsy in Lung Cancer

Numerous biomarkers present in physiological fluids like blood, urine, tissues, bronchoalveolar lavage, saliva, sputum, and cerebrospinal fluid are examined during a cutting-edge process known as a liquid biopsy. It primarily focuses on the analysis of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes in the context of lung cancer. These elements enable a thorough evaluation of the tumor's molecular profile without the need for intrusive procedures by transferring genetic data released by tumor cells into the circulation (Nooreldeen and Bach, 2021). As genetic analysis gives quantifiable feedback and tracks patient reactions, they have also been rated as a pillar in the field of precision medicine. This has allowed for a more specialized, practical, and individualized approach to individualized treatment (Casagrande et al., 2023). The potential of liquid biopsy to identify lung cancer at an early stage is one of the procedure's most important benefits in the diagnosis of the disease. Liquid biopsy, as opposed to conventional biopsies, can detect cancer-related genetic mutations and alterations when the tumor is still in its early, more curable stages. Early diagnosis can result in prompt interventions, which may enhance patient outcomes and raise the likelihood of a successful course of therapy (Casagrande et al., 2023).

All the DNA that is circulating in the bloodstream is referred to as plasma cell-free DNA (cfDNA), and even in cancer patients, the majority of it is often nonmalignant. However, within cfDNA, there exists a critical component known as ctDNA, which is directly linked to the presence of tumors. Plasma ctDNA has undergone extensive research and is now commonly employed as an alternative to conventional tissue tumor genotyping for solid tumors like non-small cell lung cancer. Its clinical use initially gained traction for the detection of EGFR mutations in NSCLC. Since the release of the initial International Association for the Study of Lung Cancer (IASLC) liquid biopsy position paper in 2018, numerous significant advancements have occurred in this field. These developments have led to changes in the decision-making process for treating advanced NSCLC and have prompted the need for an update in 2021. Currently, testing for a number of biomarkers is advised for all newly diagnosed nonsquamous, advanced-stage NSCLC cases. This transformation has been driven by the approval of a multitude of new drugs in the time span since 2018, signifying a dynamic shift in the landscape of NSCLC management (Rolfo et al., 2021).

The development of sensitive technology has made ctDNA and mutational analysis possible for patients with NSCLC. Additionally, the detection rate of ctDNA in the plasma from NSCLC patients might be higher than 80%, indicating that ctDNA analysis is a suitable substitute when sampling tissue biopsy is not a possibility (Villaflor et al., 2016). The therapy of patients with non-small cell lung cancer frequently evaluates a range of genetic mutations and modifications, such as EGFR, KRAS, ERBB2, and BRAF mutations, gene rearrangements like EML4-ALK, ROS1, NTRK1/2, and RET, exon skipping changes, and gene amplifications like MET. These molecular differences now play a crucial role in clinical practice, directing and monitoring patient care and disease progression. These mutations can be identified through PCR or NGS approaches. However, PCR-based methods are constrained to known mutations in specific genes, limiting their utility as a comprehensive ctDNA analysis tool for patients lacking these specific mutations. Conversely, NGS methods offer a broader mutational spectrum by surveying entire gene sequences (Lu et al., 2018).

Currently, larger next generation sequencing panels are being utilized more frequently in clinical settings. Examples are MSK-IMPACT, which is used for tissue samples, and MSK-ACCESS, which is used for plasma samples. Notably, circulating tumor DNA changes in 25% of the patients were present but were not found in tissue samples. This finding supports the notion that plasma samples may provide better specificity than previously believed (Gale et al., 2018; Jee et al., 2022). Targeted or untargeted NGS

can be used for ctDNA analysis. Targeted methods frequently sequence a few tens to several hundred genes, or even the full exome. To attain high sensitivity, deep sequencing is employed to amplify regions of interest encompassing clinically significant mutations, achieved through multiplex PCR or hybridization capture strategies. Due to its increased specificity and sensitivity, targeted sequencing is more appropriate for clinical diagnostics. Untargeted techniques, on the other hand, sequence the entire genome without performing the enrichment step. Whole-genome sequencing can identify novel genetic aberrations relevant to patient prognosis and therapy options despite compromising sequencing depth, making it a useful tool for fundamental biomedical research (Chen and Zhao, 2019).

The effectiveness of an NGS ctDNA profiling assay is frequently evaluated in the context of ctDNA sequencing by the precision of detecting mutant allele frequency (MAF) or variant allele frequency (VAF). They provide information about the number of ctDNAs in relation to cfDNAs that carry tumor-specific mutant alleles. As a result, a lower detectable MAF indicates greater sensitivity in an NGS assay for ctDNA analysis, allowing for the accurate identification of ctDNA despite a significant cfDNA background (Bos et al., 2021; Stewart et al., 2018).

Numerous researches and for-profit companies have already shown that NGS-based ctDNA profiling has the potential to aid in the early detection of cancer, the accurate identification of mutations that can be treated, and the prognosis of cancer patient outcomes. Therefore, molecular oncology is already transitioning to precision medicine thanks to NGS ctDNA profiling.

Liquid biopsies, which examine DNA or RNA from a patient's blood or sputum samples, can make use of NGS. Without the need for invasive treatments, liquid biopsies can be particularly useful for tracking the development of a disease, identifying minimally recurrent disease, and evaluating therapy effectiveness.

2.2.1. Limitations of liquid biopsy

Liquid biopsy has emerged as a promising method for the detection of biomarkers in NSCLC patients. This minimally invasive approach offers advantages in capturing the heterogeneity of tumors and holds potential to check for lung cancer. However, the absence of standardized protocols currently hinders the integration of liquid biopsy into clinical practice. To address this limitation, it is imperative to conduct further research involving the establishment of rigorous protocols and the inclusion of a larger, more diverse patient population. Such efforts are necessary to ensure that the

results obtained are not only accurate but also applicable across a broader spectrum of cases.

Another challenge pertains to the fragility of certain biomarkers, necessitating meticulous pre-analytical handling procedures. Moreover, controlling the intricate interplay between genetics and environmental factors poses a significant challenge. Additionally, the isolation and analysis of these biomarkers demand specific and highly sensitive methodologies due to the often low concentrations of these molecules within bodily fluids.

2.3. Next-Generation Sequencing for the Diagnosis of Lung Cancer

NGS has been employed to identify biomarkers for early diagnosis, decide on a specific course of treatment, and identify causal mutations in lung cancer patients (Wu et al., 2013). Because patients may exhibit neither symptoms nor symptoms that are comparable to those of other respiratory conditions, diagnosing early-stage lung cancer can be challenging. Additionally, due to many factors, like the quality and amount of the samples or the test's sensitivity, traditional approaches for diagnosing lung cancer frequently yield false-negative results (Hagemann et al., 2015). NGS would be advantageous at this point because it has excellent sensitivity and specificity while only requiring a minimal sample size. The NGS approach can be used to detect lung cancer-specific mutations in paraffin-embedded tissue samples more effectively than the usual PCR test since it can simultaneously detect an increasing number of alterations from the same amount of sample (Cainap et al., 2021). By providing previously unattainable insights into the molecular environment of this complicated disease, next-generation sequencing (NGS) technology has completely changed the way lung cancer is diagnosed (Esposito Abate et al., 2020). By sequencing the DNA and RNA from lung tumor samples, NGS enables clinicians to identify specific genetic mutations, alterations, and expression patterns that drive cancer growth (Cainap et al., 2021). In addition to helping with the precise classification of lung cancer subtypes, this effective tool is essential for forecasting a patient's prognosis and choosing the best course of treatment. NGS allows for the detection of targetable mutations like EGFR and ALK, facilitating the use of targeted therapies, while also uncovering potential resistance. Additionally, NGS-based liquid biopsies have become a less invasive method to track the development of the disease and the effectiveness of treatment, providing hope for more individualized and successful lung cancer management techniques. Essentially, the advent of NGS technology has ushered in a new era of precision medicine in the diagnosis of lung cancer, providing patients

and healthcare professionals with a clearer route to better results mechanisms (Karagur et al., 2023; Nooreldeen and Bach, 2021; Oxnard et al., 2014).

Specific genetic mutations in genes linked to lung cancer, such as EGFR, KRAS, ALK, PIK3CA, ROS1, and BRAF, can be found using NGS. These mutations, which are referred to as driver mutations, can direct therapy choices. Oncologists can decide whether a patient is a good candidate for targeted therapy by identifying these mutations. In addition, NGS can also detect some fusion genes that are common in lung cancer types, such as EML4-ALK, RET, ROS1, ALK, NTRK which makes the use of targeted therapies especially for these genetic abnormalities widespread (Chevallier et al., 2021).

Drug resistance is another application of NGS technology in the detection of lung cancer. NGS supports the ongoing monitoring of drug resistance mutations. Clinicians can modify treatment regimens to combat drug resistance and switch to other treatments by identifying these mutations early (Chevallier et al., 2021).

3. Conclusions

NGS has been used successfully in both research and clinical settings, and it is now a reliable method for diagnosing lung cancer. It outperforms current methods in detecting lung cancer-specific genomic and epigenetic alterations in a variety of biological samples, including blood, plasma, fresh frozen or FFPE tissue, urine, and other bodily fluids, even when conventional methods are insufficient and nucleic acid content is limited. Furthermore, liquid biopsy presents a new path with NGS for early lung cancer screening, diagnosis, and therapy, particularly in the absence of tissue samples. Circulating biomarkers may be non-invasive instruments that quickly inform medical decision-making on the need for more chemotherapy cycles or the necessity to alter the course of treatment.

In conclusion, it is expected that in the future NGS and liquid biopsy technology will play a greater role in the early detection of lung cancer, correct drug utilization, dynamic monitoring and prognosis assessment.

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Chapter 8

The Future of Ovarian Cancer Treatment: The Promise of Epigenetic Markers

Fatimeh Alghazawi[1]

Nastaran Azami[2]

Amirkasra Dehghani[3]

Elif Sibel Aslan[4]

Sajjad Eslamkhah[5]

Abstract

Epigenetics refers to inheritable DNA changes that occur without altering the DNA sequence itself. It encompasses processes like DNA methylation, histone modifications, and non-coding regulatory RNA pathways, all of which influence gene activity in mammals. The Ten-Eleven Translocate (TET) enzyme family, including TET1, TET2, and TET3, plays a role in regulating DNA methylation and gene expression. TET1 specifically converts methylated cytosine to 5-hydroxymethylcytosine (5hmC) and protects CpG islands (CGIs) from improper methylation. In ovarian cancer, TET2 gene mutations have been associated with higher tumor grade, advanced stage, lymph node metastases, and vascular thrombosis. TET2 acts as a tumor suppressor gene, and its suppression may contribute to disease development. TET3, inherited from oocytes, is linked to several diseases, including ovarian cancer. Increased TET3 expression in ovarian cancer is associated with poor outcomes and prognosis, making it a potential indicator for the disease. While vitamin C's effectiveness against ovarian cancer is still being studied, it is important to note that research is in the early stages, and further evidence

1 Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Biruni University, Istanbul, Türkiye. ORCID ID: 0009-0007-8706-2034 221106900@st.biruni.edu.tr

2 Biruni University, Istanbul, Türkiye, 0009-0006-4659-2584, 221106901@st.biruni.edu.tr

3 Biruni University, Istanbul, Türkiye, 0009-0006-3962-9908, 221106001@st.biruni.edu.tr

4 Biruni University, Istanbul, Türkiye, 0000-0002-3081-9004, easlan@biruni.edu.tr

5 0000-0001-8317-9293, sajjadeslamkhah1990@gmail.com

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is needed to establish its efficacy. Overall, epigenetic mechanisms, TET enzymes, and their mutations play crucial roles in ovarian cancer progression, providing potential targets for future therapeutic interventions.

Introduction:

Epigenetics is the study of the causal interactions between genes and their products, which create phenotypes. Humans share 99% of the same genes, making epigenetics a valuable reference for researchers and medical doctors studying diseases and disorders. C.H. Waddington's definition highlights the importance of understanding the beginnings of diseases and disorders[1]. Epigenetics involves gene expression changes without altering genetic sequence, predicting variation among humans through multiple pathways essential for maintaining tissue-specific gene expression patterns.[1]. DNA methylation and histone marks are direct mechanisms, while ncRNA is indirect. These heritable alterations are created during differentiation and preserved over cell division cycles, allowing diverse identities while maintaining genetic makeup[2]. The location of nucleosomes along the DNA, the methylation of cytosine bases in DNA, and posttranslational modifications to histone proteins are just a few examples of the epigenetic changes that are responsible for the heritability of gene expression patterns [1]. As known, a lot of diseases like diabetes, autoimmune diseases, and cancer could be affected by external factors such as aging, lifestyle, and even our prenatal development. With over 50% of new cases being identified beyond age 65 and over 70% of cancer fatalities happening in this same age group, it is well known that cancer is a disease of older individuals [3]. Ovarian cancer, the seventh most prevalent cancer worldwide, affects 4% of new cases and ranks as the ninth most common cancer-related death [3] . In the upcoming years, this ratio is expected to rise as our population ages and life expectancy rises. As a patient gets older, the results get worse and worse [3]. Epigenetics investigates the link between cancer, DNA methylation, and demethylation, in addition to how these factors influence cancer progression and chemo-resistance, particularly ovarian cancer[4]. Researchers explore using intravenous ascorbic acid (AA) for cancer treatment, with high doses recommended for OV [5]. In this review, the impact of one of the latest discovered enzymes are Ten-Eleven Translocation enzymes OV and overdoses of Vitamin C effectiveness will be reviewed.

DNA Methylation

The fundamental concept of DNA methylation is crucial to understanding the mechanism of TET enzymes. DNA methylation involves the addition of

methyl groups to the DNA molecule, which contributes to its preservation and stability [6]. This process can modulate the activity of a DNA sequence while preserving its sequence integrity [6, 7, 8, 9]. Methylation serves as an important inherited epigenetic mark. DNA methyltransferases enzymes (DNMTs), which are highly conserved among mammals, are responsible for transferring methyl groups to the DNA strand. DNMT1, DNMT3A, and DNMT3B are enzymatically active DNMTs that play key roles in initiating and maintaining DNA methylation, an extensively studied epigenetic modification in mammals [1]. In mice, the deletion of DNMT1 leads to embryonic mortality and a significant reduction in global DNA methylation levels. DNMT1 is the primary enzyme responsible for maintaining DNA methylation patterns following DNA replication. It exhibits a preference for methylating hemi methylated DNA templates, where only one DNA strand is methylated [10]. During the S phase of the cell cycle, DNMT1 is localized at DNA replication forks, indicating its role in maintenance activities. Recent studies have revealed that the accessory protein UHRF1 plays a crucial role in facilitating the proper targeting of DNMT1 to replicate DNA [11]. UHRF1 achieves this through its specific SRA domain, which enables its binding to hemi methylated DNA. An examination of DNMT1-knockout mouse embryonic DNA revealed the presence of de novo DNA methyltransferase (DNMT) activity[10]. DNMT1 primarily targets CpG regions (regions rich in cytosine-guanine dinucleotides) for DNA methylation, with the "p" representing the phosphate group [10]. DNMT3A and DNMT3B are classified as de novo methyltransferases. They play a crucial role in depositing initial methylation marks on unmethylated CpG islands during early embryogenesis and in primordial germ cells [12]. DNMT3L serves as the homolog of DNMT3A and DNMT3B, and its presence increases the number of de novo methyltransferases available for methyl group donation from S-adenosyl-l-methionine (SAM) [12]. In the human genome, approximately 70-80% of CpG regions are methylated [13]. After adding a methyl group to the base (in both strands), it's a mark to not express this gene segment which means inactivation. However, DNA methylation controls DNA replication as well by inactivation of many replication suppressors and promoters which means gene expression ([6]. Elevated methylation of CpG-rich regions is a common occurrence in various tumors, including ovarian tumors. However, it is important to note that not all CpG-rich regions associated with tumors are gene promoters [14]. Aberrant methylation of CpG islands in ovarian tumors has been linked to the repression of genes involved in crucial processes such as cell cycle control, apoptosis, drug sensitivity, and tumor suppression [15].

Ten-Eleven Translocation enzymes

Ten-eleven translocation enzymes (TET) family of dioxygenases plays an important role in the process of regulating transcription and DNA demethylation. Noteworthy that those enzymes only exist in mammalians [16]. TET1, TET2, and TET3 are large proteins and have multidomain functional sides. At 10q21.3 where TET1 located, TET2 is found on chromosome 4q24, and TET3 is found on chromosome 2p13.1 [17]. When a lack of methylation emerges in the newly produced DNA strand during replication, which may be caused by the presence of 5-hmC in the parental strand, TET enzymes are implicated in the passive DNA demethylation that takes place [17]. TET proteins share common structural features, including a conserved C-terminal region (cys-rich region) and a double-stranded β-helix (DSBH) domain. One important DNA base modification is 5-methylcytosine, which arises from the methylation of cytosine (C) and frequently plays a role in regulating gene transcription and other genomic functions [9, 18]. TET1 and TET3 contain a Cysteine-X-X-Cysteine (CXXC) domain that enables them to interact with both methylated and unmethylated CpG regions on DNA [19]. However, TET2 does not possess the CXXC domain. Instead, it forms a partnership with an independent protein called IDAX, which contains the CXXC domain [19]. As a result, the TET enzymes stop DNA's 5-methylcytosine (5mC) from being hydroxylated into 5-hydroxymethylcytosine (5hmC), and they subsequently catalyze the oxidation of 5hmC into 5-formylcytosine (5fC), and finally into 5-carboxycytosine (5caC) (Figure 1). Mutations in TET proteins and dysregulated regulation of their activity are implicated in the development of various human diseases, including cancer [13].

Antigen and cytokine receptors continuously send signals to T and B cells during immunological responses and development. These outside signals come together and are deciphered by combining transcription factors that are both widely expressed and unique to certain cell types, which work with chromatin regulators to alter the epigenome [20]. DNA and histone modifications, which enable information to be stored and/or passed down to daughter cells, are among the epigenetic changes connected to immune cell activation and differentiation [21]. As shown above, studies of the distribution of 5hmC over the entire genome show a strong connection between 5hmC and gene transcription. The quantity of 5hmC at gene bodies in thymic and peripheral T cell subsets correlates strongly positively with gene expression, RNA polymerase II occupancy, and H3K36me3 levels [21].

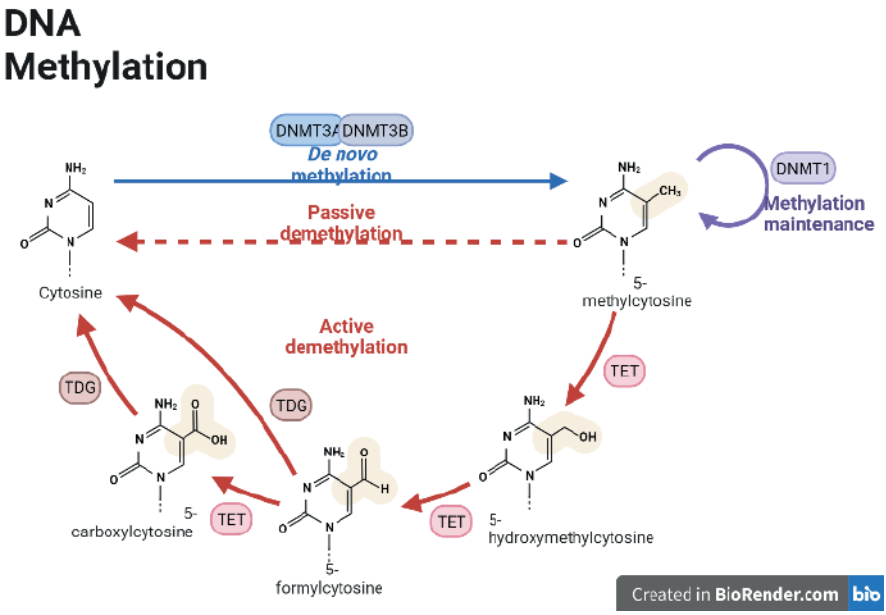

Figure 1 depicts the fundamental concept of DNA methylation/demethylation and the involvement of Ten-Eleven Translocation (TET) proteins. TET proteins play a vital role in catalyzing a series of sequential oxidations of 5-methylcytosine (5-mC) to generate 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC). Additionally, TET proteins can directly convert 5-mC to 5-fC and 5-caC [13].

TET1 role on Ovarian Cancer

TET1's role in cancer has been extensively studied and has generated significant interest in scientific investigations. Studies have revealed that TET1 plays a crucial role in regulating DNA methylation and gene expression by catalyzing the conversion of methylated cytosine to 5-hydroxymethylcytosine (5hmC) [22]. Additionally, TET1 possesses a CXXC domain that binds to unmethylated CpG islands, thereby safeguarding them from abnormal methylation. TET1 functions as a tumor suppressor and has been found to inhibit the migration and invasion of papillary thyroid cancer cells. Recent research has also highlighted its significant impact on ovarian cancer cells [22].

Studies conducted in vivo and in vitro have revealed that TET1 elevated 5-hmC levels in ovarian cancer, which in turn prevented colony formation and cell proliferation by expressing RASSF5, a tumor suppressor gene [12]. TET1's function in preserving genomic integrity and avoiding cancer is critical. TET1 deficiency, it turns out, not only causes genomic instability,

but it can also promote tumor vascular invasion [23]. TET1 overexpression, on the other hand, it has been proven to reverse the epithelial-mesenchymal transition and prevent cancer cell metastasis [24]. Chemo-resistance is caused by deregulation of DNA methylation/demethylation, and the TET family of dioxygenases (TET1/2/3) plays an important role in this. TET1 was significantly upregulated in cisplatin-resistant CP70 cells, and increased re-expression of vimentin caused partial epithelial-to-mesenchymal (EMT) [25]. TET1 was detected by immunohistochemistry in the nucleus and cytoplasm of human ovarian cancer tissues, and it was found to be positively linked with the presence of residual tumor and chemotherapy response [25].

TET2 and Ovarian Cancer

Thousands of women throughout the world are affected by the extremely aggressive and challenging-to-treat disease known as ovarian cancer. The TET2 enzyme may have a substantial influence on ovarian cancer, according to a recent study, opening new treatment and management options. Studies have shown that 5-hmC and TET2 expression are much lower in epithelial ovarian cancer than in healthy ovarian tissues [16]. Furthermore, in other studies, it was discovered that low TET2 and 5-hmC levels were linked to high tumor grade, pathologic stage, lymph node metastases, and vascular thrombosis in 130 people with epithelial ovarian cancer ([12]. TET2 and 5-hmC levels were significantly linked with poor clinical outcomes, such as a shorter time to the first recurrence, in high-grade and serous ovarian cancer [17][12]. TET2 mutations are seen in both germline and somatic ovarian cancer patients. TET2 may function as a tumor suppressor gene in ovarian cancer, and its dysregulation may help in the disease's development, according to these observations [18].In addition, studies have shown that TET2 suppression might encourage the development and invasion of ovarian cancer cells. On the other hand, new research has emphasized TET2's potential as a target for cutting-edge treatment strategies. For instance, it has been discovered that various medications, including VC, Metformin, and 5-Aza-2'-deoxycytidine, improve TET2 stability and may be utilized to prevent the growth of ovarian cancer [19]. Additionally , the discovery of germline TET2 variations in ovarian cancer patients raises the possibility that genetic testing for such variants might enhance risk assessment and direct patients' individualized treatment regimens. Overall, greater research into TET2's effects on ovarian cancer is necessary since it may have effects on diagnosis, prognosis, and therapy [20]. Another article presents our current knowledge of TET2's relationship to ovarian cancer. It provides an overview of the available studies that points to a potential involvement for

TET2 in the emergence and spread of ovarian cancer. The article also covers potential directions for novel therapies and management tactics that aim to reduce TET2 expression or stability [21].

TET3 impact on Cancer

TET3 exhibits the highest expression levels among the TET enzymes in oocytes and fertilized zygotes, while TET1 and TET2 become more predominant during early development [22]. In mouse genetic studies, TET3, inherited exclusively from oocytes, has been identified as the driver of 5mC loss and 5hmC gain in the paternal genome [23]. In addition to its association with various diseases, TET3 has been linked to several other disorders. Loss-of-function alleles of TET3 show high sensitivity in human control databases, and homozygous missense variations in TET3 have recently been connected to autosomal-recessive intellectual impairment in a consanguineous family (referred to as family 3) [9]. TET3 plays a critical role in actively reversing DNA methylation during development. Individuals with TET3 deficiency and other Mendelian disorders related to the epigenetic machinery commonly exhibit phenotypic characteristics such as developmental delay, intellectual disability, neurobehavioral symptoms, and growth anomalies [9]. TET3 has been described as an oncogene or tumor suppressor in ovarian cancer during carcinogenesis [24]. TET3 has been implicated in the inhibition of epithelial-mesenchymal transition (EMT) induced by TGF-β1, thereby exerting a protective effect against ovarian cancer [25]. Previous research has demonstrated the overexpression of TET3 in ovarian cancer tissue, although its prognostic significance and clinicopathological roles remain unclear. To elucidate the function of TET3, researchers conducted an integrated investigation using bioinformatics analysis [24]. The abundance of TET3 protein in oocytes and fertilized zygotes was confirmed [24]. TET3 expression in ovarian cancer was evaluated using the Oncomine database, as well as the TCGA and GTEx databases [14]. The relationship between TET3 gene alterations and clinicopathological features was examined through an integrative analysis of GEO datasets. Copy number alteration (CNA) and mutation analyses using cBioPortal revealed TET3 gains and diploid status, but not deletions, in ovarian cancer [14]. Furthermore, high levels of TET3 were associated with poor survival in ovarian cancer patients, as determined by the Kaplan-Meier plotter (K-M plotter) analysis [14]. This association was further validated through examination of the PrognoScan database and gene differential analyses using TCGA and GTEx data [14]. This study is the first to establish a correlation between increased TET3 expression, adverse clinicopathological outcomes, and a poor prognosis, suggesting that TET3

may serve as a diagnostic marker or therapeutic target for ovarian cancer due to its involvement in epigenetic modifications and methylation changes [14].

The Role of Vitamin C:

Numerous studies have indicated the potential of vitamin C as a treatment for ovarian cancer, as it exhibits inhibitory effects on the growth and metastasis of ovarian cancer cells. The field of cancer immunotherapy has recently garnered significant attention, leading to speculation about the potential of vitamin C supplementation to enhance immune responses and induce antitumor activity in cancer patients [26]. Moreover, clinical trials involving high-dose intravenous vitamin C administration to cancer patients have demonstrated improvements in various aspects of quality of life, including physical, mental, and emotional functions. These trials have also reported a reduction in the frequency of adverse effects such as fatigue, nausea, vomiting, and appetite loss [27].

In animal models, the co-administration of parenteral ascorbate (vitamin C) with conventional chemotherapeutic drugs carboplatin and paclitaxel has shown promising results in suppressing ovarian cancer. Furthermore, this combination therapy has demonstrated a reduction in chemotherapy-associated toxicity in ovarian cancer patients [28]. The presence of vitamin C at the reaction site is crucial for its effects. Vitamin C activates TET enzymes by reducing the embedded iron ion within TET's catalytic site. This reduction of Fe+3 to active Fe+2 restores the enzymatic activity. Interestingly, substituting vitamin C with another electron donor does not lead to a catalyzed reaction, highlighting the unique role of vitamin C in this process [29]. Vitamin C has been identified as a crucial component in the TET-mediated demethylation process, as its addition has been found to enhance TET activity [30]. Consequently, TET3 functions as a tumor suppressor. A schematic representation illustrates how vitamin C influences the TET-dependent removal of methyl groups from genomic DNA. Nevertheless, further research is required to fully comprehend the efficacy of vitamin C in ovarian cancer treatment, and it should not be used as a substitute for conventional medical interventions such as surgery, chemotherapy, and radiation [29]. Recent studies conducted on embryonic stem cells have revealed that ascorbic acid (AA), a form of vitamin C, acts as a cofactor for TET enzymes and increases their activity. In vitro experiments involving diffuse large B-cell lymphoma (DLBCL) and PTCL cells treated with intravenous AA doses demonstrated enhanced TET activity, resulting in DNA demethylation, increased expression of SMAD1 (a tumor suppressor gene), and heightened chemosensitivity of lymphoma cells [31]. Clinical

studies combining intravenous AA with chemotherapy are necessary to investigate whether AA deficiency may impact TET function and contribute to resistance in certain patients [31]. It is important to note that high doses of vitamin C can have side effects, so individuals should consult their doctor before taking large doses of vitamin C or any other supplement [30].

Conclusion:

The traditional understanding of the genetic code as the primary driver of cellular gene function and genetic changes as the main cause of human diseases has been challenged by the emergence of the epigenetic revolution in biology. In this context, TET enzymes have emerged as key players in physiology, disease, and development. The DNA demethylases belonging to the TET family have a crucial role in shaping the epigenetic landscape of tumors. While much research has focused on the direct impact of TET activity on cancer cells, it is now evident that TET involvement in the tumor microenvironment is equally critical for tumor growth and development. Recent studies have highlighted the significant role of TET1 in ovarian cancer, specifically in regulating DNA methylation patterns and gene expression. The CXXC domain of TET1 plays a protective role in preventing erroneous methylation of CGIs (CpG islands), which are important regulatory regions in the genome [32]. TET1 has an important role in maintaining genomic integrity and preventing cancer, with deficiency producing genomic instability and overexpression limiting metastasis. Recent studies suggest that the TET2 enzyme may significantly influence ovarian cancer, with studies showing lower 5-hmC and TET2 expression in epithelial ovarian cancer. These low levels are linked to high tumor grade, pathologic stage, lymph node metastases, and vascular thrombosis. TET2 mutations are present in both germline and somatic ovarian cancer patients, and their dysregulation may contribute to the disease's development. Further research is needed to understand TET2's impact on diagnosis, prognosis, and therapy. TET3 importance lies in the fact that it is the only inherited enzyme from the family of TET enzymes. Despite the lack of studies on TET3 and its relationship to ovarian cancer, the study mentioned above recommends using TET3 as a marker for ovarian cancer and, therefore, the TET3-related treatment plan. One of the most recent recommendations for cancer treatment is vitamin C. Its effect on ovarian cancer was investigated because it activates the TET enzyme, reducing cell activity and acting as a tumor suppressor. Ascorbic acid may be a potential treatment for ovarian cancer, potentially inhibiting cell growth and spreading. Cancer immunotherapy studies show potential for vitamin C supplementation to enhance immune responses in cancer patients. More research is needed to fully understand its effectiveness.

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Chapter 9

Robotics in Healthcare: A Brief Introduction

Bilge Büyükşirin[1]

Abstract

In recent years, robotics has emerged as a transformative force in the field of healthcare. As technology continues to advance, robots are playing an increasingly vital role in patient care, diagnosis, surgery, and rehabilitation. This study explores the various applications of robotics in healthcare, highlighting its potential to revolutionize the way we approach medical treatment and improve patient outcomes.

1. Introduction

Instruments used in healthcare operations have long history as medicine itself. It is known that medical instruments such as scalpels, lancets, curettes, tweezers, tubes and surgical knives made from bone, bronze, silver and iron were used in ancient Egypt and Rome. By time and technical developments medicine got developed and divided into many branches. And with new branches of medicine many specialized tools were invented for special operations. After invention of electronics and especially computers, automatized tools were started to be used in healthcare.

Robots are defined as a programmable machines which can accomplish complex mechanical tasks by itself. In medicine robots are used in rehabilitation, surgical, telepresence, drug supply, social assistance, imaging, disinfection, radiotherapy, transport and similar operations. Morgan et al. (2022) stated that rehabilitation and surgical applications are first two areas where most of the robotics studies are conducted.

In this study robotic applications used in healthcare are examined in three sections by their popularity. In first section rehabilitation and mobility robots, in second section surgical robots and in third section robots used in other operations are investigated.

1 Department of Healthcare Management, 37521 Trakya University, Edirne, Turkey. ORCID: 0000-0002-3941-7339, e-mail: bilgebuyuksirin@trakya.edu.tr

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2. Rehabilitation and Mobility Robots

Rehabilitation and mobility robots are designed and used to assist patients who have difficulty in performing physical activities, increase muscle strength and power, strengthen muscle-brain communication, and aid patients in various rehabilitation exercises. Rehabilitation and mobility robots have various designs to support body weight of the patient and to assist movement of limbs. In Fig.1 several designs are illustrated. In Fig.1a body weight of the patient is supported by a harness attached to celling and movement of the lower limbs are assisted by an exoskeleton. In Fig.1b similar to first setup body weight is supported by celling attached harness but movement of the feet is assisted by actuated programmable plates. In Fig.1c body weight of the patient is supported by a mobile robotic frame which can adapt and follow to movements of the patient. In Fig.1d body weight of the patient is supported by a mobile frame and movement of the limbs of the patient is assisted by an exoskeleton. In Fig.1e a self-balanced exoskeleton is used for both supporting body weight and movement assist. In Fig.1f and Fig.1g patient has a sitting position and treated limbs are assisted by an upper or lower limbs exoskeleton. Finally in Fig.1h a foot orthotics is shown which can be used while walking, standing or sitting.

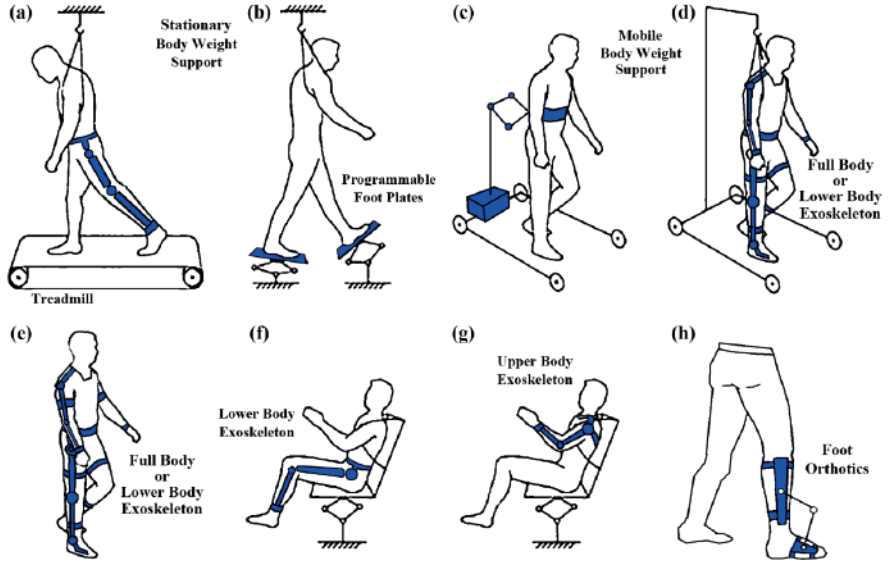

Figure 1. Rehabilitation and mobility robot designs.

It is reported that there are more than one hundred robot systems used in rehabilitation and mobility applications indexed in literature (Morgan et al., 2022). The rehabilitation and mobility robots may have the designs given in Fig.1a-h or any combination of them. In table-1 some of the common rehabilitation and mobility robots, their usages, their creators and their types are listed. These devices provide personalized therapy and can adapt to the patient's progress, making the recovery process more efficient and effective.

Table-1. Rehabilitation and Mobility Robots

Robot Name	**Purpose**	**Company/ Creators**	**Type**
Lokomat (Jezernik et al., 2003)	lower limbs rehabilitation	Hocoma AG	Fig.1a
HAL (Suzuki et al., 2007)	exoskeleton, mobility assistance, upper-lower limbs rehabilitation	Cyberdyne	Fig.1e
Hunova (Saglia et al., 2019)	rehabilitation and the sensorimotor assessment of the lower limbs and trunk	Movendo technology	Fig.1b Fig.1f
LokoHelp (Freivogel et al., 2008)	gait-training, postural training	Woodway	Fig.1a
ReoAmbulator (Calabrò et al., 2016)	gait rehabilitation, ambulation rehabilitation, coordination rehabilitation	Motorika	Fig.1a
ALEX (Banala et al., 2008)	neuromotor rehabilitation of upper limb function	Kinetek	Fig.1g
LOPES (Meuleman et al., 2015)	gait training for stroke patients	University of Twente	Fig.1a
String-Man (Surdilovic and Bernhardt, 2004)	posture balancing, gait assistance	Fraunhofer Institute	Fig.1a
Gangtrainer GT (Hesse et al., 2008)	gait trainer	Reha-Stim	Fig.1b
HapticWalker (Schmidt et al., 2008)	gait trainer	Fraunhofer Institute	Fig.1b
GaitMaster5 (Tanaka et al., 2012)	gait assistance, feet rehabilitation	University of Tsukuba	Fig.1b Fig.1h
KineAssist (Patton et al., 2008)	gait assistance	Kinea Design LLC	Fig.1c
WalkTrainer (Allemand et al., 2009)	gait assistance	Swortec SA	Fig.1d
ReWalk (Awad et al., 2020)	gait assistance	ARGO Medical	Fig.1e
NUVABAT (Ding et al., 2010)	ankle rehabilitation, measurement of ankle kinematics	Northeastern university	Fig.1b Fig.1f

3. Surgical Robots

Surgery is defined as a medical procedure which involves cutting the patient's tissues to reach the inner parts of the body, threading a health problem and closing of the wounds. Surgical operations categorized as invasive procedures where external tools are used inside the patient's body through a cut. Conventional surgical operations which also named as open surgery usually need large incisions to access the diseased area and exposes internal body cavity to outside. Because of the large incisions patient's recovery takes long time and risk of infection and complications arise. To reduce these risks during operation the size of the incision can be minimized. Which is called as minimally invasive surgery, where surgery tools got reached to the inner parts of the body through natural orifices or small holes pierced to the tissue. Using minimally invasive surgery is more advantageous for patients but still may get tiring for the operator. The advanced surgical robots specially designed for specific procedures and operations make the operator`s job much easier. These robots offer enhanced dexterity, 3D visualization, and reduced surgical trauma, resulting in shorter recovery times and less pain for patients. As a result, complex surgeries are becoming safer and more accessible.

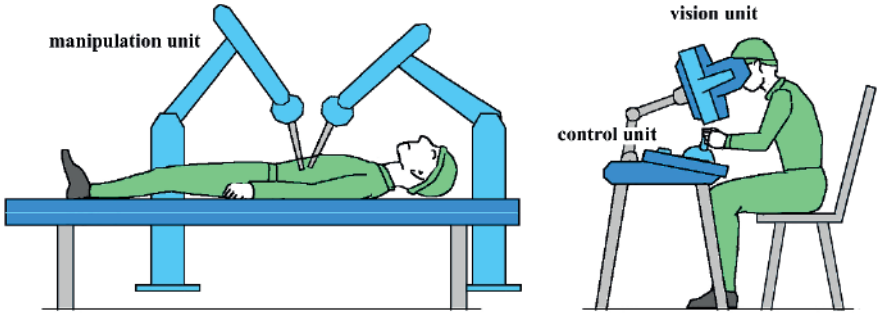

Figure 2. General units of surgical robots.

Surgical robots generally have three main units as the manipulator unit, the controller unit and the vision unit. The manipulator unit is the main actuation unit of the robot, which holds and moves the surgical tools. Manipulation unit can be made of 6 or 7 degrees of freedom robot arms or specially designed mechanisms. Surgical tools such as scalpels, forceps, retractors and clamps are got attached to end effectors of the robot arms.

Besides these tools end effectors also contain sensors and cameras to give information and vision to the operator. The operator controls the arms or mechanisms through the controller unit. The advanced controller units usually have haptic feedback property for sensing the tissue and movements. During operation the operator watches the operation through the vision unit. The operator can be in the same place with the patient or can be in another location and control the robots online. Advanced vision units provide 3d view beside conventionally 2d view.

Table-2. Surgical Robots

Robot Name	**Surgery purpose**	**Company/Creators**	**Image**
da Vinci surgical system (Freschi et al., 2013)	urology, laparoscopy, gynecology, thoracoscopy,	Intuitive Surgical Inc	Fig.2a (Intuitive, 2023)
MiroSurge (Konietschke et al., 2009)	laparoscopy	DLR robotics	Fig.2b (DLR, 2018)
Versius robotic system (Dixon et al., 2021)	gynecology, colorectal, renal, laparoscopy	CMR Surgical	Fig.2c (CMR, 2023)
Senhance (Samalavicius et al., 2020)	gynecology, laparoscopy	TransEnterix, Asesnsus	Fig.2d (Asensus, 2023)
Monarch Platform (Graetzel et al., 2019)	bronchoscopy	Auris Surgical Robotics	Fig.2e (Ethicon, 2023)
Flex® robotic system (Mattheis et al., 2017)	oropharynx, hypopharynx, larynx	Medrobotics Corp	Fig.2f (Medrobotics, 2018)
Sensei X robotic catheter system (Rolls et al., 2014)	cardiac catheter insertion	Hansen Medical Inc	Fig.2g (Hansenmedical, 2016)
NeoGuide Colonoscope (Eickhoff et al., 2007)	colonoscopy	Intuitive Surgical Inc	Fig.2h (Farnam, 2012)
Invendoscopy E200 system (Groth et al., 2011)	colonoscopy	Invendo Medical GmbH	Fig.2i (Invendo, 2015)
FreeHand v1.2 (Stolzenburg et al., 2011)	laparoscopy, thoracoscopy	Freehand Surgeon Ltd	Fig.2j (Freehandsurgeon, 2023)

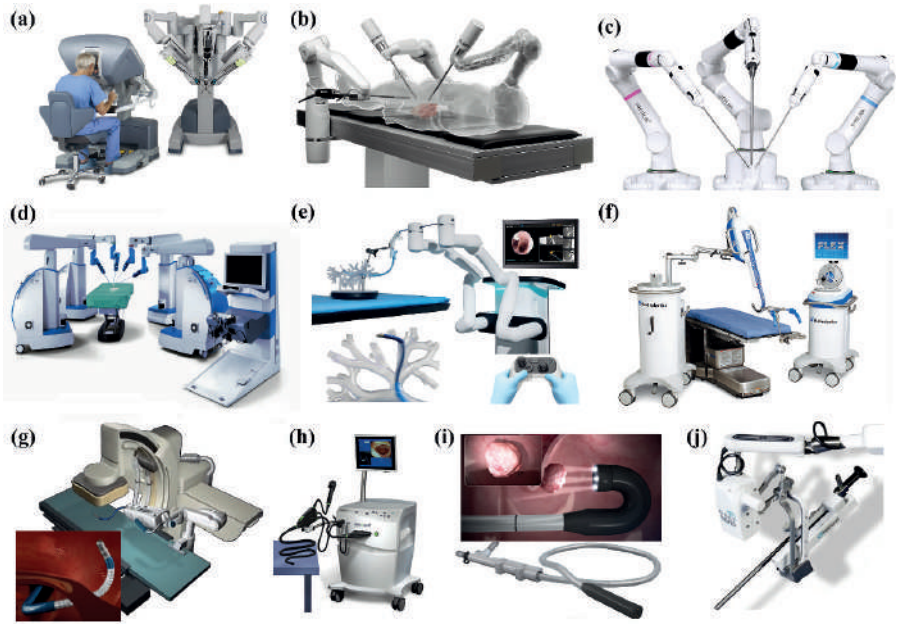

Figure 3. Commercial surgical robots.

Some of commercial surgical robots are listed in Table-2 and their images are presented in Fig.3. From these robots da Vinci, Senhance, MiroSurge and Versius robotic systems use multiple robot arms. At these systems one of the arms holds the cameras and provides information and vision to operator. While other arms are used for manupulation of the surgical tools. Other robots on the list are special mechanisms consisting of snake-like actuators with multiple degrees of freedom, used to explore body cavities such as the lungs, stomach, intestines, and main veins and to perform operations in these cavities. The FreeHand system is actually a robot assisted vision system, which helps the operator to get a clear view of the inner body.

4. Robots Used in Other Operations

4.1. Drug Delivery and Pharmacy Automation

Automation plays a significant role in drug preparation and delivery. Robotic systems are used in pharmacies to dispense medication with high accuracy, reducing the risk of errors. Moreover, tiny robotic devices are being developed for targeted drug delivery within the body, enabling precise treatment of diseases while minimizing side effects (Li et al., 2016).

In addition to these small-sized robots, mobile robots that carry equipment are among the robotic systems trying to gain a place in the healthcare industry. While these mobile robots automate routine tasks in the hospital on the other hand they are used to deliver medicine and equipment to quarantine areas where entry and exit are prohibited.

4.2. Telemedicine and Remote Monitoring

Robots are also facilitating remote healthcare delivery. Telemedicine robots equipped with cameras and sensors allow doctors to interact with patients from a distance (Koceska et al., 2019). This is particularly valuable in situations where physical presence is challenging, such as during a pandemic. Additionally, robots can monitor patients in their homes, collecting data and alerting healthcare providers to any concerning changes in real-time, thus improving the management of chronic conditions.

4.3. Socially Assistive Robots

Socially assistive robots are developed to aid humans through social interactions and guidance in various environments (Vulpe et al., 2021). These robots usually take cute animal shapes or humanoid forms. With their AI support they can take voice commands and even perform long chats with users. They are designed to guide and inform users about a procedure, location, treatment or similar. Some current research attempts to design these robots to provide psychological support and make them a part of treatment.

4.4. Radiotherapy Robots

Radiotherapy is one of the most widely used cancer treatment. Effectiveness of high energy X-rays over the cancer cells is proven by many applications. But while these rays are killing the cancer and tumor cells they are also damaging the healthy cells of the patient. This is the undesirable and most feared side effect of the radiotherapy. Fortunately, robotic radiotherapy overcame this draw back by sending concentrated rays only to the treatment area, and adapting itself to patient (Crop et al., 2015).

5. Conclusion

Robotics in healthcare is ushering in a new era of medical practice. From the operating room to remote consultations, robots are improving the quality of care, enhancing patient experiences, and increasing the efficiency of healthcare systems. As technology continues to evolve, the potential for robotics to transform healthcare is limitless. However, it is essential to strike

a balance between technological innovation and ethical considerations to ensure that the benefits of robotics are harnessed while maintaining the human touch in patient care. The future of healthcare undoubtedly includes robots as valuable partners in delivering the best possible medical services to patients worldwide.

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Chapter 10

Balance and Physical Activity in Children with Neurodevelopmental Disorders

Büşra Candiri[1]

Amine Nur Arıkan[2]

Sanem Can Çolak[3]

Abstract

Neurodevelopmental disorder is a disorder that occurs in different ways and is diagnosed in childhood involving central nervous system abnormalities. Neurodevelopmental disorder can be explained in children with autism spectrum disorder down, intellectual disability, learning disability, attention deficit hyperactivity disorder (ADHD), cerebral palsy. Children with neurodevelopmental disorder can be affected in many dimensions developmentally, experientially and environmentally. It is known that children show special conditions in various subjects from their peers due to their physical, mental, social development and brain activities being affected. It is revealed that these special conditions affect peer relations in children's social lives, cognitive issues such as problem solving, analytical thinking, language development issues such as understanding and expression, as well as the child's balance and physical activity. By processing the information coming from visual, vestibular and proprioceptive systems together, the symptoms and characteristics of physical activities in body movements with the energy available by using the muscles in the balance and skeletal system are determined to a great extent. The characteristics of children with neurodevelopmental disorders, their assessment, the balance context and the level of physical activity are described.

1 Research Assistant, PHD, Inonu University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, candiri_17@hotmail.com , 0000-0001-7413-6371

2 Research Assistant, Inonu University, Faculty of Health Sciences, Department of Child Development, arkan.amine@gmail.com , nur.arikan@inonu.edu.tr , 0000-0001-6360-2357

3 Research Assistant, Inonu University, Faculty of Health Sciences, Department of Audiology, sanemcan.colak@inonu.edu.tr, 0000-0002-7566-7964

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Children with Neurodevelopmental Disorders

Neurodevelopmental disorders are a broad category of disorders that involve some form of central nervous system abnormality [1]. Neurodevelopmental disorders occur in a certain period according to any age range and developmental areas of children. These disorders greatly affect children's social emotional development as well as physical development motor movements, physical activity and balance. Neurodevelopmental disorders are a special needs group that explains cognitive, physical development in a complex and broad dimension that interferes with children's lives and thus interferes with professional and academic skills [2].

The term neurodevelopmental disorder is frequently used to describe a brain-based phenomenon that occurs in childhood and is associated with functional impairments. While the term neurodevelopmental disorder is generally used in health and medical issues and models, the term neurodevelopmental disability is mostly preferred in social studies and social fields [3].

Neurodevelopmental disorders are also referred to as a special needs condition that causes a variety of complex symptoms in the body whose symptoms are related to brain development that cause children to experience intellectual, cognitive, communication, behavioural and psychomotor deficits [4]. However, despite this, there are many diagnostic confusions in the field.

Attention deficit hyperactivity disorder, autism spectrum disorder and children of Down Syndrome are examples of neurodevelopmental disorders' that contain both psychological and neurological abnormalities [1].

Characteristics of Children with Neurodevelopmental Disorders

Children with neurodevelopmental disorders face many difficulties in the field of explanation, diagnosis and evaluation. However, it has an important role in supporting children through the development of education and intervention programmes. Although intervention programmes are role models for the child and the family, their characteristics may vary according to the type of disorder. The phenotype of children with neurodevelopmental disorders includes ongoing health and developmental symptoms. While it may be early for parents to recognise these characteristics, it becomes more difficult at times. It is therefore of great importance for staff to knowingly recognise and identify these characteristics [1, 5].

Autism spectrum disorder is described as a neurodevelopmental disorder that causes cognitive differences, health problems, social communication and behaviours in children with environmental factors, thus causing difficulty in individual behaviour in the environment. One of the prominent features is that children with autism spectrum disorder have difficulty in adapting to the environment and experiencing behavioural problems due to conditions occurring in the brain [6]. Children with autism spectrum disorder can be diagnosed at the age of 2, starting in the first years of life. At the same time, there are characteristics such as delay or regression in social skills, cognitive development, verbal and non-verbal communication such as making eye contact, not looking when their name is called, showing stereotypical movements that show less development than their peers in social communication and interaction between people, limited interests and sensory differences [7].

Down Syndrome phenotype, motor development, balance and expressive language skills delay and these delays continue throughout their lives and they have difficulties in language and social communication interaction dimension [8]. In the cognitive dimension, situations such as inability to fulfil certain mental functions and inability to learn may also occur. However, despite cognitive and social retardation, they can improve in physical activity and balance with education and support. In this way, they can be included in the social environment with physical activity. In terms of cognition, many people have asymmetrical skill profiles, with relative strengths in nonverbal reasoning and visual processing, especially when compared to verbal and auditory processing skills [9].

Learning disability is described as a neurodevelopmental disorder that differs from its peers in the acquisition and use of listening, speaking, reading-writing or mathematical skills [10]. Learning disability is divided into 4 categories: reading disability, maths disability, written expression disability and learning disorder that cannot be named otherwise [11].

Assessments of Children with Neurodevelopmental Disorders

There are special criteria for the assessment of children with neurodevelopmental disorders according to certain areas. The evaluation process is seen as a challenging process for the child and parents. From the diagnosis of children, parents have problems in various issues such as acceptance and support [12]. There may be different methods for the evaluation of each child in neurodevelopmental disorders.

Considering the sub-dimensions, the evaluation of children with autism spectrum disorder is as follows according to DSM-5: utism Spesctrum disorder is included in DSM-5 and is used as an evaluation criterion. In DSM-5, autism spectrum disorder is described in two sections as limitation in social communication and interaction and limited, repetitive interests and activities and is stated as an evaluation element. In the dimension of limitations in social communication and interaction, difficulties in social-emotional, non-verbal communication and difficulties in initiating or maintaining peer relationships are detailed. In the dimension of limited and repetitive behaviours, repetitive motor movements, object use or speech, insistence on sameness, routines, rituals, limited interests, increased or decreased sensory interest or reactions are used as assessment tools in the diagnostic criteria [11].

Attention deficit hyperactivity disorder is one of the neurodevelopmental disorders and frequently occurs in preschool years. In addition to retardation in cognitive development and distractibility, children have additional symptoms such as anxiety disorders and separation anxiety [13].

According to DSM-5, the child can be diagnosed when the items specified for learning disability are observed in the child. The criteria considered in the evaluation process are as follows: Having difficulties in the academic field for at least six months, being slow in reading words and making frequent mistakes, having difficulty in reading comprehension, having phonological sound additions and subtractions, having difficulty in written expression, having difficulties in arithmetic, not having spatial awareness, having difficulty in understanding mathematical terms [14].

After the children with neurodevelopmental disorders are evaluated in general, the specialist staff carries out studies to support the child in cooperation with the parents. Supporting studies for balance and physical activity are carried out with education and intervention programmes. In this way, children can generally be adapted to social life [15]. Therefore, diagnosis and evaluation criteria are one of the important factors in neurodevelopmental disorders.

Neurodevelopmental Disorder and Balance

Balance is achieved by processing information from the visual, vestibular and proprioceptive systems together. A disorder in one of these systems causes loss of balance. Dizziness and imbalance are symptoms that can be seen in childhood [16]. In children, vestibular system disorder causes inability to maintain head and posture control, inability to perform

independent walking and sitting functions, poor motor development and delayed psychomotor skills [17]. This situation is more common when the information coming from the visual or sensory system becomes difficult. In children with vestibular disorders, in addition to postural complaints such as clumsiness, falls, injuries due to falls, step width, some disruptions in fine motor development may also be observed. If the vestibulo-ocular reflex, one of the most important reflexes of the vestibular system that ensures the stability of the visual field during head movement, is affected, motor dysfunctions may occur because hand-eye coordination cannot be achieved [18]. Studies show that vestibular disorders and cognitive skills are related [19, 20]. It has been reported that this situation arises from the connection of vestibular projections with the hippocampus [21].

It is known that the static and dynamic balance skills of children with neurodevelopmental disorders are worse than those of normally developing children [22]. Children with autism spectrum disorder may have difficulties in social and communication skills, as well as poor motor coordination skills and disorders in postural control. Disorders in postural control arise from problems in visual and somatosensory processing [14]. Morphological studies report that the disorders originate from the brainstem [23]. Disorders in postural control also affect the development of perceptual motor skills and social functionality [24]. When the vestibular functions of children with autism are evaluated, there is a decrease in their static and dynamic balance skills. In addition, oculomotor system disorders may occur and the vestibulo-ocular reflex may be negatively affected [25].

Visual-motor integration disorders, postural instability, oculomotor system disorders and vestibulo-ocular reflex involvement are observed in children with Down syndrome [26]. Children with Down syndrome cannot respond quickly to changes in the environment, so it takes longer to complete motor tasks [27]. Postural alignment is observed to maintain sway and balance during standing [28]. This situation is thought to be caused by cerebellar dysfunction, proprioceptive errors, biomechanical deficiencies, vestibular disorders or sensorimotor integration [29, 30]. Individuals with Down syndrome have lower neuron density, synaptic irregularities due to decreased neurotransmitters, and myelination abnormalities compared to their normally developing peers [31, 32].

Delays in motor development may be observed in children with intellectual disability. This situation limits functional abilities [33]. Disorders in postural control are observed due to the incomplete development of the central part that controls the somatosensory, visual and vestibular systems.

They have problems with motor coordination, muscle strength, endurance, fine and gross motor skills. It is also reported in the literature that vestibulo-ocular reflex dysfunction is observed in children with intellectual disability [34]. They may lose more postural control in situations where visual and somatosensory inputs change and become difficult, such as staying in the dark or walking on soft ground. These abnormalities are thought to result from processing deficiencies in the central mechanism of the vestibular system [35].

Weak motor coordination skills, postural stability, spatial orientation and oculomotor dysfunctions can be observed in children with learning disabilities [36, 37]. They show lower postural performance compared to their typically developing peers [36]. It has been reported that these disorders occur as a result of vestibulo-cerebellar dysfunction in children with learning disabilities [38]. Disorders in postural skills may result from disruptions in the integration of information from the visual, vestibular or proprioceptive systems [39]. It is known that the vestibular system is related to attention, memory, cognitive processing and visual spatial ability. For this reason, children who have cognitive problems such as learning disabilities may experience vestibular disorders [19].

In children with attention deficit and hyperactivity disorder, poor motor performance and impaired postural control are observed compared to their normally developing peers [40, 41]. Central vestibular changes in the connections between the vestibulo-cerebellum and vestibular nuclei, deficiencies in cerebellar inhibitory functions and disorders in prefrontal functions are reported [42, 43]. At the same time, it has been reported that brain imaging studies show a decrease in the volume of the cerebellar vein. For this reason, disorders in gait control may occur [44].

In cerebral palsy, which is a common neurological disorder in childhood, there are weaknesses in static and dynamic balance as well as gross and fine motor movements. They experience balance disorders as a result of problems in the visual, vestibular and somatosensory sensory systems [45]. In order to better define the disorders in cerebral palsy, a classification was made by dividing it into five levels [46]. As the level increases, the degree of balance disorder increases. It is reported in the literature that the cause of balance disorder in cerebral palsy is due to inadequate motor and somatosensory deficiencies. At the same time, oculomotor dysfunctions are also observed in children with cerebral palsy [47].

As a result, postural instability, motor coordination disorder and oculomotor system dysfunction may be observed in children with

neurodevelopmental disorders. Children with neurodevelopmental disorders may not be able to express themselves, have difficulty communicating with their parents or caregivers, and may not be able to describe their symptoms. Vestibular disorder also negatively affects postural control, development of fine motor skills, academic success and cognitive development, emotional and social behavior skills. For this reason, children should be observed carefully, and in case of any suspicion, an expert should be consulted. Additionally, vestibular assessment tests can be challenging in children because they require good cooperation. Therefore, it is necessary to make appropriate procedural changes in evaluation tests, continue testing by taking breaks, and use a game-based approach. Vestibular rehabilitation and physical activity, after early detection of disorders through a comprehensive evaluation, is an effective approach in children with neurodevelopmental disorders and balance problems [48].

Neurodevelopmental Disorder and Physical Activity

Physical activity is body movement that results in energy expenditure using skeletal muscles [49]. Physical activity in the first years of life is important for growth and development [50]. High levels of cardiorespiratory endurance in childhood are closely related to lower cardiometabolic risk in adulthood [51]. In addition, physical activity has important benefits in terms of preventing secondary health problems that disabled children may experience and supporting communication skills, self-confidence, and psychosocial development [52]. Physical activity in the form of walking has been shown to have beneficial effects in terms of physical and subjective health (health satisfaction) in a population consisting of children with visual, hearing and physical disabilities, autism, learning disabilities and emotional disorders [53].

It is possible to talk about the different benefits of physical activity in children with various neurodevelopmental disorders. Physical activity has been found to be effective in increasing academic performance [54], decreasing anxiety and depression levels [55], and social communication [56] in children with autism. Physical activity increases self-esteem and prevents chronic diseases in children with Down syndrome [57]. In a comprehensive systematic review in 2023, 35 scientific studies conducted since 1990 investigating the effects of physical activity on children with Down syndrome were examined. Water sports, aerobic exercises, strength exercises and game training practices have been identified to increase physical activity. It has been determined that these practices generally cause improvements in maximum oxygen consumption, maximum heart rate,

upper and lower body strength, body weight and body fat percentage [58]. In individuals with intellectual disabilities, physical activity causes beneficial results in general health perception and physical fitness parameters such as strength, endurance, and body mass index [59]. In children with learning disabilities, physical activity increases their social roles, improves physical and mental health, improves academic success and reduces irritable behaviors that occur due to learning disabilities [60, 61]. In individuals with attention deficit hyperactivity disorder, physical activity supports increasing self-esteem as well as physical and general health-related parameters [62]. Other benefits include reducing impulsivity and hyperactivity, increasing attention and improving executive functions [63]. It has been shown that physical activity in children with CP (cerebral palsy) is beneficial for reducing chronic pain, fatigue and osteoporosis, supporting mental and social development, improving physical function [64], and increasing quality of life and happiness [65].

Physical Activity Deficiencies

Physical activity participation of disabled children and adolescents is affected by many factors. These; communication, transportation, costs, lack of awareness, lack of social support [66], type of disability, insufficient time, inadequate facilities, factors related to parents (fear, labeling) [67], child's lack of interest and behavioral problems [68]. In a systematic review investigating the physical activity levels of disabled children between the ages of 0-5.99, it was observed that they had low physical activity levels as a result of 21 studies included [50].

Motor and behavioral disorders and communication problems in children with autism affect physical activity participation [69]. In a study in which the physical activity patterns of children with autism were examined with the help of an accelerometer, it was determined that the children showed insufficient physical activity and had too much sedentary time. Additionally, physical activity deficiencies were found to be more pronounced at older ages [70]. In another study conducted on a large population, it was determined that male children with autism, especially between the ages of 6 and 11, had insufficient physical activity levels compared to children in the general population [71]. In the meta-analysis in 2021, it was aimed to investigate the differences between children with autism and typically developing children in terms of moderate and vigorous physical activity. As a result of 9 studies that evaluated with the help of an accelerometer, it was analyzed that children with autism have 30 minutes less daily moderate physical activity level than normally developing children. Children also have

been shown lower physical activity performance during physical education and break hours during school education [72]. As a result of 28 studies and a comparison of the data of 805 autistic children with 1573 healthy children in 2023, it was determined that their moderate to severe physical activity levels were less than their peers [73].

In children with Down syndrome, disease-specific characteristics such as hypotonia, obesity and congenital heart problems, parental factors, coordination disorders and accessibility to physical activity affect physical activity participation [57]. The results of a systematic review conducted in 2019 have shown that the physical activity levels of participants with Down syndrome under the age of 21, assessed with the help of an accelerometer, were significantly lower than their healthy peers. It has also been determined that lack of physical activity has an impression that is inversely proportional to age [74]. It has also been documented that they have higher weight and lower physical activity performance than their healthy peers [75].

In a study conducted in Iceland, the physical activity behavior of children with mild and severe intellectual disabilities has been measured with the help of an accelerometer. It has been determined that children with intellectual disabilities are 40% less active than typically developing children matched for age and gender. Additionally, they have been found to spend 9% more sedentary time. None of the children with intellectual disabilities met the recommended daily average of 60 minutes of moderate-intensity physical activity [76]. More than 70% of children with visual, hearing, physical and mental disabilities and social development problems have been shown insufficient levels of physical activity at school. The most physical activity deficiencies were observed in children with severe mental disabilities [77].

A study conducted on young people with learning disabilities and attention deficit hyperactivity disorder found that they were significantly more obese than their healthy peers. In addition, in the examination of cases where both pathologies were seen separately and together, it was found that physical activity levels were significantly low. However, it has been determined that only individuals with learning disabilities are better at meeting their physical activity levels [78].

Reasons for the deficiencies in physical activity of children with CP include advanced age, female gender, and the presence of hip dysplasia [79]. From a general perspective, personal factors such as the child's physical abilities and psychological factors, parental factors (such as acceptance of disability), opportunities for participation in sports (such as lack of opportunity and awareness, time-related problems, financial situations), social environment

(acceptance among peers, bullying) Environmental factors such as) are obstacles to the physical activity performance of children with CP [80]. In a study examining the physical activity levels of children with CP, children's activities were recorded using an accelerometer for 4 days. As a result, it was determined that only 25% of children met the recommended daily physical activity levels [64].

Evaluation of Physical Activity

In the results of the literature research on physical activity in children with autism, evaluation methods are basically grouped as questionnaires and accelerometers. Accelerometers are mostly used with 3 axes. Surveys that provide subjective evaluation are stated as the Physical Activity Survey for Children, Godin-Shephard Leisure Survey, activity diary or surveys based on daily activity reports created by the authors [69].

No comprehensive research has been found on the methods used to evaluate physical activity in children with Down syndrome. However, it has been determined that accelerometers are mostly used as an objective method [81, 82].

Methods to evaluate physical activity in mentally retarded children aged 5-18 years are summarized.

Accordingly, classification was made as objective and subjective methods. Direct observation methods (Activity Level Observation Program, Children's Physical Activity Form, etc.), accelerometers, pedometers, accelerometers and double-labeled water method are given as objective methods. Subjective methods were based on activity diaries, surveys and parent reports. [83].

A new tool has been developed to evaluate physical activity in children with learning disabilities due to communication-related problems. Important features of the Learning Disability Physical Activity Questionnaire are that it is easy to read, has visual content, and is based on self-report. It was found to be welcomed by the participants and was also important in promoting physical activity [84].

Psychometric properties of physical activity measurements used to evaluate the physical activity level of children with cerebral palsy were examined and summarized. Basically, pedometers, accelerometers, heart rate monitors, activity monitors, armbands with sensors (based on body temperature, galvanic skin response, and accelerometer data), and monitors that can track energy expenditure have been identified. Subjective methods were investigated, including the Physical Activity Questionnaire, questionnaires

based on the Physical Activity Summary, and multimedia activity recall questionnaires for children and adults. Questionnaires have been shown to be easy to use in the clinic. However, it was also found that the correlation of these surveys with step and accelerometers was weak. In addition, the reliability of accelerometers in children with CP has not been demonstrated. There is evidence that energy expenditure monitors, activity monitors, heart rate monitors, and the Physical Activity Questionnaire are reliable [85].

The accelerometer used in the objective evaluation of physical activity must be used for at least 10 hours a day. For appropriate evaluation in children and adolescents, it is recommended that follow-up be done between 4-9 days [86].

Promoting Physical Activity

World Health Organization guidelines encourage an average of 60 minutes of moderate-intensity physical activity per day, including for children with disabilities [87, 88].

A recent systematic review examined the impact of digital health applications on promoting physical activity in children with autism and intellectual disabilities. It has been observed that there are active video games, e-learning methods and social media-based interventions. Positive results have been reported for many of these interventions [89].

Physical activity has an important place in children with Down syndrome due to the high potential for weight gain [90]. Providing cycling training to children, increasing strength training during adolescence, encouraging participation in special Olympics, adapting sports programs, providing educational programs by physiotherapists including families, community programs and practices aimed at improving physical activity in schools are encouraging practices in terms of increasing physical activity [91].

It is generally reported that practices to encourage physical activity in children with attention deficit hyperactivity disorder are limited. For this purpose, studies have been carried out on cooperation through various organizations and revealing a common recipe. Further studies are needed to promote physical activity [92].

A systematic literature review in children with CP found that leisure-time physical activity procedures resulted in beneficial outcomes for musculoskeletal strength, cardiorespiratory fitness, quality of life, spasticity, participation, and physical function. It has been stated that the most effective interventions are exercise training, active video games, recreational activities, behavioral coaching and motor skill training [93].

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Chapter 11

Circumcision Complications

Feride Mehmetoğlu[1]

Abstract

Circumcision, a common surgical procedure characterized by the excision of the penile foreskin, traditionally integrates cultural, religious, and occasionally, medical contexts, continues to spark extensive discussion and research due to its spectrum of potential complications. Although generally considered safe, the complication rate varies between 2% and 10%. This study explores and categorizes the complications into common issues such as bleeding and infection; less common, yet notably impactful issues like injuries to the glans and urethra; and potential long-term psychological effects. A notable focus is directed toward procedural and post-operative aspects, examining different techniques like the Plastibell, Gomco clamp, and Alisclamp, each presenting varied complications and success rates. Furthermore, the exploration delves into specific cases, exemplifying potential catastrophic results like necrotizing fasciitis and significant urethral damage. Through a lens that balances clinical outcomes with ethical considerations, the discourse further ventures into the psychological and quality-of-life implications for affected individuals and their caregivers. This comprehensive analysis aims not only to highlight the physical and psychological risks associated with circumcision but also to catalyze a continual, multifaceted discussion among healthcare professionals to refine practice protocols, elevate patient safety standards, and examine the ethical contours enveloping non-medical circumcisions in pediatric populations.

Introduction

Circumcision is a surgical procedure involving the removal of the skin covering the tip of the penis. Often performed shortly after birth or during childhood, its reasons can range from religious and cultural rites to personal choice or medical necessity. Advocates highlight potential health benefits,

1 Pediatric Surgeon, Department of Pediatric Surgery, Dortcelik Children's Hospital, ORCID: 0000-0002-8387-5296

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such as a reduced risk of certain infections and conditions. On the other hand, concerns arise about the procedure's necessity, potential complications, and ethical considerations, especially when performed on minors without consent. Its prevalence varies worldwide, influenced by traditions, beliefs, and medical recommendations, making circumcision a subject continually explored in medical, ethical, and sociocultural discussions. Circumcision boasts an ancient history, remaining pivotal in diverse religious, cultural, and societal contexts. Archaeological discoveries from Egyptian tombs circa 4000 BC offer evidence of early circumcisions. The first documented instance hails from Egypt during the Sixth Dynasty, where artwork depicts the circumcision of young boys. Jewish traditions, tracing back to 1800 BC, dictate circumcision on the eighth day after birth, a ritual rooted in the Abrahamic covenant described in Genesis 17. Although not explicitly referenced in the Qur'an, circumcision is customary in Muslim societies, emulating the Sunnah of Prophet Muhammad; however, the age of circumcision varies widely within these communities. In parts of Africa, such as among the Xhosa tribe in South Africa, circumcision is deeply entwined with religious and cultural rites of passage. Meanwhile, certain Indigenous Australian communities historically practiced unique circumcision rites, including the sub-incision of the urethra, a procedure marked by its potential complications. Yet, this tradition, showcased in rock art at the in Kakadu National Park, underscores the profound cultural significance of circumcision practices throughout history (1-8).

Complications of the Circumcision

The complication rate of circumcision is between 2% and 10% when performed by experienced hands under sterile conditions. Circumcision, a surgical removal of the foreskin, can entail various complications. Issues like infection, excessive bleeding, and improper healing can occur. Sometimes, dissatisfaction with cosmetic outcomes arises. In rare instances, more serious complications like damage to the urethra or glans can happen, necessitating further medical intervention. Complications of circumcision can be summarized under the following headings (9-10):

1. Common Complications of Circumcision: Bleeding, Infection, Improper healing and scar formation
2. Less Common Complications: Injury to the glans, Urethral complications, Excessive or insufficient removal of the foreskin
3. Long-Term Complications: Post-circumcision strictures (narrowing), Regrowth of the circumcision skin, Loss or changes in sensitivity

4. Psychological Impacts

Common Complications of Circumcision

Bleeding is the most common complication following circumcision, occurring in approximately 1% of cases according to a large retrospective review (7-9). **Figure 1.** This bleeding may manifest along the skin edges between sutures or originate from a specific blood vessel, frequently at the frenulum. Attentive hemostasis during the procedure and sufficient skin edge compression during newborn circumcisions can prevent most incidents. However, blood clot displacement or cautery eschar dislodging can occur. Most post-circumcision bleeding is controllable with direct pressure. Rarely, wound exploration and suturing might be necessary. Hematologic evaluations are recommended only for patients who bleed persistently or significantly. A retrospective review of 48 patients from the Mayo Clinic Pediatric Hemophilia database, who possessed various coagulopathies and underwent circumcision, revealed 11 bleeding complications. Of these, three were severe, requiring red blood cell transfusions despite preoperative factor replacement. For hemophiliac patients requiring circumcision, preoperative and perioperative factor replacement is imperative. Fibrin glue has also proven effective in reducing the need for recombinant factor replacement without significantly impacting bleeding complications (11-14).

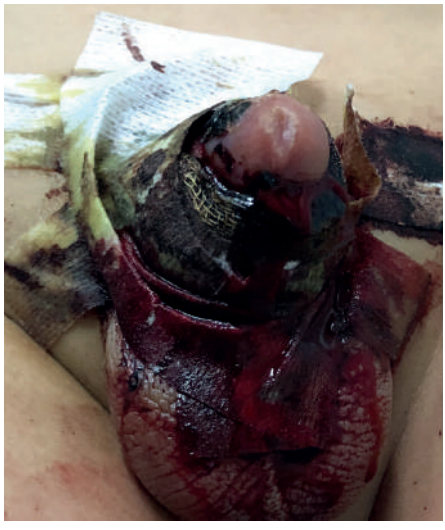

Figure 1 Bleeding after circumcision

Photograph Source: Feride Mehmetoğlu, Dortcelik Children's Hospital

Infections following circumcision are relatively rare thanks to the penis's robust dual blood supply. A study involving 5,521 circumcisions, comparing the Plastibell technique to the Gomco clamp, indicated a mere 23 (0.4%) infections, with the Plastibell group experiencing significantly more. All responded positively to topical and oral antibiotic treatments. The typical causative organisms are skin flora, but due to the diaper's unhygienic environment, colonic flora might also be implicated. Proper patient preparation, adhering to sanitary protocols, and diligent wound care, including cleaning and applying antibiotic ointment during diaper changes, can prevent most infections (15). The patients who underwent circumcision with Alisclamp also have several complications such as swelling, infection, edema and bleeding **(Figure 2-3).**

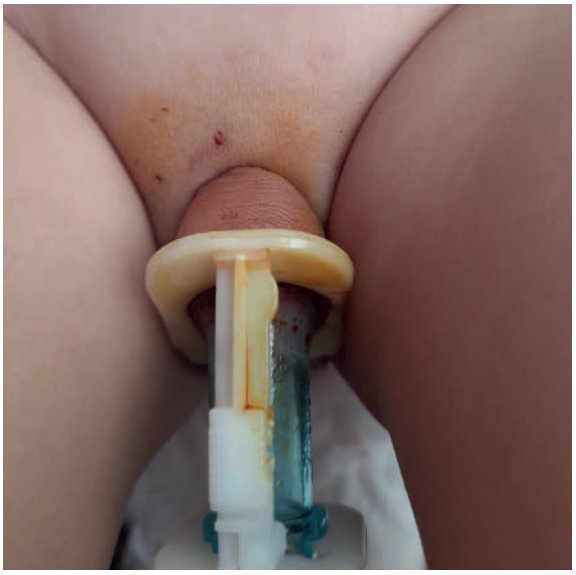

Figure 2: Circumcision with Alisclamp device

Photograph Source: Feride Mehmetoğlu, Dortcelik Children's Hospital

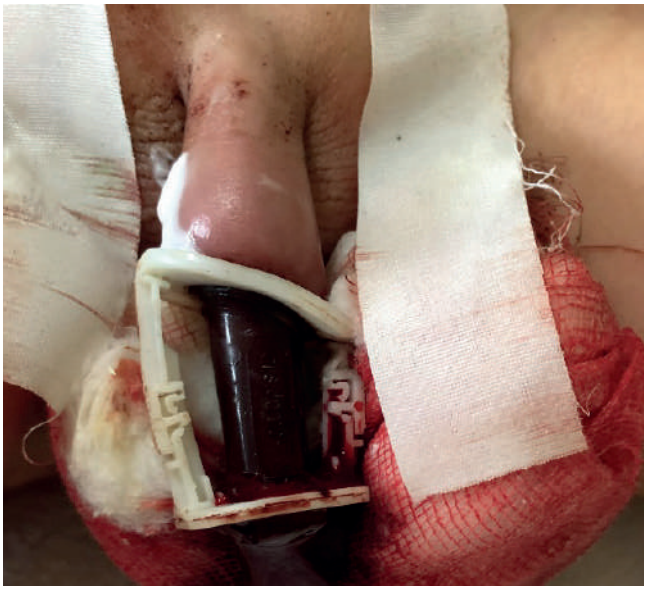

Figure 3: Bleeding after circumcision with Alisclamp device

Photograph Source: Feride Mehmetoğlu, Dortcelik Children's Hospital

However, severe infections, including necrotizing fasciitis, have been reported following Plastibell circumcisions. Symptoms like erythema, induration, pain, tachycardia, leucocytosis, or bandemia, especially when exceeding physical findings, have been noted. This polymicrobial infection, similar to adult cases, necessitates empiric broad-spectrum antibiotics to cover Gram-negative, Gram-positive, and anaerobic organisms. A recommended regimen might include an aminoglycoside, nafcillin, vancomycin, and clindamycin. Quick surgical assessment and assertive debridement of necrotic tissue are crucial (4-14-16).

Following neonatal circumcision, regardless of the utilized method, wound dehiscence and degloving injuries may arise. In instances of degloving, unintended entrapment and amputation of excess skin into the clamp can occur. There's also a less frequent risk of misjudging the amount of skin to remove during free-hand circumcision. Commonly, such injuries are addressed through diligent local wound care, enabling healing through secondary intention. Occasionally, reports highlight the success of autografting excised skin, achieving satisfactory cosmetic results. Ensuring meticulous technique and sharp focus during the procedure is crucial to mitigating these risks (8-16).

Less Common Complications

Glans injury during circumcision represents a distressing and noteworthy complication, with impacts that may transcend the immediate physical trauma, having potential psychological implications for both the individual and their caregivers. **Figure 4** This complication is especially disconcerting given that circumcision, often performed in neonatal stages, is intended to be a straightforward, low-risk surgical procedure. Glans injury can range from superficial damage to more grave injuries, which could, in severe instances, impact urinary function and sexual health in the long-term. Various factors, such as the surgical technique utilized, practitioner experience, and the anatomical specifics of the penis, may influence the risk of glans injury **Figure 5** (16-18).

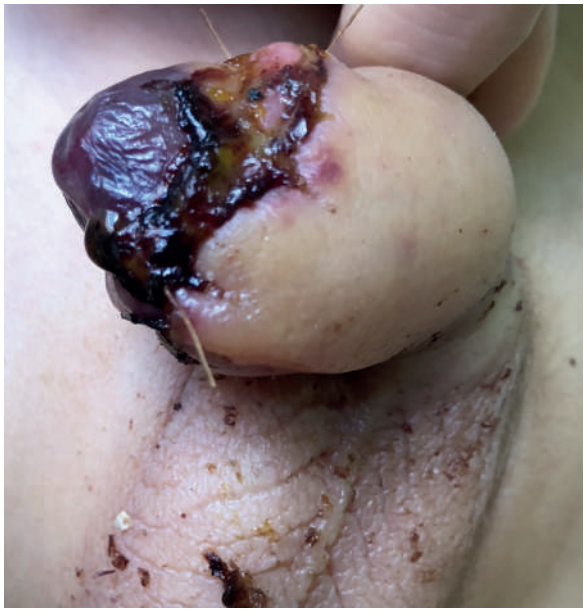

Figure 4 Glans injury during circumcision

Photograph Source: Feride Mehmetoğlu, Dortcelik Children's Hospital

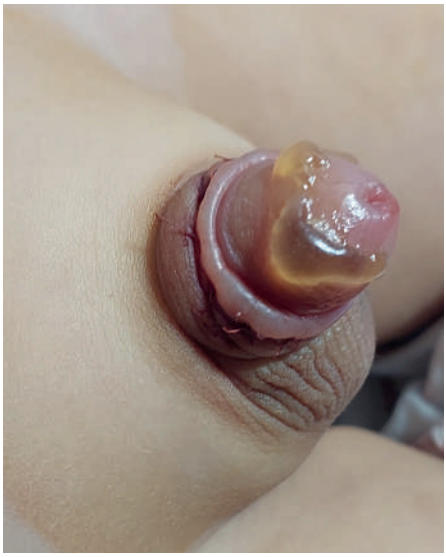

Figure 5 Bullous dermatitis of the glans after circumcision

Photograph Source: Feride Mehmetoğlu, Dortcelik Children's Hospital

Whether employing methods like the Plastibell, Mogen clamp, or Gomco clamp, or undertaking a freehand technique, the cruciality of safeguarding the glans during the procedure is unequivocal. Adequate training, thorough preoperative assessment, and judicious procedural care are imperative to mitigate risks. In the unfortunate event of glans injury, comprehensive management, encompassing immediate surgical intervention, potential long-term therapy, and psychological support, becomes vital. The scrutiny of this complication underscores the need for continuous evaluation and potential enhancement of existing circumcision protocols and practitioner training to safeguard against such traumatic eventualities. The emphasis must persistently linger on optimizing safety in this common procedure (14,15,17).

Urethral injury subsequent to circumcision warrants a profound exploration and critical analysis due to its potential to impart long-lasting ramifications on urinary function, sexual health, and overall quality of life. In a procedure, predominantly executed in neonates and often for non-medical, cultural, or religious reasons, the safety and minimization of complications should be paramount. Urethral injuries, though rare, are grave complications, embodying a spectrum from minor urethral meatus damage to complete urethral severance. These occurrences could be due to an assortment of factors including, but not limited to, the surgical technique,

practitioner expertise, adherence to safety protocols, and patient anatomy (14,15).

It's imperative to underscore the necessity of comprehensive preoperative planning and scrutiny of the patient's anatomy to mitigate the risk of urethral injuries. Selecting an appropriate technique, perhaps favoring those with a robust track record of safety, and ensuring the practitioner is well-versed and proficient in executing the procedure, are pivotal. The discourse around urethral injury also brings to light the question of the ethicality and appropriateness of performing non-medically indicated circumcisions, particularly in pediatric populations unable to provide informed consent (13-16).

In instances where urethral injury does occur, the management thereof is multifaceted, often necessitating immediate surgical repair and potentially, ongoing interventions to preserve urinary function. Not to be eclipsed by the physical repercussions, the psychosocial implications of urethral injury, encompassing potential self-esteem and body image issues, necessitate a compassionate and holistic approach to management, involving both physical and psychological therapeutic strategies (16-19).

This facet of post-circumcision complication demands continued research, enhanced practitioner training, and perhaps a revisitation of guidelines pertaining to circumcision. Constructive discourse and continual scientific inquiry into optimizing surgical techniques and post-operative care are essential to safeguarding the well-being of patients undergoing circumcision, ensuring that both their immediate and long-term health is uncompromised (14-19).

Long-Term Complications

Meatal stenosis, characterized by the narrowing of the urethral opening, is one notable adverse outcome. This condition can cause a myriad of urinary symptoms, including painful urination and a deflected urine stream. Its prevalence is believed to arise from the chronic irritation of the exposed meatus in a post-circumcision setting. **Figure 6** Another significant concern is the potential for insufficient or excessive foreskin removal, leading either to continued phimotic symptoms **Figure 7** or excessive exposure of the glans, respectively. Both scenarios can produce discomfort, aesthetic concerns, or functional problems during sexual activity. Scarring, though not universal, is another potential drawback. Acquired penile epidermoid cysts may present as an early or late complication after circumcision and hypospadias surgeries. (20) **Figure 8.** Poor cosmetic results and recurrent dermatitis are also common complications. **Figures 9-10**

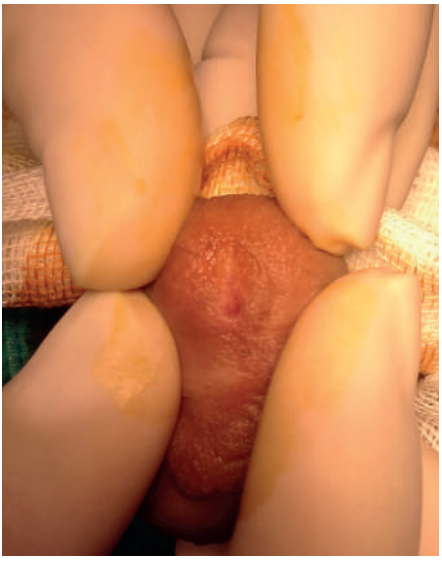

Figure 6: Meatal stenosis after circumcision

Photograph source: Dortcelik Children's Hospital, Feride Mehmetoğlu

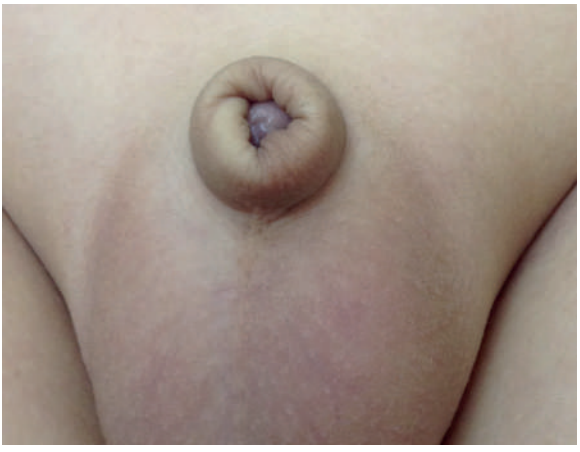

Figure 7: Secondary phimosis caused by insufficient removal of the preputium

Photograph source: Dortcelik Children's Hospital, Feride Mehmetoğlu

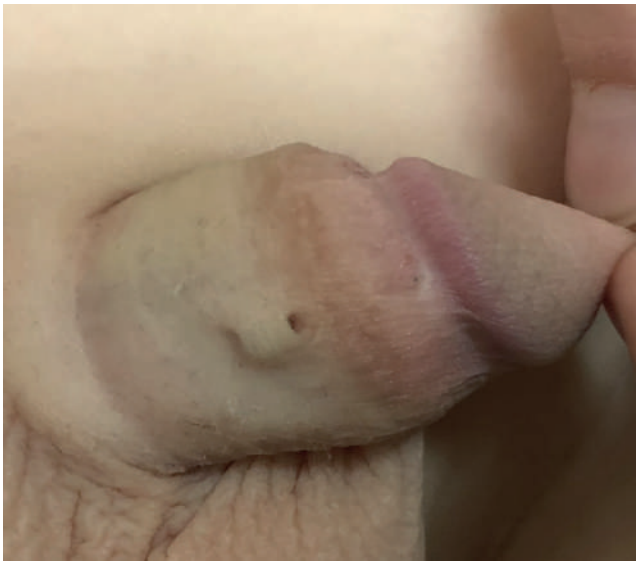

Figure 8 Skin bridge with inclusion cyst after circumcision

Photograph source: Dortcelik Children's Hospital, Feride Mehmetoğlu

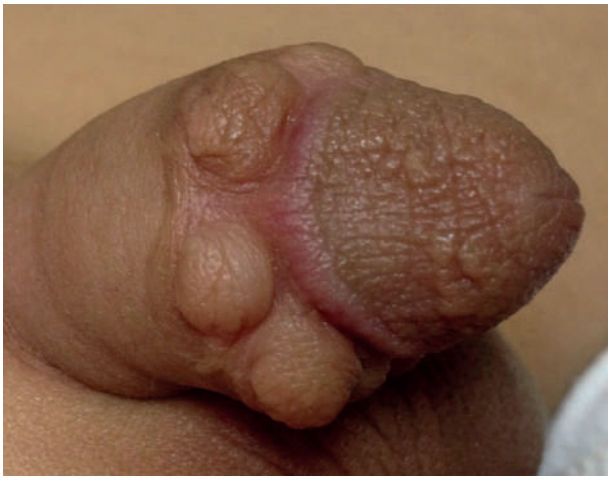

Figure 9 Poor cosmetic outcome presumed to be suture-related after circumcision

Photograph source: Dortcelik Children's Hospital, Feride Mehmetoğlu

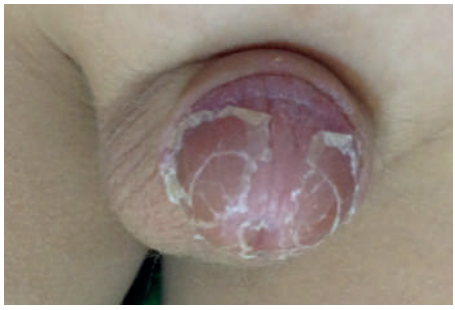

Figure 10 Healing after the development of glans dermatitis

Photograph source: Dortcelik Children's Hospital, Feride Mehmetoğlu

Depending on the healing process and technique used, some men experience cosmetically displeasing or functionally limiting scars (14,15,21-25). Moreover, there's the risk of altered penile sensitivity. While studies on this topic are varied, some men report reduced sensitivity, which they attribute to the loss of the protective foreskin and the keratinization of the exposed glans over time. Lastly, psychological and emotional repercussions cannot be discounted. Some men express feelings of loss, anger, or even resentment, particularly if the circumcision was performed in infancy without their consent. They may perceive their circumcision as a violation of bodily autonomy. While these feelings are subjective and not experienced by all circumcised males, they underscore the importance of informed decision-making processes. In conclusion, while circumcision offers specific health advantages, it's imperative that medical professionals and parents are cognizant of the potential long-term complications and weigh them against the benefits when considering the procedure (14,15,25).

Circumcision, a common surgical procedure involving the partial or complete removal of the prepuce (foreskin), has been practiced across cultures for diverse reasons, encompassing religious, cultural, and medical grounds. Nevertheless, an often-overlooked long-term complication might emerge when an extended length of the prepuce is left post-operatively: a condition occasionally referred to as redundant or excessive foreskin. The matter is not merely aesthetic; the implications may permeate various facets of an individual's life, spanning from psychological impacts to substantive medical complications.

When an extended prepuce is left, it may instigate issues related to hygiene, potentially amplifying the risk of infections, such as balanitis and urinary tract infections, especially in pediatric patients. Additionally, sexual function and satisfaction could be jeopardized due to the retained sensitivity or paradoxical desensitization of the glans. Psychologically, perceptions of normalcy and aesthetic concerns may also weigh heavily on individuals, potentially inducing anxiety or issues related to self-esteem and body image. Moreover, for parents who decide to circumcise their children due to cultural or religious practices, concerns regarding whether the procedure was performed 'correctly' or in alignment with cultural norms may emerge (18).

Medically, addressing this complication may necessitate further surgical interventions, which, aside from the inherent risks of any surgical procedure, might bring about additional psychological stress and financial burdens for the patient or their caregivers. As such, the healthcare community must engage in an encompassing dialogue concerning standardized post-circumcision outcomes and possible interventions to mitigate the impacts of an extended prepuce, ensuring the wellness of the individual across their lifespan (21).

It is paramount that adequate attention is paid not only to the immediate, short-term outcomes of circumcision but also to the lingering, long-term implications that may substantially affect an individual's quality of life. Consequently, forging an integrative approach that amalgamates surgical precision, cultural sensitivity, and a comprehensive understanding of long-term outcomes is imperative to circumvent the potential physical and psychological implications of an extended prepuce (14,15,25)

Psychological Impacts of Circumcision

Existing knowledge about male circumcision often hinges on accounts from individuals reaching out to organizations like the Circumcision Resource Center (CRC). Many of these men, circumcised as infants, report feelings of anger, loss, shame, and a sense of violation. Despite being unaware of their early-life circumcision, some correlate these negative emotions to the procedure. In societies where circumcision is less common, recognizing their altered state might lead to traumatic realizations for some circumcised individuals (26-28).

There are several reasons why circumcised men might not vocalize their feelings (26-30):

- Cultural beliefs about circumcision, often perceived as beneficial, deter introspection.

- The intense emotions linked to circumcision are distressing, prompting suppression.
- Fear of ridicule or rejection inhibits open expression.
- Early-life traumas, typically unconscious, manifest non-verbally, affecting attitudes and behaviors.

This internalized trauma might influence perceptions of masculinity. A prevalent fear among American men, potentially tied to circumcision, is the anxiety about penis size. This concern has been commercialized, with advertisements for enlargement methods prevalent. Interestingly, research suggests men might overemphasize the importance of size in attracting partners, though the influence of circumcision on this perception remains unclarified (28).

Negative feelings about the penis are intricately linked to body image, which encompasses judgments about the body's appearance to others and can deeply affect a man's day-to-day life. The notions of self-worth and body image are closely tied, influencing one's psychological well-being. A poor body image can hinder one's social and intimate connections. Individuals who have experienced bodily loss, such as through a mastectomy, often grapple with feelings of decreased attractiveness, desirability, and sexual satisfaction post-procedure. This diminished body image can also sap motivation, lower feelings of competence, power, and status, and even lead to depressive and suicidal tendencies (29).

Though the specific circumstances and age at the time of loss can vary, the emotional aftermath of feeling that a crucial part of one's body is absent is a shared experience among those who have undergone procedures like mastectomy and, for some, circumcision. This sensation, especially in the context of circumcision, can lead to profound distress, as the penis is traditionally associated with masculinity. An injury or alteration to this part can create not only a physical scar but also a psychological one, often manifesting in decreased self-esteem (30).

Over time, such symptoms might manifest as longer-term psychological repercussions. For instance, there's a potential connection between adult circumcision, decreased sensitivity, and impotence. Furthermore, infant circumcision, known to reduce sexual sensitivity, might play an overlooked role in the high rates of impotence observed among American men. In a study involving men between the ages of 40 and 70, over half reported varying degrees of impotence. This prevalence increased with age and was

linked to heightened levels of anger, depression, and decreased self-esteem (29-31).

Conclusions

Circumcision, while globally practiced, isn't without risks, and discussions surrounding it are often fraught with contrasting perspectives on cultural norms, ethics, and health benefits. Its complications range from common ones like bleeding and infection to rarer, severe ones like glans or urethral injuries. These physical complications not only present immediate health threats but also potential long-term psychological impacts. Preventive strategies, including meticulous technique and rigorous practitioner training, play pivotal roles in reducing risks. This practice demands ongoing scrutiny and debate, considering its ethical dimensions, particularly concerning non-medical circumcisions in minors. The alignment of cultural practices, medical ethics, and patient safety remains imperative, advocating a balanced approach that prioritizes the well-being of the individual above all.

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Chapter 12

Advanced Treatment Techniques In Radiotherapy

Ayse Gulbin Kavak[1]

Abstract

Radiotherapy is used to destroy cancer cells or control their growth. The main purpose of radiotherapy is to protect healthy organs while destroying the tumor. Ionization radiations such as x-rays, gamma rays and proton particles are used in radiation therapy. Nowadays, advanced treatment techniques are used when applying radiotherapy to cancer patients. Advanced treatment techniques have been developed to target cancer cells and protect healthy tissues, increase the effectiveness of radiotherapy and minimize side effects. Treatment techniques applied in radiotherapy largely depend on the development of technology. With the developments in computer technology, imaging methods have improved, and parallel to these, there have been developments in radiotherapy techniques. There are many advanced treatment techniques used in radiation therapy. The main ones are intensity-modulated radiation therapy, image-guided radiation therapy, stereotactic radiosurgery and stereotactic body radiation therapy, adaptive radiotherapy, brachytherapy, hypofractionation and proton therapy. Intensity modulated radiation therapy (IMRT) is a treatment technique that uses different beams of light to the targeted volume, destroying cancer cells while sparing surrounding tissues. Image guided radiation therapy (IGRT) uses real-time image guidance during treatment. Patient position and tumor movements are observed so that the target can be treated more accurately. Stereotactic Radiosurgery and Stereotactic Body Radiotherapy are basically based on treating small lesions by focusing on them with very high doses of radiation. Adaptive radiotherapy is a radiotherapy technique that takes into account the patient's anatomical and physiological changes during cancer treatment. This technique makes it possible to update the radiotherapy plan in response to changes in the patient's body and to provide a treatment that targets the radiation dose more precisely. Brachytherapy is a treatment technique that

1 Assistant Professor, Gaziantep University, Medical Faculty, Radiation Oncology Department, kavakgulbin@gmail.com, https://orcid.org/0000-0003-1995-1642

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involves placing radioactive sources directly or very closely into or near cancer cells. Hypofractionation involves giving higher doses to the patient in a shorter period of time compared to conventional radiotherapy fractionation. Proton therapy is a treatment that targets cancer cells using proton-charged particles instead of x-rays or gamma rays. Advanced treatment techniques in radiotherapy offer more options and higher success rates in cancer treatment. However, the type of cancer for each disease differs from each other, so it is very important to determine the most appropriate treatment approach for the patient.

Radiotherapy

Radiotherapy is a medical procedure used in the treatment of cancer. The goal of radiotherapy is to use high-energy radiation to eradicate cancer cells or control their growth. While targeting cancer cells for destruction, radiotherapy aims to minimize damage to the surrounding healthy tissues. The objectives of radiotherapy can be listed as follows: to destroy cancer cells or control their growth, reduce the tumor size, alleviate or eliminate cancer symptoms (Jaffray et al., 2007)

Radiation therapy is divided into external radiotherapy and internal radiotherapy:

In external radiotherapy, high-energy X-rays are applied from the outside to the tumoral area. High-energy radiation beams are generated by a machine for external radiotherapy and directed externally at the patient's body to target and treat cancerous cells or tumors (Jaffray et al., 2007).

In internal radiotherapy which is called brachytherapy , the radiation source is placed directly inside the tumor or body cavities (Ragde, 2004).

The process of radiotherapy starts with imaging. Firstly, a Computed Tomography (CT) images of the patient are obtained in DICOM format. The CT image is then transferred to the treatment planning system. The images transferred to the treatment planning system are fused with magnetic resonance imaging (MRI) and/or positron emission tomography (PET) images to contour the target volume. Precise planning is essential for the effectiveness and safety of radiotherapy. In treatment planning, the aim is to deliver the desired dose to the tumor while protecting the surrounding healthy organs. The radiation dose and treatment duration to be administered to the patient are determined based on the type of cancer and the stage of the disease. Patient treatment planning in radiotherapy is done by selecting the appropriate device, energy, and treatment techniques for the tumor. The most current treatment techniques applied to patients are described in detail below (Schneider et al., 1996).

1. Intensity Modulated Radiation Therapy (IMRT)

Intensity-Modulated Radiation Therapy (IMRT) is one of the significant developments in the field of radiation oncology. IMRT aims to deliver radiation to the tumor with precision, minimizing damage to the surrounding healthy tissues. One of the primary goals of radiation therapy is to minimize the damage to healthy tissues while delivering a therapeutic dose of radiation to the tumor. IMRT represents a paradigm shift in radiation oncology treatment, allowing different dose distributions to different target volumes. The origins of IMRT can be traced back to three-dimensional conformal radiation therapy, which aimed to improve the accuracy of radiation delivery (Ezzell et al., 2003). While the concept of modulating radiation intensity was proposed in the 1980s, it was the development of computer technology and advanced treatment planning systems that made clinical IMRT possible. The first clinical IMRT treatments began in the early 1990s and marked a significant milestone in radiation therapy (Jaffray et al., 2007).

IMRT is based on inverse treatment planning, where the desired dose distribution is determined, and the treatment planning system calculates the optimal intensity pattern for each beam to achieve this distribution. This approach is in contrast to conventional radiation therapy, which relies on forward planning (Jaffray et al., 2007).

IMRT divides each treatment field into multiple beams, each with different intensities. By modulating these beams according to intensity, an optimal dose distribution is achieved that conforms to the shape of the tumor. The accuracy of the dose delivered to the patient is just as important as the planning of IMRT. When planning in IMRT, a reverse optimization plan is made by entering the objectives and priority values for the target volume and critical organs into the system. Additionally, an IMRT plan can be made by using the multicriteria optimization algorithm found in some treatment planning systems. (Kavak et al. 2022). Multileaf collimators (MLCs) are lead plates composed of a series of thin leaves located inside the treatment device head. IMRT heavily relies on MLCs, which can shape the radiation field during treatment. This technology allows precise and rapid modulation of radiation intensity, enhancing dose conformity. With the development of MLCs, Volumetric Modulated Arc Therapy (VMAT) has also become applicable. It is a more advanced treatment technique than IMRT. VMAT treatments can be delivered accurately and effectively with single or multiple arcs. In Volumetric Modulated Arc Therapy, unlike IMRT, VMAT delivery is more complex than IMRT because the gantry rotation speed, dose rate and MLC shape are constantly changing at the same time. The primary

advantage of Volumetric Modulated Arc Therapy is that it can treat the patient much faster than fixed gantry IMRT. (Surucu et al., 2012). Before starting IMRT, a patient-specific quality control test is required. Once these quality control tests meet the eligibility criteria, IMRT or VMAT treatment can be approved. IMRT is generally applied to cancer types such as head and neck cancer, prostate cancer, and brain tumors, among others. IMRT is one of the methods that maximize therapeutic effect in cancer treatment (Jaffray et al., 2007).

2. Image-Guided Radiation Therapy

Image-Guided Radiation Therapy (IGRT) represents a revolutionary approach in the implementation of radiation therapy, offering a dynamic and adaptable approach to cancer treatment. IGRT is a significant advancement in the field of radiation oncology and plays a central role in providing precise and personalized radiation therapy while minimizing the impact on healthy tissues (Xing et al., 2006).

The roots of IGRT may date back to the 1990s when it emerged as an extension of traditional radiation therapy. The development of IGRT has progressed in parallel with advances in medical imaging that enable the integration of real-time imaging and treatment delivery (Simpson et al., 2010).

The fundamental principle of IGRT is precise target localization. IGRT ensures that the tumor or target volume is accurately positioned and monitored in real-time during radiation delivery, not only during the treatment planning phase. This allows for adjustments to be made based on the target's position, shape, and size at any given moment. IGRT enables adaptable treatment planning that can accommodate changes in the patient's anatomy or tumor response during treatment, making it particularly valuable in managing anatomical changes caused by factors such as weight loss, tumor regression, or organ motion (Bissonnette et al., 2012).

With technological advancements, IGRT makes use of various imaging techniques such as cone-beam computed tomography (CBCT), positron emission tomography (PET), magnetic resonance imaging (MRI), and ultrasound (Simpson et al., 2010).

IGRT has a wide range of applications in clinical practice for various cancer types, including prostate cancer, lung cancer, head and neck cancer, gynecological cancers, brain tumors, and pediatric tumors (Perkins et al., 2006). The ability to precisely target tumors in IGRT enhances the effectiveness of this treatment method. Ensuring the accuracy and safety

of IGRT is crucial. Regular equipment calibration, image registration, and staff training are essential. Quality assurance measures are also important for minimizing errors and ensuring patient safety. IGRT enables personalized treatment plans, advanced patient comfort, and convenience (Bissonnette et al., 2012).

3. Stereotactic Radiosurgery (SRS) and Stereotactic Body Radiation Therapy (SBRT)

Stereotactic radiosurgery (SRS) is a radiation therapy used to treat functional abnormalities and small tumors in the brain. In SRS treatment, a higher dose of radiation is applied in fewer fractions compared to conventional treatment, which helps better preserve healthy tissue (Combs et al., 2005). When used to treat body tumors, it is referred to as stereotactic body radiation therapy (SBRT) (Okunieff et al., 2006). SRS and SBRT can be considered groundbreaking treatment methods in radiation oncology. These concepts emerged in the early 20th century with the development of stereotactic frames for brain surgery (Combs et al., 2005). However, their application in radiation therapy began in the 1980s and has rapidly advanced since then. Technological advancements and clinical evidence have facilitated their adoption.

The main principles of SRS and SBRT are high precision and conformity (Schefter et al., 2005). They deliver high doses of radiation to the target with sub-millimeter accuracy while minimizing exposure to surrounding healthy tissues. This is achieved through the best targeting and immobilization techniques of the tumor. In SRS, a typical treatment involves delivering a single high dose in a single session, while the SBRT concept allows for the treatment of larger and more irregularly shaped tumors by extending it to several or more fractions (Vergalasova et al., 2019).

SRS and SBRT heavily rely on advanced imaging methods such as Cone Beam Computed Tomography (CBCT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET) for real-time guidance in treatment planning and delivery.

SRS and SBRT are applied to conditions such as brain metastases, primary brain tumors, lung cancer, spine and bone metastases, liver tumors, and prostate cancers. Precise dose optimization algorithms are used for SRS and SBRT to ensure the target receives a therapeutic dose while minimizing normal tissue toxicity (Samlowski et al., 2007). Robust quality assurance measures, including device calibration, image registration, and personnel training, are necessary to guarantee the safety and accuracy of SRS and

SBRT treatments. Studies have shown high local control rates, reduced side effects, improved quality of life, and increased survival rates with SRS and SBRT. With ongoing technological advancements and expanding clinical indications, SRS and SBRT will continue to play an increasingly prominent role in modern oncology (Okunieff et al., 2006).

4. Adaptive Radiation Therapy

In the constantly evolving field of radiation oncology, Adaptive Radiation Therapy (ART) has emerged as a significant advancement. ART represents a dynamic, patient-centric approach to cancer treatment that harnesses the power of precise medicine and technology to minimize potential harm while maximizing therapeutic efficacy. It is an innovative approach to delivering radiation therapy to cancer patients. Traditional radiation therapy methods often rely on a one-dimensional approach where treatment plans are designed based on initial imaging and clinical evaluations (Sonke et al., 2019). However, tumors are not static and can change in size, shape, and location during the course of treatment. This variability poses a significant challenge in ensuring that the intended radiation therapy reaches its target while sparing healthy tissues (Sonke et al., 2019).

ART is designed to address this challenge by adapting the radiation treatment plan to these changes. It integrates advanced imaging techniques like Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET) into the radiation therapy process. In ART, continuous monitoring of the tumor's response to treatment and real-time adjustments when needed are essential. ART often utilizes intensity-modulated radiation therapy (IMRT) or volumetric modulated arc therapy (VMAT) to further customize the radiation dose distribution (Aydogan et al., 2011). ART's versatility allows for the implementation of various plans in cancer treatment (Castelli et al., 2018).

One of the key applications of ART is monitoring tumor regression and adapting to it. As radiation therapy progresses, tumors can shrink or change shape. By acquiring new patient images, treatment plans are adjusted to ensure that the tumor remains within the radiation field, allowing the therapeutic dose to be delivered to the tumor while minimizing damage to surrounding healthy tissues (Roth et al., 2020). Conversely, for tumors exhibiting growth or changes in size, ART is necessary to continue treatment. Detecting these changes early allows for the readjustment of the treatment plan to encompass the tumor volume and prevent low doses within the tumor.

Another critical aspect of ART is its ability to protect at-risk organs. Continuous monitoring allows for adjustments to the treatment plan when critical structures near the tumor are at risk of receiving excessive radiation. This feature improves the protection of critical organs like the spinal cord, brainstem, heart, and lungs when they are in close proximity to the tumor (Sonke et al., 2019).

ART also helps optimize fractionation schedules. In some cases, treatment may need adjustments based on the tumor's response. For instance, if a tumor responds well to radiation therapy, the treatment plan can be adjusted to shorten the treatment duration, thereby reducing the patient's overall radiation exposure. ART is one way to minimize the risk of complications arising from re-irradiation for patients who have previously undergone radiation therapy and require retreatment. By avoiding previously irradiated areas, the treatment plan can be precisely adapted to enhance the feasibility of re-treatment (Keall et al., n.d.).

Due to the dynamic nature of ART, it provides increased treatment accuracy and effectiveness by aligning with changes in tumor position and size. It minimizes exposure of healthy tissues to radiation, thereby reducing treatment-related side effects and improving the patient's quality of life. The dynamic nature of ART can be attributed to better tumor control and increased likelihood of successful treatment outcomes (Keall et al., n.d.).

While ART represents a revolutionary approach, it comes with its challenges and considerations. Implementing ART requires a skilled technical team and technical expertise. The application of ART relies on advanced imaging and specialized treatment planning techniques. Continuous monitoring and plan adaptation can be time-consuming in terms of both time and equipment. ART is not suitable for every patient and cancer type, as its application depends on individual circumstances and the feasibility of real-time adaptation. To effectively harness the full potential of ART, ongoing research and education are essential. As technology continues to advance, ART will become even more precise and widely accessible. Artificial intelligence and machine learning are likely to play a significant role in automating and facilitating the adaptation process. The future of ART holds promises for further improving cancer treatment outcomes, reducing side effects, and enhancing the patient experience.

In conclusion, ART represents a monumental shift in the approach to cancer treatment. By acknowledging the dynamic nature of tumors, it adapts treatment plans accordingly. With numerous benefits such as increased

precision, reduced side effects, and improved tumor control, ART is at the forefront of modern radiation oncology (Keall et al., n.d.).

5. BRACHYTHERAPY

Brachytherapy, commonly referred to as "internal radiation therapy," is a highly effective and localized approach in the treatment of various cancer types. This treatment technique involves the direct placement of sealed radioactive sources into or near the tumor, allowing for precise radiation delivery while minimizing exposure to surrounding healthy tissues. Brachytherapy has a rich history that spans over a century, with its roots tracing back to the pioneering work of early physicists like Pierre and Marie Curie, who discovered radioactive elements radium and polonium (Chargari et al., 2019). These discoveries laid the foundation for the development of brachytherapy techniques, with the first clinical applications reported in the early 20th century. Since then, significant advancements have been made in brachytherapy (Chargari et al., 2019).

Brachytherapy is a treatment technique that utilizes various radioactive sources. The selection of appropriate sources depends on the type, size, and location of the tumor. Precise dosimetry is crucial in brachytherapy to ensure the safe and effective delivery of radiation. The application of brachytherapy involves special equipment and procedures, including applicators, catheters, and after loaders (Nag et al., 2000).

Brachytherapy is categorized as interstitial, intracavitary, and surface brachytherapy. Interstitial brachytherapy involves the direct placement of radioactive sources into or near the tumor. Examples include prostate implants and breast cancer. Intracavitary brachytherapy entails the placement of radioactive sources into natural body cavities such as the cervix or esophagus. Surface brachytherapy is used to treat skin cancers and other superficial lesions (Ragde, 2004).

Brachytherapy plays a significant role in the treatment of gynecological cancers, including cervical, uterine, and vaginal cancers. It can also be considered as an alternative method for delivering radiation to the entire breast in breast cancer patients. Over the years, brachytherapy has evolved into a precise, effective, and well-tolerated cancer treatment method. Ongoing research and technological innovations continue to offer hope, promising better outcomes and quality of life for cancer patients. The potential for brachytherapy to play a more important role in cancer treatment in the future is both exciting and promising (Chargari et al., 2019).

6. Hypofractionation

Radiation therapy has become the cornerstone of cancer treatment, applied before or after surgery, in conjunction with chemotherapy. Radiation therapy provides an effective tool to target and destroy cancer cells. Conventional radiation therapy programs typically involve giving conventional doses of radiation therapy 5 days per week. However, the scope of radiation therapy is evolving and a new approach called hypofractionation is gaining importance (Jones et al., 2000).

Hypofractionation is a departure from traditional radiation therapy techniques. Hypofractionation involves giving larger doses of radiation in fewer treatment sessions, rather than giving smaller doses of radiation over a long period of time. This approach offers several advantages for both patients. The most important of these is the shortening of treatment time in terms of patient comfort. With traditional radiation therapy, patients often need to visit the treatment center for several weeks; This can be financially and spiritually difficult and disrupt their daily lives (Jones et al., 2000). Hypofractionation, on the other hand, allows for a significantly shorter treatment period. Patients can complete their treatment within a few days or weeks, reducing the time and effort required for travel and treatment. Shorter treatment programs mean fewer clinic visits and reduce the financial burden on both patients and healthcare systems (Hunter et al., 2018). Hypofractionation is therefore critical in the context of rising healthcare costs and limited resources. Hypofractionation not only reduces the number of treatment sessions but also eases transportation difficulties for patients (Hunter et al., 2018). This is especially helpful for people who live far from the treatment center. The intensified treatment program of hypofractionation generally results in fewer side effects and a more comfortable experience for patients. This can be said to have a positive impact on the overall quality of life during and after radiation therapy (Jones et al., 2000).

Hypofractionation is a versatile technique that can be applied to various types of cancer, including breast, prostate, lung and brain cancer. Its effectiveness is supported by extensive clinical research showing that larger radiation doses delivered in fewer fractions can provide the same level of tumor control as conventional fractionation (Hegemann et al., 2014).

Hypofractionation has begun to be used very frequently in breast cancer treatment. Clinical experience has shown that administering higher doses in fewer fractions after breast-conserving surgery is not only safe but can also provide equivalent or even better results compared to conventional radiation therapy (Hickey et al., 2016).

Hypofractionated radiation therapy has become widely used in the treatment of prostate cancer. It offers a balance between effective cancer control and patient convenience. Research shows that this approach can be as effective as traditional radiation therapy, with the advantage of shorter treatment time (Hegemann et al., 2014).

Hypofractionation is advantageous for lung and brain cancer patients. These cancers are often found in areas close to critical organs. Delivering higher radiation doses in fewer sessions provides better tumor control and minimizes damage to surrounding healthy tissues (Hegemann et al., 2014).

Although hypofractionation has its benefits, it also has its challenges and considerations. Careful patient selection, advanced imaging techniques, and precise treatment planning are crucial to maximize the benefits of this approach while minimizing the risk of side effects (Haffty, 2010).

In spite of hypofractionation is an exciting development in the field of radiation therapy, offering patients a shorter and more cost-effective treatment option, it should be applied with caution due to the high dose delivered. As technology continues to advance, radiation therapy will become more precise and personalized, leading to better outcomes for cancer patients. Hypofractionation represents a significant advance that has the potential to improve the lives of countless cancer patients worldwide. In the future, hypofractionation techniques will continue to advance and improve (Jones et al., 2000).

7. Proton Therapy

In recent years, advances in medical technology have revolutionized the field of cancer treatment. The most important of these is proton therapy, a cutting-edge method that offers significant advantages over traditional radiation therapy. Proton therapy uses protons, positively charged particles, to target cancer cells with extraordinary precision (Bussière & Adams, 2003).

Proton therapy is a form of radiation therapy that uses charged particles, protons, to treat cancer. Unlike traditional X-ray or photon therapy, which uses high-energy photons, proton therapy uses protons that can be precisely controlled in terms of their speed and energy. This makes it possible to deliver the radiation dose more accurately, minimizing damage to healthy tissues and vital organs surrounding the tumor (Sánchez-Parcerisa et al., 2014).

The Particle Acceleration process begins with a particle accelerator, which produces protons and accelerates them to the desired energy level. Protons are then directed into the treatment chamber through an array of magnets.

One of the most important advantages of proton therapy is the precise control of the energy of protons. This allows protons to adjust the range and depth of penetration into the body, allowing protons to deliver the maximum radiation dose precisely into the tumor, sparing healthy tissues (Bussière & Adams, 2003).

Before treatment begins, detailed imaging such as CT scans and MRIs are used to create a treatment plan that outlines the exact location of the tumor and the path the protons will take. The medical team creates a treatment plan to configure the accelerator and magnets for each patient's unique situation. After the treatment plan is created, protons are directed to the tumor in a highly controlled manner. This minimizes radiation exposure to surrounding healthy tissue and critical structures.

Proton therapy has shown promising results in various types of cancer, especially in cases where preserving surrounding healthy tissue is crucial. Some notable applications include:

Pediatric Cancer: Children's developing bodies are very pediatrically sensitive to radiation, making proton therapy an excellent option for cancer cases. It minimizes the risk of long-term side effects and secondary cancer (Roth et al., 2020).

Brain Tumors: Proton therapy is highly effective in treating brain tumors because it allows precise targeting near critical structures such as the optic nerves and brainstem (Skaarup et al., 2021).

Prostate Cancer: Proton therapy has become a popular choice for treating prostate cancer due to its ability to protect the rectum and bladder from radiation exposure (Skaarup et al., 2021).

Head and Neck Cancers: Cancers in the head and neck area can be difficult to treat without damaging important structures such as salivary glands and vocal cords. Proton therapy reduces secondary damage. It reduces the side effects of Proton Therapy. Proton therapy minimizes radiation exposure to healthy tissues, often leading to reduced short-term side effects such as nausea and skin irritation. Proton therapy is associated with a reduced risk of long-term complications and improves patients' quality of life after treatment. The accuracy of proton therapy results in a higher dose of radiation being delivered to the tumor, potentially increasing the effectiveness of the treatment. It may reduce the risk of secondary cancers caused by radiation therapy because healthy tissues are exposed to less radiation (Skaarup et al., 2021).

Proton therapy is a remarkable advance in cancer treatment; it offers better sensitivity and less collateral damage compared to traditional radiation therapy. As technology continues to advance, it holds great promise for a broader range of cancer types and patients. The field of proton therapy is still evolving, and ongoing research and development are expected to further advance this innovative approach to cancer care (Vanderwaeren et al., 2021).

8. Radiomics and Artificial Intelligence (AI) in Radiation Therapy

Radiation therapy plays a crucial role in treating cancer patients, allowing precise targeting of tumors while sparing healthy tissues. The integration of radiomics and artificial intelligence (AI) into radiation therapy has led to a transformative paradigm shift in the field. Radiomics is an emerging field in medical imaging that focuses on the extraction of high-dimensional data from radiological images (Lohmann et al., 2020). These data include not only standard anatomical information but also textural and quantitative features that describe the heterogeneity, shape, and spatial distribution of tumor and healthy tissues. Radiomics can provide a basis for more precise treatment planning and response assessment by extracting valuable information normally imperceptible to the human eye. The role of radiomics in Radiation Therapy is important. Radiomics offers the ability to predict a tumor's response to radiation therapy. By analyzing pretreatment imaging data, radiomics features can provide insights into tumor behavior and help radiation oncologists tailor treatment plans to the specific characteristics of the tumor. Radiomics during and after radiation therapy can help evaluate response to treatment. By measuring changes in tumor tissue and shape over time, radiomics analysis can provide early indicators of the effectiveness of treatment or the need for treatment modification (Arimura et al., 2019).

Artificial intelligence, especially machine learning and deep learning, is beginning to become an important component of radiation therapy. Artificial intelligence algorithms can aid in treatment planning, decision-making, and real-time adaptation by processing and analyzing large amounts of radiomics data (Fu et al., 2022). AI algorithms can optimize radiation treatment plans by considering radiomics data to determine ideal beam angles, dose distribution, and fractionation schedules. This leads to more effective tumor control and reduced damage to surrounding healthy tissues. The automation of contouring and segmentation tasks in radiation therapy has been greatly improved thanks to artificial intelligence. Significantly reduces the time and variability associated with manual identification of structures, increasing efficiency and consistency in the treatment planning process. AI-powered adaptive radiation therapy allows real-time adaptation of treatment plans

based on daily imaging. This ensures that treatment remains accurate even if there are anatomical changes in the patient between sessions (Lohmann et al., 2020).

Although radiomics and artificial intelligence have great potential in radiation therapy, they present some challenges. In artificial intelligence, data quality and standardization are important. High-quality and standardized imaging data is required for radiomics and AI analyses. Variability in imaging protocols and equipment can pose challenges to accurate and reproducible results. Translating radiomics and AI tools from research to clinical application requires rigorous validation and integration with existing treatment protocols. Patient data privacy, algorithm transparency, and regulatory approvals are critical issues that must be addressed as AI technologies are incorporated into clinical practice (Fu et al., 2022).

The synergy between radiomics and artificial intelligence in radiation therapy is an evolving field with ongoing research and development. In the future, AI may be able to provide personalized treatment planning based on radiomics and genomic data. It can perform patient-specific treatment adaptation using real-time imaging and artificial intelligence. It may integrate with other methods, such as genomics, for a comprehensive understanding of cancer behavior (Arabi & Zaidi, 2020).

Ultimately, radiomics and artificial intelligence have the potential to revolutionize radiation therapy by providing more precise and personalized treatment options. As technology continues to advance and research progresses, the integration of these tools into clinical practice will improve patient outcomes and reinforce the essential role of radiation therapy in cancer treatment (Huynh et al., 2020).

These advanced treatment techniques in radiotherapy aim to optimize the balance between tumor control and healthy tissue preservation, reducing side effects of treatment and improving overall treatment outcomes. The choice of technique depends on the specific cancer type, stage, location and patient characteristics and is determined by a multidisciplinary approach including radiation oncologists, medical physicists and dosimetrists (Lohmann et al., 2020).

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Chapter 13

Evaluation of Eating Disorder Within the Scope of Addiction Concept

Merve Gördağ[1]

İ. İlknur Uysal[2]

Abstract

Nutrition is the consumption of adequate amounts of food necessary for the body to sustain life. Nutrition is an emotional as well as a physical need and this has led to the creation of the concept of emotional eating. This form of eating, which emerged out of physical need, gave birth to eating disorders and the related concept of addiction. Eating disorders were not a topic that was emphasized much until the 20th century and were categorized and collected in the publication of DSM-5. Although eating addiction is not included in the DSM as a diagnostic criterion, it is widely used as a concept. Current research supports the concept of eating addiction and research is still ongoing.

1. GENERAL INFORMATION

1.1. Eating and Nutrition

The food substances that are essential for our bodies are called nutrients. Our main nutrients are carbohydrates, proteins, fats, minerals and vitamins. These nutrients are divided into macro and micro nutrients. Macro nutrients (carbohydrates, fats, proteins) provide energy, while micro nutrients (vitamins and minerals) contribute to and help the regulation of the events in the body. In order for the body to benefit from these nutrients, they must be consumed in sufficient amounts, digested and utilized by the cells (Aksoy, 2008).

1 Uzm. Psk., Necmettin Erbakan University, Faculty of Medicine, Department of Neuroscience, Konya, Orcid: 0000-0001-5487-4887, E-mail: merveatceken@yahoo.com

2 Prof. Dr., Necmettin Erbakan University, Faculty of Medicine, Department of Anatomy, Konya, Orcid: 0000-0003-2939-9767, E-mail: uysali@yahoo.com

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In the following years, researches concluded that a balanced diet is necessary for health and the amount to be consumed should be moderate, which led to the birth of the concept of nutritional guidance. Accordingly, nutrients were researched and analyzed, and standards showing the amount of nutrients that should be consumed daily were established. The values included in the nutritional guidelines, which vary according to countries, were prepared under the leadership of Hacettepe University in 2002 in Turkey and were made available under the name of "Turkey Specific Nutrition Guidelines" by the Ministry of Health in 2004. According to this guide, food s are divided into 4 groups (Milk Group / Meat Group / Vegetable and Fruit Group / Bread and

Grains Group) and foods from all four groups should be available at every meal in order to have an adequate, healthy and balanced diet. While consumption below the recommended amount impairs health in growth and development, consumption above the recommended amount causes chronic diseases and obesity (Merdol, 2015).

Nutrition is the consumption of the required amount of nutrients needed for the continuation of life in good health. Nutrition is a compulsory need for our life and it should start from the womb for our immunity (Baysal A, 2004).

Nutrition is important for physical and sensory needs. Because health in the physical sense depends on meeting the biochemical needs of tissues and cells in the body with important nutrients obtained from food. The concept of health in the sensory sense is the understanding that food affects the mental and physical structure of people (Aksoy, 2008).

Adequate and balanced nutrition is one of the basic and most important elements for both the society and the individuals who make up the society to live in a healthy and strong way, to develop economically and socially, to increase the level of welfare, and to survive in peace and security (Sevim & Güldemir, 2019).

In prehistoric times, nutrition was limited to filling the stomach, but with the transition to settled life, table setting and an increase in food varieties emerged. As different foods started to be used together, the concept of "flavor" came to the fore. In 400 BC, Hippocrates emphasized the relationship between nutrition and health and stated that healing could be achieved with food and that medication should not be used when healing could be achieved with food. This sentence has passed into the international literature as *"let food be your medicine and medicine be your food"* (Merdol, 2015).

1.2. Emotional Eating

Psychological characteristics of the person affect eating attitudes emotionally or cognitively. This is seen not only in overweight or obese individuals but also in individuals with ideal weight (Özkan & Bilici, 2018).

The concept of emotional eating is defined as an eating disorder developed in response to the emotions felt. All positive and negative emotions can affect this behavior (İnalkaç &

Arslantaş, 2018). It is seen that people who have negative emotions and have difficulty overcoming this emotion consume more food than necessary in order to use the comforting effect of food (Macth, 2008). While this concept was initially associated with the factor that causes bulimic patients to engage in binge eating behavior, later research has discovered its relationship with binge eating attacks. It has been found that this overeating behavior in response to negative emotions is seen in obese individuals, overweight individuals who are on a diet, and women with eating disorders. Individuals who tend to be thinner due to external factors tend to diet strictly and may exhibit emotional eating behavior in order to combat the resulting negative emotions. Emotional eating becomes more important in these groups showing restriction behavior related to eating (Sevinçer and Konuk 2013).

The characteristics that distinguish emotional eating from hunger are; emotional eating desire comes suddenly, especially high-calorie foods are preferred, it is difficult to reach the feeling of satiety, and as a result of eating, regret and guilt occur. While satisfying foods are preferred in normal hunger, hunger develops slowly and guilt is not felt as a result of satiety (Gürdöl, 2018).

Emotional eating behavior has started to be the subject of new research and has not yet been included as a diagnostic criterion in DSM-5. However, it is thought to be considered as a form of bulimia nervosa (BN) and will be effective in the treatment of eating disorders by taking place as a different diagnosis with its inclusion in new research (İnalkaç & Arslantaş, 2018). It can lead to more advanced eating addiction or binge eating disorder. It is a fact that many morbid obesity patients have emotional eating habits (Ünal 2018).

In a study conducted with female patients diagnosed with restrictive Anorexia Neurosis (AN) and Bulimia Neurosis (BN) for emotional eating behavior, it was determined that mood affects eating behavior and these patients have deficiencies in emotion regulation. According to the results of the study, individuals with both disorders reported that their moods were

negative and that they used dysfunctional emotion regulation methods. In addition, BN patients exhibited more eating behaviors in case of unhappiness, while AN patients ate less than normal. In cases where they felt happy, BN patients reported less eating behavior than normal, while AN patients reported eating more than normal (Adrian et al., 2021). Considering all these results, we can say that mood is effective on eating, and the eating behaviors of patients diagnosed with eating disorders are affected by mood.

In order to control emotional eating behavior, the factors that cause overeating should be identified and people should be asked to keep an eating diary if necessary. In the next step, a therapeutic activity (such as sports, massage, music and warm showers) should be substituted in order to relieve the emotional state/feeling of emptiness that triggers eating. In addition, the biochemical aspect of eating should be examined to see whether the centers related to appetite are transmitting the correct message (Gürdöl, 2018).

2. EATING DISORDERS

Eating disorders are the occurrence of physical and psychosocial deterioration in a person's eating habits, appearance and thoughts about the amount of weight. Wrong thoughts that occur in the person affect eating behavior (Merdol, 2015).

Eating and feeding disorders are a group of psychiatric disorders with a chronic course caused by the interaction of many different factors. It also develops comorbidity with disorders such as depression, anxiety disorders and obsessive-compulsive disorders, which may also complicate the treatment process. For this reason, there is no single treatment method and a multidisciplinary team work is needed. Since it can be detected in line with the information provided by people, a smaller amount of diseases can be detected than the actual rate (Yılmaz, 2019).

In a study conducted on eating disorders, it was found that the rate of eating disorders was higher in women than in men, the group between the ages of 18-30 and 31-40 had a higher rate of eating disorders compared to the group between the ages of 46-60, and the rate of satisfaction with their body decreased by 14% in those with eating disorders (Cengiz et al., 2022).

2.1. Eating Disorders and Emotions

The relationship between lifelong eating behavior and emotions has been researched for a long time and this situation causes problems such as eating disorders as well as enjoying food. Eating behavior, which is affected

by many factors, is associated with positive and negative emotions such as stress, sadness, excitement, happiness and joy (Özkan & Bilici, 2018).

The emergence of eating disorders involves multiple factors and eating behavior can be influenced by many factors. One of these factors is emotions, and many emotions are experienced in this process. Although people exhibit eating behavior to avoid sadness in the short term, they feel shame and guilt due to eating in the long term. In this sense, emotion regulation is important, and it has been found that individuals who learn to regulate emotions move away from eating attacks (Faraji & Fırat, 2022). Sadness also has the effect of increasing or decreasing eating behavior, and individuals show eating behavior only to regulate emotion without enjoying food (Russ, 1998).

Eating disorders and inadequate emotion regulation cause the inability to control impulses and exhibit purposeful behaviors in the process of experiencing negative emotions. In this context, studies have shown that people diagnosed with eating disorders have difficulty coping with negative emotions (Özsoy, 2021).

In a study on Binge Eating Disorder, it was found that eating attacks increased with negative emotions, only unhealthy foods were consumed during the attacks, and the feeling of sadness caused by being alone triggered the depressive mood accompanying the disease and increased the number of attacks (Güven et al., 2020). It is seen that negative mood causes both eating attacks and unhealthy eating behaviors.

In those diagnosed with bulimia nervosa disorder, the shame they feel in the eating cycle is felt excessively and causes the repetitive display of disordered eating behavior (Faraji & Fırat, 2022).

2.2. Classification of Eating Disorders

The World Health Organization (WHO) defines health as a state of well-being in physiological, psychological and social aspects, not limiting it to concepts such as disease or disability. While all these aspects complement each other, disruption in any one of them leads to deterioration in the other. In addition, mental and behavioral problems constitute the basis of psychiatric disorders. Nutrition plays an important role in the majority of psychiatric disorders. Nutritional problems seen in this group of patients, the relationship between food and nutrients and diseases are addressed in order to be treated (Akbulut 2015).

Classifying diseases and combining similar characteristics makes them easier to assess and treat. In this sense, there are two basic classifications:

International Classification of Diseases (ICD) and Diagnostic and Statistical Manual of Mental Disorders (DSM) (Akbulut 2015).

Eating disorders were not studied much until the 20th century and no specific classification was made. With the DSM published by the American Psychiatric Association, eating disorders started to be mentioned. With the publication of DSM-5 in 2013, eating disorders were grouped into 8 subcategories. These categories are anorexia nervosa, bulimia nervosa, binge eating disorder, pica, rumination disorders, and avoidant/restrictive eating disorders (Demirer & Yardımcı, 2020). Anorexia neurosis, bulimia neurosis and binge eating disorder cover adolescence and adulthood. Pica, Rumination Disorder and Avoidant/Restricted Food Intake Disorder are disorders that occur especially in childhood (Morrison, 2016). Therefore, these disorders are discussed in more detail in order to understand disorders that cover adolescence and adulthood:

2.2.1. Anorexia Nervosa (AN):

The 3 main indicators of anorexia nervosa are excessive reduction in food intake, excessive concern about weight gain, and a distorted body perception that they are overweight (Morrison, 2016). In these individuals, eating behavior is obsessed with food. Although they do not eat much, food-related thoughts are present in a large part of their minds and they spend most of their time talking, thinking and preparing food for others. They also suffer from hyperactivity and think that they can deserve to eat by practicing heavy and rigidly prescribed exercises. This is how they can combat guilt about eating (Smolin et al., 2020). It is a disorder characterized by an intense effort not to gain weight despite being underweight as a result of perceiving the body differently in terms of shape and weight. The treatment is long-term and hospitalization is necessary due to impaired physiological functionality (Merdol, 2015).

The diagnostic criterion of amenorrhea (cessation of menstrual bleeding) in anorexics has been removed in DSM-5 and it is stated that this diagnostic criterion has many reasons that are not related to weight loss. In addition, blood pressure is generally lower, skin becomes dry, hormone levels drop, anemia and decreased bone density are observed (Kring and Johnson, 2017). The mortality rate is 6 times higher in people with this disease and is accompanied by depression and anxiety. It is also more common in women, and the proportion of men is one-third of women. Unlike other disorders, no time period is specified for the diagnostic criteria, and it is evaluated by looking at subtypes and body mass index (Morrison, 2016).

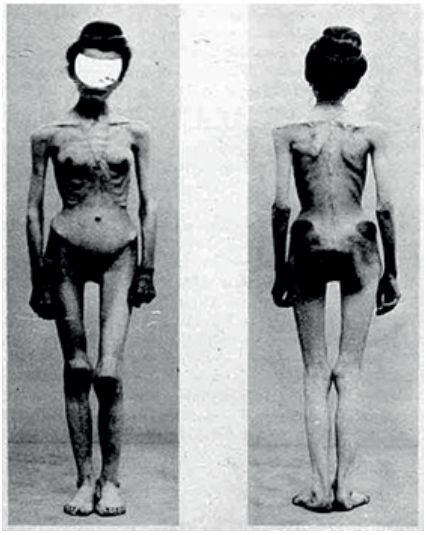

Figure 1: Physical appearance of an individual diagnosed with anorexia nervosa, https://tr.m.wikipedia.org/wiki/Anoreksiya_nervoza Date of access: 04.12.2022

2.2.2. Bulimia Nervosa (BN):

These people overeat, gain weight as a result of eating and at the same time try to stop gaining weight. Unlike anorexics, body weight is not low. Bouts of binge eating are seen and they prefer excessive calorie foods at such times (Merdol, 2015). A certain proportion of these patients are obese, but surprisingly their weight is usually normal. They also do not eat regularly and use laxatives as well as fasting and excessive exercise after eating. Due to the lack of control, shame and regret are the emotional state after binge eating. This is why they usually eat when they are alone. Depression and stress trigger overeating behaviors. In addition, the act of vomiting is the critical behavior in receiving this diagnosis, almost all of them exhibit vomiting behavior (Morrison, 2016). While these patients are aware that their behavior is wrong, anorexics have no such awareness. In the treatment of bulimics, cognitive behavioral therapy methods are followed with the aim of replacing overeating behavior with normal eating behavior (Merdol, 2015).

They exhibit compensatory behaviors such as vomiting, not eating and excessive exercise to prevent weight gain. In these people, binge eating is of 2 types; the first is eating more than a normal person would eat in a short time, and the second is that the person loses control and does not know where to stop. DSM-5 defines bulimia as a period of binge eating and

compensatory behaviors at least once a week within 3 months. In DSM-4-TR, the frequency was two times a week. In addition, the non-exclusion subtype was removed from the DSM-5 because it was difficult to distinguish between non-exclusion and binge eating disorder (Kring & Johnson, 2017).

Bulimia nervosa is more common than AN, with a higher prevalence in women than in men, as in AN. In addition, lower tooth enamel is damaged to the point of destruction due to vomiting. It is comorbid with mood disorders, anxiety disorders, impulse control disorders and substance abuse. The person is diagnosed after exhibiting inappropriate behavior at least once a week for 3 months (Morrison, 2016). Attacks are followed by days of self-starvation and people exhibit more antisocial behaviors. Complications are many and electrolyte disorders, pharyngitis and esophagitis can be seen due to the use of laxatives and diuretics (Sencer & Orhan, 2005).

2.2.3. Binge Eating Disorder (BED):

These individuals usually exhibit rapidly recurring binge eating behavior when they are alone and cannot control themselves (Merdol, 2015). As in bulimia nervosa, binge eating behavior is observed in a short period of time, but unlike bulimia nervosa, laxative use and vomiting behavior are not observed (APA 2013). It is usually initiated after a failed diet, typically when feeling sad or anxious, and high-calorie/ tasty foods are preferred. Due to fast eating, the feeling of fullness is realized much later and guilt follows (Morrison, 2016). People with BED are usually obese, but not all obese people have the disorder (Kring & Johnson, 2017).

The main feature of this disease is the excessive amount of food eaten and loss of control on overeating behavior. It is seen in 2% of adults and the incidence in women is 2 times higher than in men. This disorder can also be seen in people with Type 2 diabetes. In addition, these people find it very difficult to lose weight. Within 3 months, binge eating behavior at least once a week is sufficient for the diagnosis (Morrison, 2016). Complications include obesity-associated diabetes, high blood pressure and cholesterol, gallbladder and heart diseases (Smolin et al., 2020).

Table 1: Comparison of eating disorder types in terms of certain characteristics, (Morrison, 2016) Date of access: 01.12.2022

	Anorexia Nervosa	BulimiyaNervosa	Binge Eating Disorder
Binge Eating	No.	Yes	Yes
Self-Perception	Abnormal (perceives as overweight	Affected by the weight and shapeof the body	Not much affected
Compensatory Behaviors	Yes	Yes	No.
Body weight Low	Yes	No.	No.
Feeling a Lack of Control	No.	Yes	Yes

3. NEUROBIOLOGY OF EATING

Neural and endocrine systems play a role in the regulation of eating behavior. While the neural system interacts with the hormones secreted from the digestive system and regulates food intake, the Arcuate nucleus of the hypothalamus also plays a role in food intake. These effects on food intake appear as appetizing or unappetizing (Orhan, 2021).

Brain regions that affect appetite, peptides synthesized from these regions, molecules such as glucose and fatty acids that provide information about the amount of fuel in the body, some neurotransmitters (such as neuropeptide Y, serotonin, dopamine, noradrenaline) and some hormones (such as ghrelin, leptin, peptide YY) are involved in the energy homeostasis that the hypothalamus regulates (Gürdöl, 2018). Neurons forming a network create a feeling of hunger and satiety in line with the messages they receive from surrounding tissues, Nucleus Tractus Solitarii (NTS) and higher centers of the brain. There are neural extensions and communication between the NTS and the hypothalamus (Sencer & Orhan, 2005).

Table 2: Hormones and neurotransmitters affecting eating behavior, https://www.facebook.com/fizyolojisayfasi/photos/a.464963570202091/954453451253098/?type=3 Access Date: 01.12.2022

HORMONES AND NEUROTRANSMITTERS AFFECTING EATING BEHAVIOR	
ENJOYMENT INCREASING	**APPETITE SUPPRESSANT**
Neuropeptide Y (NPY)	Alpha Melanocyte-stimulating hormone
Agouti-related Protein (AGRP)	Leptin
Melanin concentrating hormone (MCH)	Serotonin
Oreksin	Norepinephrine
Endorphins	Corticotropin-releasing hormone (CRH)
Galanin	Insulin
Glutamate- GABA	Cholecystokinin (CCK)
Cortisol	Glucagon-like peptide
Ghrelin	Cocaine-Amphetamine-regulated transcript (CART)
Endocannabinoids	Peptide YY (PYY)

Ghrelin is a neuropeptide secreted from the stomach and its presence in the circulation affects feeding. It increases the release of growth hormone, Adenocorticotropic (ACTH), neuropeptide Y and insulin and thus eating behavior increases (Aksoy, 2008). This hormone stimulates the hunger center of the hypothalamus, and its secretion ends when the stomach responds full (Orhan, 2021).

Leptin is an antagonist of ghrelin. It acts on food intake, gastric emptying and energy balance by modulating neuropeptides in the hypothalamus. It also suppresses eating behavior by acting through cholecystokinin and vagus. When the hormone level increases with weight gain, resistance develops and a vicious cycle occurs, resulting in obesity (Aksoy, 2008). It suppresses appetite by increasing metabolic rate. It also suppresses neurons that synthesize Neuropeptide- Y (NPY), an appetite stimulating hormone. NPY has effects just like the hormone Ghrelin. Like leptin, insulin hormone also reaches the arcuate nucleus via circulation and inhibits NPY synthesis (Gürdöl, 2018). Leptin is secreted from fat cells and transmits the satiety signal to the brain. The increase in leptin level in the blood is related to the amount of fat, and the occurrence of an increase provides signal transmission to the hypothalamus and causes appetite suppression and energy burning

(Orhan, 2021). While leptin is known as satiety hormone with this feature, Ghrelin is called hunger hormone because it is secreted in case of hunger. The balance between these two hormones is important and if it is disrupted, eating disorders will occur.

Neuropeptide Y is the most important neurotransmitter in internal messages and is the strongest hunger stimulant. In order for the feeling of satiety to occur as a result of messages from the environment, it chooses to suppress NPY (Sencer and Orhan, 2005).

Peptide YY (PYY) hormone is stimulated by foods rich in fat and protein. The reason why protein-based nutrition is preferred in diets is to try to activate this hormone. It is an appetite suppressant and slows stomach emptying. It is the antagonist of NPY hormone (Gürdöl, 2018).

Eating addiction may be thought to be caused by disruptions in gene expression of hormones such as Agouti's relationship peptide (AgRP), Proopiomelanocortin (POMC), leptin, ghrelin at the hypothalamic level or in communication between neurons (Uzbay, 2015). In order to understand weight control and eating disorders, it is important to understand the neuropeptide and hormonal changes of appetite. Thus, it provides new opportunities in the treatment process (Zincir, 2014).

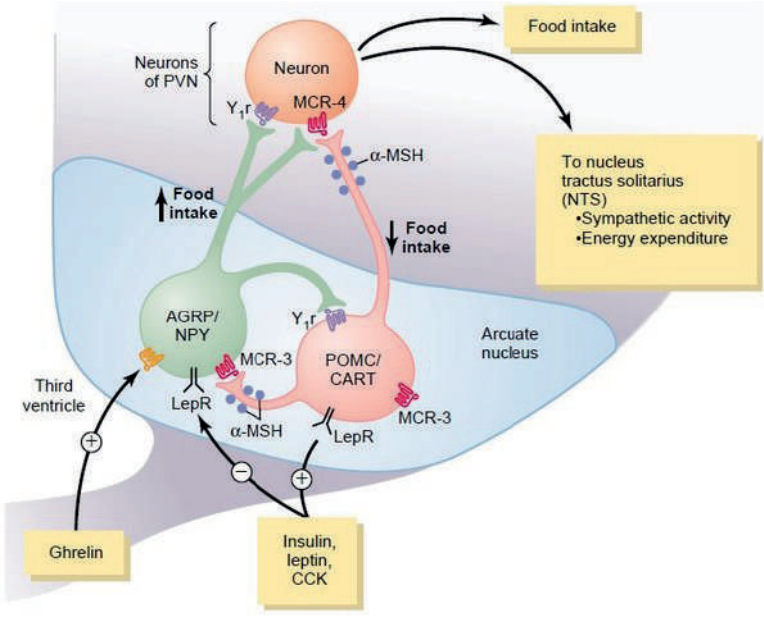

Figure.2: Arcuate Nucleus and Hormones Regulating Energy Balance in the Brain, https://www.quora.com/How-does-brain-chemistry-affect-nutrition

Access Date: 10.10.2023

CRH: Corticotropin releasing hormone; MCH: Melanin concentrating hormone; MC4R: Melanocortin

4 receptor; αMSH: Alpha melanocyte stimulating hormone; NPY: Neuropeptide Y; POMC: Propiomelanocortin; TRH: Thyrotropin releasing hormone.

Nutrition is a natural need and the nutrients needed are taken into the body in this way. When insufficient nutrients and energy are taken, nutrient stores are used through various mechanisms. Starvation caused by nutrient deficiencies affects many systems in metabolism and leaves permanent traces. These inadequacies affect hormone levels, immune and nervous systems and create disorder in the body. This can even lead to behaviors such as aggression and violence. Food intake low in tryptophan and low cholesterol levels affect the amount of serotonin neurotransmitter, resulting in a decrease in the amount of serotonin; accordingly, it causes aggressive behaviors (Özenoğlu & Ünal, 2015). Accordingly, changes in mood, decrease in food consumption, increase in energy consumption and weight loss occur (Akbulut, 2015). In addition, serotonin and dopamine are interrelated, and low serotonin levels cause an increase in dopamine levels, and vice versa, an increase in dopamine levels and a decrease in serotonin levels can cause unwanted aggressive behaviors (Özenoğlu & Ünal, 2015).

When we look at the relationship between eating and stress, a strong link has been found between obesity and stress. In acute stress, energy is needed in both cases due to the "fight or flight" alarm in the organism, and acceleration of metabolism with oxygen use is given in response to stress. In this case, appetite decreases and oxidation increases. In chronic stress; insulin resistance develops and glucose transport decreases accordingly. Negative nitrogen balance is observed with acceleration of protein degradation. Activation of the hypothalamic- pituitary-adrenal (HPA) axis is activated, resulting in an increase in cortisol level and thus appetite, resulting in weight gain (Gürdöl, 2018). It has also been found that ATP axis activity in obese people is 25% lower than non-obese people. Accordingly, it was found that 22% less energy was consumed in total erythrocytes compared to non-obese people (Sencer & Orhan, 2005).

4. THE CONCEPT OF ADDICTION

Addiction is an important disease characterized by the development of tolerance, withdrawal, failures to quit, loss of control over use, material and moral losses, and disruption of work and social life (Kring & Johnson, 2017). It is a widespread modern disease that directly or indirectly affects

every stage of the lives of both the individual and their relatives. The disease is widespread throughout the population (Dinçer, 2019).

Addiction can be generally defined as an unstoppable desire for an object, individual or entity and a pathological behavior associated with the mind. Although it harms the person mentally, physically and socially, people cannot stop this obsessive state and want to continue. While we think of a chemical or herbal substance when we think of addiction, today there are also behavioral addiction types where behaviors are at the forefront and medical treatment is needed (Uzbay, 2015).

The term "addiction" is a common term in today's society, although there is no consensus on an established clinical definition. In clinical practice, there is no formal diagnosis for addiction. Instead, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) states that the term can be used to describe severe substance use disorders (APA, 2013).

4.1. Behavioral Addiction

Reward pathways and the dopamine neurotransmitter are associated not only with addictive substances but also with basic needs such as hunger, thirst and sexuality (Uzbay, 2015).

It is thought that the perception of behaviors in behavioral addictions as rule-breaking or egodystonic with excessive desire to define the behavior is important in making a diagnosis. In the version before DSM-5, the removal of the criterion of "legal problems related to the substance" and the replacement of the criterion of craving can be interpreted as a harbinger that behavioral addiction types may be included in the diagnostic classification in the future (Evren, 2020).

The reason why behavioral addictions are derived with many names (e.g. gaming addiction, digital game addiction, obsessive-compulsive gaming) is that they include different views. According to one view, these behaviors can be interpreted as addiction, compulsion according to another view, or impulsivity according to another perspective. However, one of the reasons for considering these behaviors as addiction is that they are initially egosyntonic or hedonic, but then the pleasure received may decrease over time (Potenza et al, 2009).

4.2. Eating Addiction

Recently, dietary habits have changed and people no longer consume food for survival, but also engage in eating behavior for other reasons. As a

result of the changes in foods due to the development in the food industry, there has been a transition to foods that are high in energy; contain sugar, fat and additives; have low nutritional value and can be easily taken. Thus, the food eaten served as a reward and started to be preferred due to its accessibility (Dimitrijević et al., 2015).

The symptoms included in the DSM criteria for addiction are also seen in eating addiction. Loss of control is also present in eating addiction. They eat faster than individuals without eating addiction, show eating behavior even though they are not hungry, and as a result of overeating, feelings of shame, guilt, depression and disgust arise. Despite their anxiety, they cannot stop themselves from eating. Fatty and sugary foods are thought to trigger this condition. This condition can also be seen in the non-clinical population. 9% of people of normal weight and 21% of people diagnosed with obesity show this behavior. Another common symptom is unsuccessful attempts to stop consumption. This is indicated by high annual expenditure on diet products. Another symptom is that rather than engaging in activity and eating healthy foods, people prefer unhealthy ones and prefer eating to activity. When food cravings come, all other activities can be reduced, and consuming food brings happiness (Dimitrijević et al., 2015).

Eating addiction was first used in the literature by Theron Randolph in 1956 (Adrian & Gearhardt, 2014). After the researches, scales were developed at Yale to evaluate this concept as a clinical diagnosis. In 2009, Gearrhardt and colleagues developed the Yale Eating Addiction Scale (YEAS) consisting of 27 items with 1440 university students (Gearhardt et al., 2009). The Turkish validity and reliability study of the scale was conducted in 2012 by applying it to 156 clinical and non-clinical individuals (Bayraktar et al., 2012).

The concept of eating addiction is complex, previous studies were unreliable in defining eating addiction, but after the development of the standardized Yale Eating Addiction Scale (EES), it has shown more promising results. Research in this area has primarily focused on the distinction between obesity and eating addiction, as eating addiction is also seen in individuals with low body mass index or normal weight (Dimitrijević et al., 2015).

Although binge eating has not been included in the DSM-5 diagnostic criteria like gambling addiction due to insufficient empirical evidence, the debate about the addictive potential of food has continued. Organizations such as the American Society of Addiction Medicine (ASAM) have chosen to include "food addiction" in the list of possible addictive disorders. It states that behavioral addictions involve similar brain changes and neural pathways

as in substance addiction (American Society of Addiction Medicine: Definition of addiction - Long version, 2011).

In 2006, it was found that patients receiving inpatient addiction treatment in Michigan had a higher rate of bariatric surgery than the normal population. In addition, when we look at the history of these patients, they did not have any previous addiction. It was found that they had tried the substance after the surgical procedure. This event was interpreted by Dr. Hopper and Dr. Saules as the decrease in the amount of food eaten by people with eating addiction after the stomach reduction operation and the substitution of another addictive substance instead of food. It can be said that food activates the reward pathway of the brain like a substance (Uzbay, 2015). In another study conducted with people who underwent the same surgical operation, it was found that people who previously reported more problems with high glycemic index and high sugar/low fat foods were more likely to develop a new substance addiction after the operation, and they stated that this created a cross-sensitivity (Fowler et al., 2014).

For decades, critics have delayed the inclusion of nicotine in the concept of addiction and its prevention, arguing that its addictiveness is different from that of other drugs (e.g. alcohol, opiates) because tobacco use does not cause visible intoxication and there is no physical distress as with morphine. Whether eating addiction is valid or not is an empirical question, but criticism can lead to mistakes such as the delay in defining tobacco as a substance addiction. The next steps should focus on identifying specific addictive substances and examining individual differences that may increase the risk of addiction (Schulte, 2015).

Considering the sociocultural effects; praising thinness and promoting this thinness in the virtual environment has a significant effect on people's body perception. In addition, according to gender, the incidence of eating disorders is higher in women than in men. Another effect is the objectification of the female body, where women are known for their bodies while men are known for their achievements. When the personality characteristics of these individuals are analyzed, it has been observed that they have negative affectivity, perfectionism, and low self-awareness (Kring & Johnson, 2017).

Eating behavior is also regulated by hedonic systems. This system is the excessive desire for food that is considered delicious and the pleasure derived from eating. Individuals with this type of nutrition consume pleasure-oriented consumption rather than adequate and balanced nutrition. It is suggested that dopamine has an effect on the formation of this desire and there are also studies showing that delicious foods activate the reward

system. Accordingly, when sugar, fat and carbohydrate consumption is high, these foods increase the amount of dopamine and opioids (Orhan, 2021).

4.2.1. Studies on Eating Addiction

In a related study conducted at Yale University in 2009, brain regions associated with eating addiction were identified. Forty-eight women participating in the study were given milk shakes with milk, after an eating addiction test and brain imaging study were conducted. Looking at the brain images of individuals with high test results; higher activation was detected in the caudate nucleus, medial orbitofrontal cortex, anterior cingulate cortex and amygdala. These regions are associated with substance addiction. In the same study group, increased activation in the dorsolateral prefrontal cortex, which is known to be related to willpower, was detected when the pleasant food was resisted, and decreased activation in the lateral orbitofrontal cortex after drinking milkshakes, as in substance addicts. According to the results of this study, increased activation in reward-related areas of the brain and decreased activation in inhibitory brain regions were similar to substance addiction (Gearhardt et al., 2011). The orbitofrontal cortex plays a role in the regulation of impulses and emotions, and in case of decreased activation, impulsive behaviors emerge (Yener, 2002). This part also causes behavior in the form of craving. According to the results of this study, it was determined that low activation of the orbitofrontal cortex makes it difficult for the person to stop himself/herself against the pleasant food, and it can be said that this region has an important role in eating addiction (Uzbay, 2015). In another study conducted with the EEG system, it was found that people who were found to have eating addiction by scoring high on the NADS had increased functional connectivity in the frontal and parietal areas, similar to people with substance addiction (Imperatori et al., 2015).

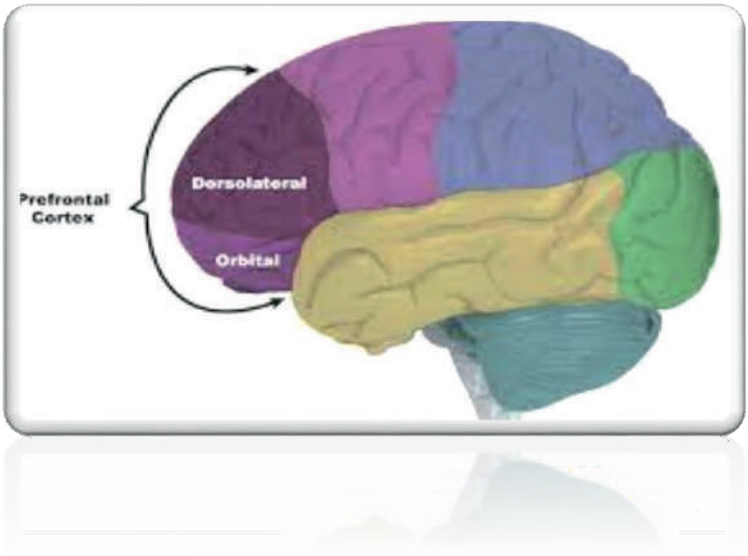

Figure 3: Parts of the Prefrontal Cortex. (These parts are located in the prefontal region and are involved in volitional functions through the reward system), McGee, 2004 Date of Access: 26.11.2022

The brain's reward system interacts with the limbic system, which controls behaviors such as eating and drinking. When hunger occurs, this control system tries to fulfill this need for survival and begins to search for food. The information generated as a result of hunger- induced imbalance in the organism stimulates the ventral tegmental area (VTA), from which nerve axons reach the Nucleis Accubens. From this area, dopamine is released. This mesolimbic pathway is also seen in the imbalance caused by the absence of a chemical substance. When the mesolimbic system is activated, food is sought if it was previously unknown how to obtain it. For example, if the experimental animal in the cage has found the food by pressing the pedal, it will place it in the hippocampus and the next time it is hungry, it will go directly to the area where it will obtain the food and press the pedal. However, it should be kept in mind that experimental animals can exhibit pedal pressing behavior and spend time in this area without being hungry (Uzbay, 2015). A study conducted on primates proves this. It was found that in the case of permanent conditioning of chocolate in primates, they spent more time in the environment where they received chocolate, while no such behavior was encountered in the control group and they did not make a place preference. It was concluded that chocolate can act like a chemical substance by conditioning (Duarte et al., 2014).

One animal study showed that rats conditioned on sugar-sweetened pellets no longer responded to the reward they were conditioned to when given naltrexone, an opiate antagonist (Le Merrer and Stephens, 2006). However, in another study, rats fed high-fat food did not show opiate-like withdrawal when given naloxone, an opiate antagonist, as with sugar. This suggests that food composition is related to food addiction and that the opiate system in the brain may be differentially affected by fatty food compared to sugar (Corwin, 2011). There have been many studies investigating the relationship between food addiction and the opiate system, and although the results cannot say that food is an addictive substance, the results obtained against opiate antagonists suggest that the opiate system plays a role in food-related reward (Gordon et al., 2018).

Colantuoni et al (2001) compared brain chemistry changes in rats with intermittent and excessive glucose intake to rats given a normal food diet. They found that exposure to highly palatable food in an intermittent eating pattern resulted in increased activation of dopamine D1 and μ-opioid-1 receptors, as well as reduced binding of dopamine D2 receptors in the dorsal striatum.

In another study, subjects were given an appetite suppressant that blocked dopamine function, and the blockade was not effective in adults who scored higher for addiction on the NADL test compared to the control group. This suggested that dopamine signaling strength was altered in adults who ate more food and were thought to have an eating addiction, similar to that seen in adults with substance use disorders. It was also found that high-scoring participants reported more food cravings after tasting their favorite delicious foods (such as potato chips, chocolate and cookies) (Davis et al., 2014).

In a study conducted to define the relationship between food addiction and anxiety level, it was found that when the amount of high-calorie (high-fat and sugary) products given to obese mice was reduced, they exhibited excessive food seeking behavior, and in the open field test, they spent more time at the edges and less time in the middle area and exhibited anxiety behavior (Pickering et al., 2009). In a similar study, it was observed that mice fed high- fat foods showed increased cortisol levels and more anxiety symptoms after the diet was stopped (Sharma et al., 2013).

The nuclei of the hypothalamus are directly involved as hunger and satiety centers. These centers determine our state of eating. The area responsible for the hunger center is defined as the lateral nucleus, while the part responsible for the satiety center is the ventromedial nucleus. The hypothalamus thus controls the cycle of hunger and satiety (Aksoy, 2008). However, in the

studies conducted on this subject, the lesions in these regions do not show a complete picture of Aneroxia nervosa or Bulimia nervosa. For example, as a result of a lesion in the lateral nucleus, the animal does not feel hunger and behaves indifferent to food. On the contrary, anorexics are very interested in food and starve themselves even though they feel hungry. Accordingly, a dysfunctional hypothalamus cannot be mentioned. In addition, other studies have found that serotonin and dopamine neurotransmitters are associated with anorexic and bulimic individuals (Kring & Johnson, 2017).

In another study, a relationship was found between appetite levels of emotions such as sadness, stress, happiness and excitement and body mass index. In negative emotions and situations, the eating tendency of students with eating addiction was found to be higher compared to students without eating addiction. In addition, in the research conducted with students with eating addiction, restrictive eating, emotional eating and external eating scores were found to be higher than students without eating addiction (Dinçer, 2019).

In the study looking at the relationship between eating addiction and impulsivity, it was concluded that those who met the criteria for eating addiction were more impulsive than the other group according to the score of obese and Binge Eating Disordered individuals on the Yale Eating Addiction Scale (YED). In addition, in another study comparing eating addiction, impulsivity and Body Mass Index results, a significant relationship was found between the three topics (Murphy et al., 2014). In another study looking at the relationship with impulsivity, a statistically significant relationship was found between eating addiction and impulsivity subscales and it was found that impulsivity subscales increased eating addiction (Kandeğer, 2016).

In a study examining the relationship between insomnia and eating addiction in university students, the prevalence of eating addiction was found to be 12% and was found to increase compared to the rates in previous studies. A high degree of significance was found between insomnia severity and eating addiction, and it was interpreted that they were risk factors for each other. The researcher stated that circadian rhythms were disrupted as a result of individuals starting to sleep at late hours with technology, which led to obesity and other disorders related to metabolism (Kandeğer, 2016).

In a study aiming to conduct a comprehensive meta-analysis on food addiction, a total of 31 articles and 47 studies were selected from Pubmed and PsychInfo, filtering out those containing quantitative and empirical studies. The results of all these systematic reviews support the validity of food addiction as a diagnostic construct in general, especially as it relates

to high-energy foods in terms of added sweeteners and refined ingredients. Most studies in the current review reported evidence of symptoms related to neurological changes and impaired control, with fewer studies assessing preoccupation, chronicity, relapse, social impairment and risky use. They concluded that behavioral and substance-related aspects of food addiction appear to be intertwined, but that the substance (very tasty food) component may be more prominent than behavior (eating) in the diagnostic classification of this phenomenon. The meta- analysis also found that the most common foods associated with addiction symptoms were those high in added fats and/or refined carbohydrates such as sugar. These findings are consistent with previous literature. As a result of all screening studies, it was suggested that more studies like this should be conducted for the treatment of eating addiction (Gordon et al., 2018).

In a study conducted with adults with obesity who had high scores on the NADS scale, it was found that they reported significant craving behavior, hedonic feeding, and sweet food cravings. In addition, the same high-scoring obese group showed more impulsivity than the control group (Davis et al., 2011).

In their study with rats, Johnson and Kenny (2010) found that rats that were given unlimited access to high-calorie foods (pork, sausage, cheesecake, chocolate) continued to consume the food even though they were given a deterrent conditional stimulus (electric shock to their feet). On the other hand, rats that were given limited eating rights or fed normal food decreased food consumption when the aversive stimulus was presented.

5. CONCLUSION

Although eating addiction is not included in the DSM as a diagnostic criterion, it is widely used as a concept. Eating and nutrition is a mandatory behavior for our daily life and a balanced diet is extremely important for healthy development. Eating behavior is not only the result of a physiological need, but can also be emotionally driven, resulting in the concepts of emotional eating and hedonic consumption.

Studies with animals have started to increase as well as studies with humans. As a result of the development of the Yale Eating Addiction Scale (YEAS) and the completion of the validity and reliability study, researches have started to gain more momentum. However, although there is still no conclusive evidence that food is addictive, studies have found that excessive consumption of sugar/carbohydrate/fatty foods in the macronutrient group has an addictive effect similar to the chemicals in substance addiction and

activates/inhibits similar brain regions. Research also shows that eating addiction is not only a concept for the group diagnosed with obesity and binge eating disorder. Continuing research on the subject is important in terms of discovering curative treatments for people.

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Chapter 14

Biopolymer Bacterial Cellulose Produced by Bacteria and its Use in Health

Aytül Bayraktar[1]

Abstract

Bacterial cellulose is frequently used because it is economical and suitable for various production areas. Bacterial cellulose (BC) is a pure, crystalline material with superior properties, synthesized by aerobic bacteria. BC is produced by some bacteria, such as *Gluconacetobacter xylinum*, which stores abundant amounts of fibrils in 3D networks. Bacterial cellulose (BC) is a very comprehensive biomaterial. It is used in many areas such as the food industry, pharmaceutical industry, industrial and agricultural sectors. By producing bacterial cellulose from waste materials, it reduces costs and allows the use of environmentally friendly materials. BC can be used practically in different scientific researches and studies, especially in medical devices. Due to its excellent nanostructure and properties, bacterial cellulose is used in many medical treatments and textural applications. The search for new and active BC-producing microbial strains provides an impressive boost to BC production processes. Membrane types prepared with BC accelerate the wound healing process and prevent complications. Bacterial cellulose composites containing various materials have been designed to increase their applicability to living tissue. BC allows biocomposites to regulate cell adhesion for scaffolds and grafts. Bacterial cellulose, which is used to replace or support drug treatment, is increasingly being investigated. This study includes biocompatible and biodegradable bacterial celluloses, current biomedical applications, exploratory studies, and low cost BC production methods.

1. Introduction

Cellulose, one of the most important components of the primary cell wall of green plants, is a natural biopolymer that is quite common in nature (Updegraff, 1969). It forms the main structural component of the plant cell

1 Doç. Dr., Süleyman Demirel Üniversitesi, aytulbayraktar@sdu.edu.tr, 0000-0002-1725-6315

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wall and is frequently used in the production of paper, texturing and paper clay (Huber et al., 2012; Klemm et al., 2005, Gandini, 2008). The type of cellulose synthesized by tunicates, an ocean animal, is called astunisin (Zhao and Li, 2014). Regardless of the source, cellulose has the same chemical compositions but can differ in structure and have different physical properties (Brown, 1886; Ross et al., 1991).

Biocompatible natural biopolymers used in various materials and devices have become the preferred choice in research in medicine and related fields. Researchers' studies on the subject have led to the discovery of new systems, and this closely concerns the complex structures of tissue (De Oliveira Barud et al., 2016).

Cellulose (PC) formed by plants contains hemicellulose and lignin in its structure. Depending on the plant source used, the separation process may require toxic chemicals that are not environmentally friendly and have high costs (Vasconcelos et al., 2017).

Bacterial cellulose, which is a very promising material as well as a very pure natural exopolysaccharide, is produced by aerobic bacteria (*Gluconacetobacter, Agrobacterium, Aerobacter, Achromobacter, Azotobacter, Rhizobium, Sarcina and Salmonella*) (Jonas and Farah, 1998; Chawla et al., 2009). BC lacks lignin and hemicellulose and consists of microfibrils (Moniri et al., 2017). These microfibers will be arranged in a three-dimensional patterned structure that provides a porous geometry and high mechanical strength (Khan et al. 2015a; Mohite and Patil 2014). BC has high crystallinity (>80%) (Keshk, 2014), high water retention (Saibuatong and Phisalaphong, 2010) and degree of polymerization compared to plant cellulose varieties (Dahman, 2009). Due to these properties, it is used in biomedical and other related fields.

Nowadays, materials produced by tissue engineering are widely used in biomedical devices and products, wound & burn dressings, and treatment and healing of damaged tissues. Due to its excellent nanostructure and properties, microbial cellulose is a notable candidate for numerous medical and tissue engineering applications (Cherian et al., 2013).

Specially made materials that regulate environmental conditions and increase cell proliferation, growth, migration and modifications, thus increasing wound healing rates and allowing the wound closure process to occur more quickly, pave the way for the future of developing medicine (Lucchesi et al., 2008). The desired features of such devices are that the area where such devices are located is a humid environment, can be

absorbed from the blood, has important features such as gas exchange, heat permeability and minimum tissue adhesion (Boateng et al. 2008). Due to mediation, the interface plays a crucial role in wound healing, scaffolds (Nge et al., 2010), implants (Svensson et al., 2005), drug delivery systems and in vivo performance of biomaterials developed for medicine (Piatkowski et al., 2001; Martina et al., 2001).

BC medical applications werein skin repair treatments for burns, scars and ulcers. BC membranes have features such as speeding up the epithelialization process and preventing infections. Biocomposite materials prepared with Bacterial Cellulose have the capacity to significantly regulate cell adhesion in scaffolding and grafting processes, and very thin films of bacterial cellulose can be used in the application of diagnostic sensors that can be developed to neutralize many antigens (Picheth et al., 2017).

The aim of the chapter entitled "Biopolymer Bacterial Cellulose Produced By Bacteria And Its Use In Health" to focused on we have been explaining biomedical products that have been used in the health field up to now and may have potential for future use. Studies on BC have proven that it is a biomaterial that serves many areas. At the same time, its extraordinary molecular assemblies network structure and valuable properties in many biomedical applications have opened it to extensive international studies. Thanks to its natural and ultra-thin three-dimensional bacterial cellulose network structure with different properties, it can be used biomimetically in the production of materials similar to human and animal tissues. This study specifically aimed to expand the knowledge in this field and promote the practical application of BC and BC composites. From a scientific and material perspective, the most important task of these unique biopolymer cellulosic materials shows that extraordinary activities can be achieved using nanoscale materials.

2. Properties of The Bacterial Cellulose

The chemical structure of bacterial cellulose consists of chains of D-glucopyranose connected by β-1,4 glycosidic bonds (Picheth et al., 2017). Geometric state of the material; It is defined by forming parallel chains with hydrogen and van der Waals interactions between molecules. The structure containing large groups of molecules held together by intermolecular forces is called cellulose microfiber (Koizumi et al., 2008). Treatment of BC with sodium hydroxide creates an anti-parallel packing stabilized by a hydrogen bonding pack forming a significantly lower energy three-dimensional arrangement (Cellulose Type II) (Kolpak et al., 1978; Batenburg and Kroon., 1997).

Cellulose is an insoluble molecule with a molecular weight ranging from 2000 to 14,000. Studies on bacterial cellulose have shown that it is chemically similar to plant cellulose, but its macromolecular structure and characteristics are different from PC (Keshk and El-Kott., 2017).

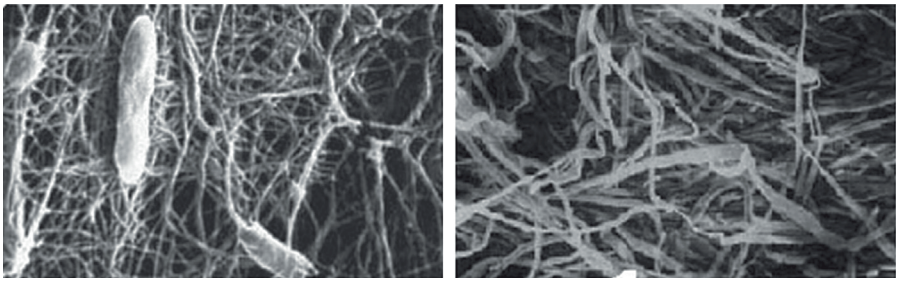

Figure 1: Scanning electron micro images of bacterial cellulose(BC) and plant cellulose(PS) (Keshk and El-Kott., 2017).

Bacterial cellulose is a natural biopolymer composed of glycosic units and mostly water, but its mechanical behavior is comparable to other synthetically produced artificial polymers and fibers, making bacterial cellulose as strong as synthetics. The tensile strength of BC is 200-300 MPa and the Young's modulus is 15-35 GPa (Ruka et al., 2014).

In general, the features of the BC research in the literature can be summarized as in the Figure 2.

Figure 2: Properties of The Bacterial Cellulose

The most important features that make bacterial cellulose superior are; It can be listed as providing mechanical strength even in moist state, showing biocompatible behavior, being non-toxic to living things, being environmentally friendly, having low density, and being biodegradable. When viewed from these perspectives, all these features make it suitable for all kinds of medical, tissue engineering, etc. making it a unique material in technological fields (Czajaet al., 2006; Hu et al., 2014; Klemm, et al., 2001; Svenssonet al., 2005; Shah, et al., 2013).

3. Sythesis of Bacterial Cellulose

BC was first published in 1886 by A.J. It was introduced by Brown from the extracellular cellulose synthase of the bacterium *Gluconacetobacterxylinus*. It has been found that the cell wall formed on the cellulosic surface during vinegar fermentation gives a chemically equivalent gelatinic matte structure (Keshk and El-Kott, 2017).

Bacterial cellulose production was characterized by Histrin and Shramm. HS developing the medium, HS medium; Made with glucose, peptone, yeast extract, disodium phosphate, citric acid and pH adjusted to 6. Variable nitrogen source, pH, and indicators affect the productivity of BC (Castro et al., 2015). Cellulose was then found to form on samples containing the cell-free extract of *Gluconacetobacter xylinus*, glucose and ATP, adhering to the traditional HS method. Starting from glucose, Gluconacetobacter xylinus produces cellulose in pellet form at the air/liquid interface of the culture medium in static culture (Hestrin and Schramm, 1954).

Many polysaccharide materials are secreted by gram-negative bacteria, but these bacteria are unable to produce more than a few types of cellulose. *Acetobacterxylinum* is a Gram-negative, aerobic, rod-shaped organism, but it has become the most studied BC source due to its ability to produce polymers at high levels and even under difficult conditions (Steinbüchel and Rhee, 2005, Ross, 1991).

Depending on the physiological state of the cell, bacteria that can produce gluconeogenesis and cellulose work together in the pentose-phosphate cycle or Krebs cycle (Ross et al. 1991). The glucose-cellulosic conversion mechanism, which includes cellulosic biosynthesis processes, occurs through *Acetobacterxylinum*. Bacterial cellulose synthesis is a precisely and specifically regulated multistep pathway involving large amounts of single and catalytic as well as regulatory protein clusters. Therefore, its supramolecular structure is not yet well defined (Bielecki et al., 2005).

Cellulose synthesis in microorganisms and plants consists of two steps; formation of the glucan chain by polymerization of glucose units; and synthesis and crystallization of the cellulose chain (Czaja, 2007). A schematic representation of this two-step pathway is shown in Figure 3 (Moniri et al., 2017). The cell forms BC between the outer and cytoplasmic membranes (De Ley et al., 1984). After the cellulose molecules are synthesized in bacteria, they are passed through the export components to form fibrils with an average diameter of 3 nm, and microfibers are assembled from these protofibrils in lines of approximately 80 nm (Iguchi et al., 2000).

Changes made in the culture medium of bacterial cellulose affect the productivity. Changes such as pH, nitrogen amount and carbon amount are the most important. *Komagataeibacter xylinum*, a most universally used strain, was grown in a liquid medium with many different carbon sources (e.g. amylose, maltose, rhamnose, glucose, etc.) (Ruka et al., 2014). If we think that glycerol is the main waste in biodiesel production, it may be economically attractive to reduce the cost with carbon stock. In addition, the authors encountered glucose as a carbon source and (Jung et al., 2010)

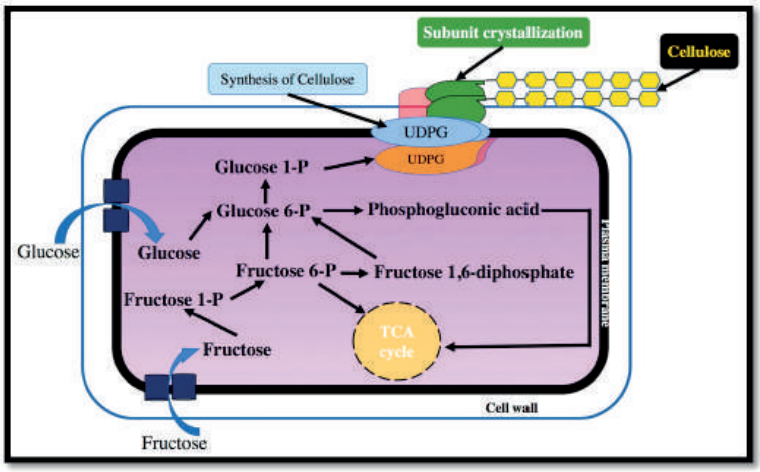

Figure 3: Two-step biosynthetic pathway of cellulose in organism cells (Moniri et al., 2017)

The production of biofuels and other chemicals with the structures formed as a result of the compact combination of cellulose, hemicelluloses and lignin is limited due to the inadequacy of enzymatic hydrolysis. Penttilä et al., (2018) used BC and wood-containing hemicellulose material in the composite material they used to examine the effect of enzymatic hydrolysis.

Hemicelluloses in particular found that BC synthesized in the presence of xylan was more sensitive than enzymatically hydrolyzed, hemicellulose-free bacterial cellulose. He reported that easier enzymatic hydrolysis is achieved with BC produced in this way and that it may offer new pursuits to come from the peak of biomass recovery through genetic engineering.

Additionally, various methods have been developed to obtain BC cost more economically. Islam and his colleagues applied these methods in their review studies; BC production from fruit juices (Kurosumi, 2009; Jagannath et al., 2014; Andrade et al., 2015; Hungund et al., 2013, Ha et al., 2011), sugarcane molasses (Bae and Shod, 2004), 2005; Keshk and Sameshima, 2006), agricultural and industrial wastes (Khan et al., 2015b; Shah et al., 2013; Hong and Qiu, 2008; Goelzer et al., 2009, Kuo et al., 2010; Shezad et al., 2009), reported as food waste (Khan et al., 2007; Wu and Liu, 2012; Tsouko et al., 2015).

BC production is basically carried out by two methods; It is a static and agitated method. Methods have different benefit and harm rates. While genetic stability is better in static culture, variants may occur in agitated culture. However, according to research, the physiological properties of BC produced by static culture were found to be more efficient compared to the properties of BC produced by agitated culture. (Deshpande et al., 2023).

3.1. Static Fermentation

A liquid-gas interface is used to produce BC via static fermentation. Microorganisms are added to the containers containing the medium and incubated for about 2 weeks under optimum conditions (28°C, pH: 4-7) until the desired film layer is formed. The resulting BC is then washed with sodium hydroxide and purified water until the desired pH value is reached (Sharma et al., 2021). In order for BC to reach its most efficient state, growth medium is added intermittently to the culture container. After BC reaches a certain size, growth stops due to depletion of resources such as nutrients in the environment.

3.2.Agitated Fermentation

The shaking culture method follows the same steps as the static method, but the shaking method uses an orbital shaking incubator. In static culture, as the amount of cellulose on the surface increases, oxygen transfers decreases. Therefore, sufficient oxygen cannot pass to the cultured bacteria. These limitations are eliminated because the shaking method provides sufficient oxygen and nutrients to the entire culture. With this method; The speed of

agitation and the nutrients provided are responsible for the efficiency of the BC. Figure 4 depicts the two main fermentation methods for BC synthesis (Deshpande et al.,2023).

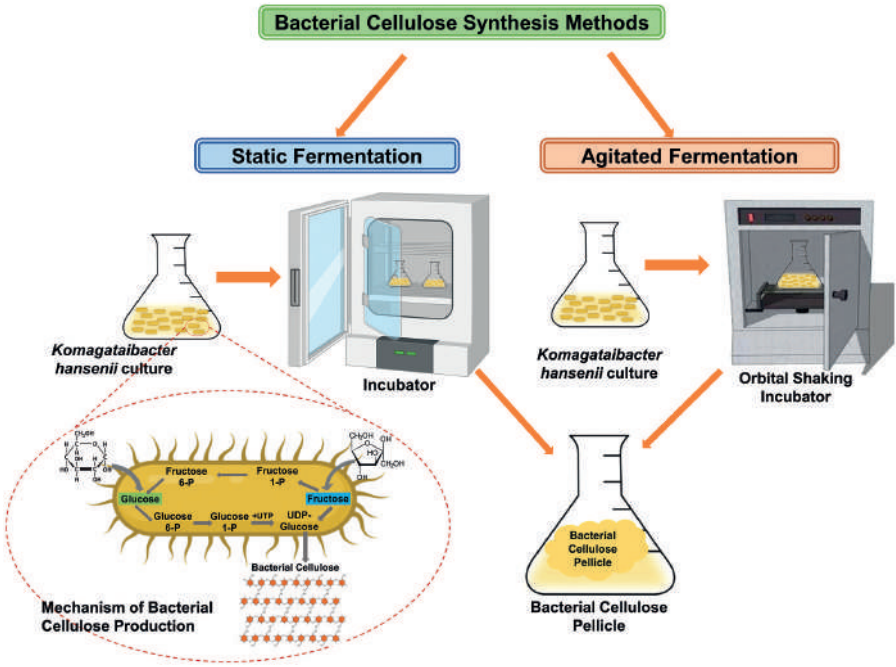

Figure 4: BC Fermantation methods (Deshpande et al.,2023)

4. Biomedical Application Areas of BC

BC is rapidly gaining great attention in the biomedical field because it is biocompatible, provides biodegradable properties, promotes cell epithelialization, is non-toxic, has the ability to trap moisture, has minimal tissue adhesion and has good pharmacological findings.

4.1. Wound Dressing

In an ideal wound dressing; It is expected to have properties such as ensuring gas exchange of substances such as oxygen, not causing infection or even preventing possible infections, keeping the area moist, not causing allergies to the person, providing epithelialization, absorbing exudates, and being able to separate from the surface without causing any irritation. So far, studies have been carried out on many types of biopolymers (chitin, chitosan, collagen, etc.) such as cellulose as wound dressings.

The most valid reasons for using bacterial cellulose as an artificial skin are; Features such as showing high mechanical properties in moist or wet state, providing the necessary permeability under optimum conditions, and minimizing irritation to the skin tissue can be given. (Choi S et al., 2022).

Almeida et al., (2014) in their study; They evaluated the potential irritation that BC may cause on human skin. By dividing BC patches into two types, with and without glycerin, they ensured that the types remained on the skin surface for 2 and 24 hours, and after the elapsed time, the patches were removed and measured trans epidermal water losses (TEWL). There was no significant difference in terms of the absence of barrier disruption after the measurements. They found similar results for erythema. The inclusion of glycerin in the study resulted in a reasonable skin moisturizing effect in the treatment of dryness-induced skin lesions such as dermatitis and psoriasis.

Studies have shown that bacterial cellulose can be used in the treatment of second and third degree burns and lesions in the skin tissue (Fontana et al., 1990). These studies have been tested on more than 300 patients and it has been documented that BC has many advantages during treatment, such as adhesion to the wound, decreased infection rate, ease of observation, and pain relief (Keshk and El-Kott, 2017). This has proven its feasibility both in terms of the patient's recovery time and in terms of economy. Biofill and gengiflex have used bacterial cellulose products in many areas such as the medical, dental and pharmaceutical sectors (Jonas and Farah, 1998).

In the study conducted on the resulting wound dressing (DermafillTM) product, it was observed that healing was achieved in a 75% shorter time with the use of bacterial cellulose.

One of the most famous bacterial cellulose composites used as wound dressings is the silver-containing BC-AG composite material. This is because the BC-Ag composite prevents the proliferation of bacteria without killing them and has bactericidal effects (Maneerung et al., 2008).

4.2. Artificial Blood Vessels

Artificial blood vessels are obtained especially with materials such as polyurethane and DACRON. BC has opened a new path in tissue engineering with its excellent mechanical properties for obtaining artificial blood vessels (Choi S et al., 2022). BC can replace arteriosclerotic vessels, is sufficient for use, has mechanical properties close to small diameter (<5) vessels (Klemm et al., 2001), has high burst pressure, has good water retention, has a pure fibrous structure and is environmentally friendly. It is a suitable biopolymer for artificial blood vessels because it does not damage tissues.

Another idea put forward by Fink (2009) is; reported that bacterial cellulose may pose less risk of blood clotting than other synthetic types. This means that BC is an ideal material for artificial blood vessels. When real blood vessels are examined, they are seen to have an inner cell that enables blood clotting.

Studies have shown that the carotid artery was successfully implanted in animal experiments and that the developed material and stability has shown that it is preserved for a long time (Schumann et al., 2009).

Zang et al., (2015) produced artificial blood vessels from bacterial cellulose using *Gluconacetobacter xylinum*. The artificial blood vessels created were shaped like tubes. It was determined that there was no toxic effect on the cells cultured in BC tubes and the surrounding tissues. BC tubes, which proved their effectiveness in vitro, were later implanted into New Zealand rabbits (Figure 5), and complete endothelialization was observed in the in vivo study (Choi S et al., 2022).

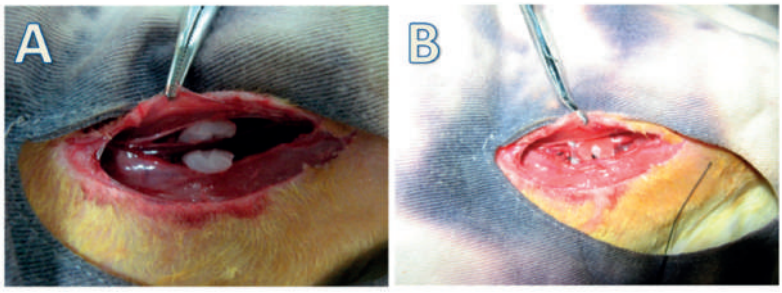

Figure 5: BC graft implanted in rabbit (Choi S et al., 2022)

4.3. Bone and Cartilage Tissue Engineering

Bone disease can be difficult to heal and can lead to major tissue disorders that require bone grafts to support the healing process. It may be possible to treat the bone loss by replacing it with material that is transplanted from another human or another species as an alternative (Palsson and Bhatia, 2004; Deng and Liu, 2005).

To find an alternative method of bone structure in the living body, many researchers have worked on biomaterials that mimic bone. Bone tissue in general; It consists of collagen and calcium hydroxyapatite. Studies have shown that bacterial cellulose and hydroxyapatite can form scaffolds by combining them in harmony (Choi S et al., 2022).

The BC scaffold obtained from *Acetobacter xylinum* X-2 loaded with BMP-2 was found to have a suitable ossification feature in fibroblast cells of mice in in vitro studies. In a mouse study of BMP-2 coated BC scaffold, it was observed that bone formation increased and calcium reached high levels within 4 weeks after implantation (Shi Q. et al.,2012).

In another study, Codreanu et al., (2020) developed scaffolds for rats using BC-modified polyhydroxyalkanoates, salt and tributyl citrate. When the modified BC scaffold was examined 4 weeks after implantation, osteoblast differentiation was observed. When examined again after 20 weeks, more ossification was observed (Figure 6).

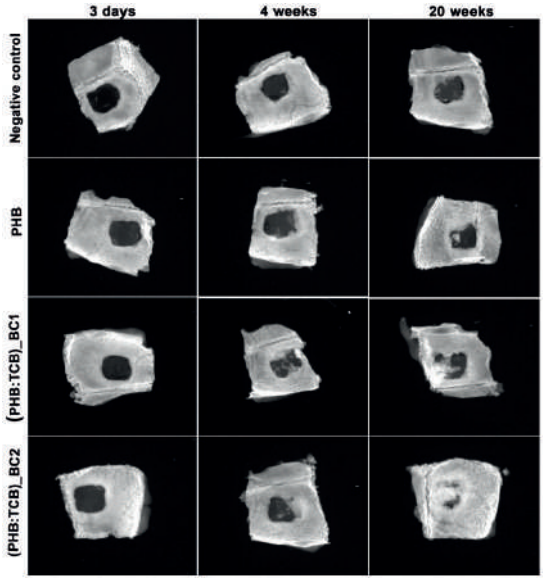

Figure 6: Bone treated with scaffolds (Codreanu et al., 2020)

Since articular cartilage is not a tissue that can fully renew itself, a lot of research is being done to repair the cartilage. Studies have been developed specifically on a structure in which the biomaterial can bind to chondrocytes and proliferate. It has been seen in studies that bacterial cellulose obtained from bacteria contributes to the proliferation of chondrocytes (Svensson, 2005). In the same study, it was observed that unmodified BC showed higher efficiency than its modified form. For these reasons, it has been determined that bacterial cellulose is a suitable scaffold model for cartilage repair (Choi S et al., 2022).

In another study, nanofibers consisting of bacterial cellulose (<1%) containing PVA nanocomposites were prepared and subjected to thermal cycling to examine their mechanical properties. PVA-BC nanocomposites have been investigated as potential materials for articular cartilage, and PVA-BC nanocomposites have tunable elastic modulus sizes similar to that of original articular cartilage. showed compressible mechanical properties (Millon et al., 2009).

4.4. Artificial Cornea and Retina

The cornea is a curved and curved tissue that is specialized to protect the eye's vision and protect it from external factors. Problems occurring in the cornea are an important cause of vision loss. It is a technique that replaces damaged corneas with keratoplasty to reverse vision loss (Foster, 2003; Whitcher et al., 2001). Techniques developed in the field of tissue and bioengineering are applied to provide cornea-like tissue, and these techniques make artificial cornea synthesis possible (Ullah et al., 2016). In this context, BC has pioneered the discovery of this remarkable material with light transmittance and biocompatibility as an innovative scaffold (Hui et al., 2009).It has been shown that corneal stromal cells can be maintained, replicated and reproduced in BC scaffold. It is argued that BC stands support inward growth of corneal stromal cells, which suggests that the potential for engineering corneal production. (BC-PVA-nHA) composite (Kharaghani et al., 2015) showed that the water content (82-84%) of BC-PVA-nHA composites resembles almost natural human cornea (78%).

Various BC biocomposites have been described to fully adapt the properties of the material used for eye treatment. For example, Wang et al., (2010) increased the light transmittance and UV absorption of BC by adding polyvinyl alcohol to BC; Goncalves et al., (2015) have reported that they show an RPE proliferation using improved chitosan and carboxymethyl cellulose by increasing the hydrophilicity of BC by surface modification. In addition, different functional structures have been reported to produce three-dimensional structures that are more suitable for cell growth to relieve artificial cornea or glaucoma (Chiaoprakobkij et al., 2011; Zaborowska et al., 2010). Therefore, many BC composites can support the development of corneal stromal cells while preserving the person's vision. Materials such as poly(methyl)methacrylate and hydroxyapatite, which are available in clinics and have a higher probability of irritation compared to BC composites, are expected to be replaced by BC in terms of their use as eye scaffolds (Dutton 1991).

4.5. Dental Implant Material

Compatibility of dental implants used in dental health with the surrounding tissue is a challenging problem. Osseointegration is especially necessary between the dental bone tissue and the implant. The high mechanical properties of bacterial cellulose, its compatibility with the surrounding tissue, its ability to not lose its properties under wet conditions and its absorption capacity are proof that it can be used in dental health applications (Choi S et al., 2022).

Yoshino et al. (2013) evaluated the use of BC as a root canal treatment material for intradental abscess formation. In this study; Cellulose strains were prepared using BC membranes produced by *Acetobacter hansenii* (ATCC 700178 and ATCC 35059). The mechanical properties and drug release efficiency of BC were found to be higher under simulated conditions, thus indicating that bacterial cellulose has high potential for root canal treatments.

Olyveira et al., (2014) reported that the use of BC as dental biometry produced significant results. In addition, biomimetic precipitation of calcium phosphate, a biological concern on bacterial cellulose, has been studied by mimicking body fluids. Chondroitin sulfate activity in bacterial cellulose was analyzed by characterization studies and confirmed that calcium phosphate participates in the uniform spherical form of bacterial cellulose nanocomposite surface and calcium phosphate particles. They point out that future work will lead the cell adhesion and vitality feature.

4.6. Drug Delivery Systems

In recent years, chitosan, alginate, cellulose, etc. have been used for drug delivery systems. A lot of biomaterial research has been carried out including natural biopolymers. Especially biomaterials containing BC; It is frequently used in drug delivery system applications due to its high pore size, non-toxicity to living beings, fine structure and biodegradability. Drug delivery materials prepared using BC; It can provide drug release in a controlled manner through impregnation by providing thin film layers.

Amin et al., (2012) carried out studies to determine the usage areas of BC in drug delivery systems, hydrogel syntheses and drug applications. First, for the production of hydrogels, BC was exposed to electron beam at different rates and then acrylic acid (AA) was added. Characterization analysis determined that AA was successfully impregnated onto cellulose fibers and the reaction mechanism in hydrogel synthesis was predictable. Other analyzes showed the formation of thermally stable hydrogels with AA

content and the pore size determined by the irradiation dosage. The results of swelling and in vitro drug release studies revealed that hydrogels are both thermo and pH sensitive. In addition to the morphological properties of these thermo and pH responses, these BC / AA hydrogels are promising for future use of controlled drug delivery systems.

Silva et al., (2014) investigated the roles of BC membranes in the transdermal drug delivery system for anti-inflammatory drug. Bacterial cellulose films loaded with diclofenac sodium salt as anti-inflammatory were prepared using plasticizer (glycerol) and characterization tests were performed for its morphology. In vitro studies performed with Franz cells showed that the incorporation of diclofenac into bacterial cellulose membranes provided penetration rates similar to those achieved with other commercially available membranes. This widespread profile facilitates the incorporation of drug loading membranes to ensure ease of administration and excellent potential for the use of membranes for transdermal delivery of diclofenac.

4.7. Other Researchs

Evans et al., (2003) BC contrary to plant cellulose, it is beneficial to catalyze the accumulation of metals to form a finely disunited homogeneous catalyst lamina. Empirical data in the literature have shown that BC, aqueous solution of palladium, reducing groups has the capacity of initializing gold and silver precipitation. In the research conducted by Evans et al., (2003) BC was dehydrated to a thin membrane texture acceptable for the construction of membrane electrode assemblies (MEAs), since it contained water equivalent to at least 200 times the dry weight of cellulose. The results of working with palladium-cellulose have shown that when incubated with sodium dithionite, they can catalyze the formation of hydrogen and form an electric current from the hydrogen in an MEA containing a natural cellulose as a polyelectrolyte membrane (PEM). The advantages of using natural and metallized bacterial cellulose membranes in an MEA compared to other PEMs such as Nafion 117® are reported to be the higher thermal stability of the gas passage at 130 °C and lower. Gadim et al., (2016) described the characterization of a Nafion® / bacterial cellulose (BC) nanocomposite prepared by impregnating a nanofibrillar BC membrane with Nafion®. Such a nanocomposite membrane is crack-free and has a thickness close to 100 μm and has been shown to be applied in an air / hydrogen fuel cell membrane Nafion® / BC membrane.

Meniscus lesions due to alternating cell damage after a trauma or in the absence of any trauma, damage or tumor are a frequently seen problem in

society. Once the meniscus is removed, it cannot regenerate. Lesions grow and cause osteoarthritis. Collagen meniscus implants have been used in clinical applications to structure the meniscus tissue and provide function. Bodin et al., (2007) matched the properties of BC with porcine meniscus using a collagen material. The collagen meniscus implants were used in clinical practice to reconstruct the meniscus tissue after partial meniscectomy. the swine meniscus is clearly stronger in the higher compaction strain due to the regular and ordered structure of the collagen fibrils in the meniscus. BC can be produced as a meniscus that is cheap and easy to combine and promotes cell migration, making it an attractive material for meniscus implants.

Silver has been the most commonly exploited and used inorganic metal against infections since antiquity. Several ways in which silver ions kill bacteria have been mentioned in the literature as follows. First is the interaction of silver ions with thiol groups of enzymes and proteins important for bacterial respiration and cell wall and transport of the parent substance in the cell. and the binding of silver ions to the bacterial cell membrane and the outer bacterial cell by altering the function of the bacterial cell wall (Cho et al., 2005; Sondi et al., 2004; Percival et al., 2005). The silver metal is converted into silver ions slightly with the physiological system and interacts with the bacterial cells. Knowledge exists that silver nanoparticles with effective antibacterial, antifungal and antiviral properties are attractive antibacterial agents (Rai et al., 2009). Maneerung et al., (2008) used silver nanoparticles to create BC antimicrobial activity. The findings indicate that freeze-dried silver nanoparticle-impregnated BC is important for gram-negative and (*Escherichia coli*) gram-positive (*Staphylococcus aurous*) bactericides.

Acasigua et al., (2014), they modified the bacterial cellulose fermentation process by adding hyaluronic acid and gelatin (1% w / w) before the bacteria were inoculated. Characterization of bacterial celluloses affected by hyaluronic acid and gelatin was analyzed and adhesion and viability studies were performed with human female pulp stem cells using natural bacterial cellulose / hyaluronic acid as a skeleton for regenerative medicine. MTT viability assays have reported higher cell adhesion over time in bacterial cellulose / gelatin and bacterial cellulose / hyaluronic acid scaffolds with differences in fiber agglomeration in bacterial cellulose / gelatin. Thus, the use of bacterial cellulose in stem cell cells has been reported for the first time in this study.

There are a number of studies that have been in use and continue to be investigated. These studies are shown in Table1.

Table 1. Application areas of BC composites

Biomedical and Pharmaceutical Applications of BC-Based Composites	
Artifical Blood Vessels	Contact Lenses
Bionalysis	Transdermal Patches
Meniscus Implants	Biosensors
Cardiovascular Tissue Replacement	Bone Tissue Regeneration
Artifical Endocranium	Anti- viral Film
Hemodialysis	Vertabral Disc Replacement
Drug Delivery and Enantiomer Separation	Ligaments and Tendons Substitues
Stem Cell Teraphy	Anti-microbial Wound Dressing
İmmobilization of Enzymes and Cells	Dental Root Canal Treatment
Tissue Engineering of Cartilage	Artifical Cornea

Conclusion

Bacterial cellulose is a naturally occurring, renewable polymer with a wide range of uses. This type of polymer is obtained from the bacterium *Gluconacetobacter xylinus*. BC; It is a polymer that does not contain lignin and hemicellulose and has high mechanical properties, purity, crystallinity and an unchanging structure. Unique properties such as high water retention capacity and good chemical stability make BC unique. BC can be produced in almost any shape due to its high malleability. BC is a truly interesting, emerging biomaterial that has proven to be useful in various aspects in biomedical application. BC has a structural appearance that is far superior to plant cellulose.

In this article review, BC and BC composite materials were examined in general and the use of BC-related nanocomposites such as collagen, gelatin, fibroin, chitosan, silver, alginate, hydroxyapatite, BC nanocomposites were examined along with the examined materials. Therefore, this paper revisited and presented a number of different BC and BC composite materials designed for biomedical applications (wound dressings, cell scaffolds, drug delivery systems), among other descriptions. Based on this, we concluded that BC composites have many unique properties such as strength in their mechanical structure, high water retention capacity, in vitro and in vivo biocompatibility, and biodegradability. These include different composite BC membranes, wound dressings, dental prosthetics, skeletal and cartilage implants, and especially in biomedical fields such as drug administration.

BC exhibits excellent material properties alone or in composite form and can therefore be used as drug carriers, especially in topical and transdermal delivery systems. We also hope that this chapter can be shortened to bring together high-quality information from the literature to inspire the development of new materials on bacterial cellulose.

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