INTERNATIONAL RESEARCH AND REVIEWS IN HEALTH SCIENCES

EDITORS
PROF. DR. HASAN AKGÜL
PROF. DR. ZELİHA SELAMOĞLU

DECEMBER 2023

SERÜVEN YAYINEVİ

Genel Yayın Yönetmeni / Editor in Chief • C. Cansın Selin Temana

Kapak & İç Tasarım / Cover & Interior Design • Serüven Yayınevi

Birinci Basım / First Edition • © Aralık 2023

ISBN • 978-625-6760-93-6

Serüven Yayınevi / Serüven Publishing
Türkiye Adres / Turkey Address: Kızılay Mah. Fevzi Çakmak 1. Sokak
Ümit Apt No: 22/A Çankaya/ANKARA
Telefon / Phone: 05437675765
web: www.seruvenyayinevi.com
e-mail: seruvenyayinevi@gmail.com

Baskı & Cilt / Printing & Volume
Sertifika / Certificate No: 47083

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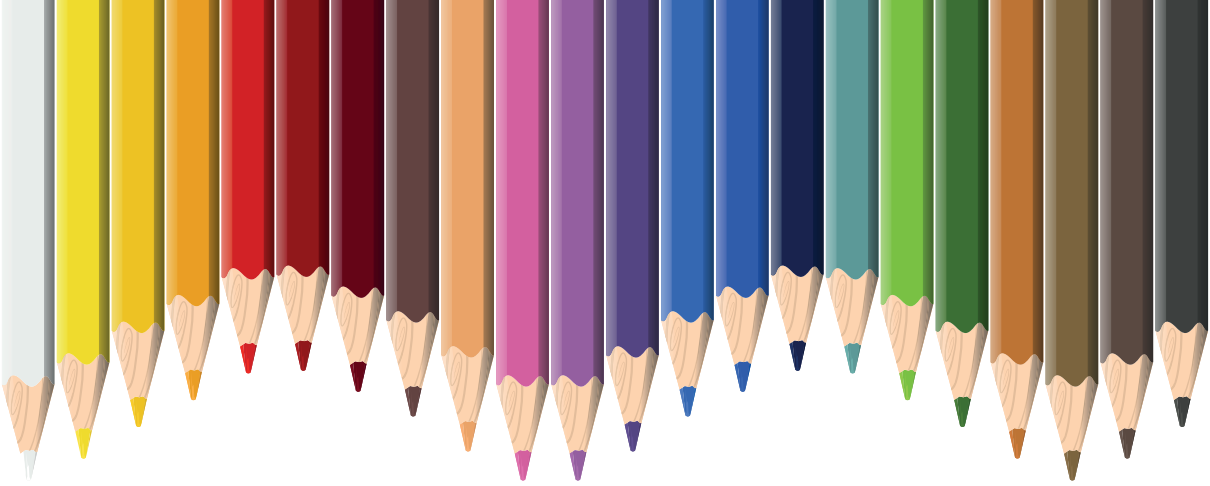

Chapter 1

DIAGNOSIS AND TRADITIONAL-CURRENT TREATMENT METHODS OF TRIGEMINAL NEURALGIA

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1.Trigeminal Neuralgia

Trigeminal neuralgia (TN) is characterised by attacks of sudden and severe pain affecting a specific branch of the trigeminal nerve (Maarbjerg, Di Stefano et al., 2017). The name tic douloureux is also used for TN (Kheirallah & Ozzo, 2020). This neuropathic pain syndrome most commonly manifests as paroxysmal pain attacks localized to one side of the face, and these attacks typically occur in short durations, often ranging from seconds to minutes (J. M. Zakrzewska & Linskey, 2014). 91-99% of patients report triggerable attacks and this is pathognomonic for the disease (Lambru, Zakrzewska et al., 2021). These pain attacks resulting from abnormal excitation in the maxillary and mandibular divisions of the trigeminal nerve are often defined by patients as sensations of electric shocks or a burning feeling. The intensity of this pain can significantly impact patients' quality of life (I. H. Society, 2018; J. M. Zakrzewska & Linskey, 2014). The ophthalmic branch of the trigeminal nerve is affected in fewer than 5% of TN cases (Obermann, 2010). The reported annual incidence of TN a is 4.3 cases per 100,000 individuals. In addition, the majority of patients are between 60-70 years of age and it is rare to be seen before the age of 40 (Krafft, 2008). In a study of 1040 patients in a paediatric headache clinic in 2021, 5 children aged 9.5-16.5 years were diagnosed with TN (Brameli, Kachko et al., 2021). The risk of TN in hypertensive patients is also higher than in the general population (Oliveira, Baaklini et al., 2009).

The precise etiology of TN remains partly elusive; however, it is frequently associated with neurovascular compression, wherein blood vessels exert pressure on the trigeminal nerve, leading to irritation and ensuing pain (Maarbjerg, Di Stefano et al., 2017). Some individuals may have an underlying medical condition, like multiple sclerosis, which can affect the myelin sheath of the trigeminal nerve and contribute to TN (Cruccu, Finnerup et al., 2016). Additionally, genetic inheritance has also been established as a factor in TN (Panchagnula, Sularz et al., 2019).

TN is usually treated with medication. Antiepileptic drugs such as gabapentin, carbamazepine, baclofen, clonezepam are used in pharmacologic treatment (Miloro, Ghali et al., 2011). Failures in medical treatments lead to the need for some surgical treatments. Surgical treatment includes peripheral injections of neurolytic agents, peripheral neurectomy, cryotherapy, microvascular decompression of the affected vascular loop (Janetta procedure), gammaknife radiation surgery, balloon compression of the root entry site (Erdem & Alkan, 2001; Hupp, Tucker et al., 2013).

1.1.Symptoms, Diagnosis Process

The symptoms of TN are often known as paroxysmal pain, unilateral pain (96%) (right>left), absence of pain between attacks, absence of dental factors, and pain triggered by mild superficial stimuli (trigger points). In these patients, local anesthesia applied to the trigger point temporarily stops the pain (Hupp, Tucker et al., 2013). The higher occurrence of pain on the right side in TN is linked to the right-side foramen rotundum and foramen ovale being narrower (Love & Coakham, 2001). Most patients do not experience pain during sleep (Türp & Gobetti, 1996).

Sweet diagnostic criteria defined in 1969 are also used for the diagnosis of TN. Sweet's diagnostic criteria include mild touch-onset (at trigger points), paroxysmal, unilateral pain localized to the innervation area of the trigeminal nerve and a normal clinical neurosensory test (Miloro, Ghali et al., 2011). Furthermore there are diagnostic criteria recommended by the International Headache Society for classic TN (Table 1), and the classic type accounts for 75% of TN. Nevertheless, all of patients doesn't have these conditions. Atypical TN is used for patients who do not meet these criteria (Jurge, 2016).

1.Paroxysmal attacks of facial or frontal pain, lasting from few seconds up to 2 minutes
2.At least 4 of the following characteristics are fulfilled: **a. Pain is distributed in one or more divisions of the trigeminal nerve** **b. Sudden, intense, sharp, stabbing, superficial or burning quality** **c. Severe intensity** **d. Presence of trigger areas or pain can be provoked by daily activities such as** **talking, eating, washing the face, or brushing the teeth** **e. Symptom-free periods between pain attacks**
3.No neurological deficit
4.Pain attacks are stereotyped in individual patients
5.Other causes of facial pain are excluded by history, physical examination and special investigations, if required

Table 1. *Criteria for classical trigeminal neuralgia determined by the International Headache Society.(T. I. H. Society, 2004).*

In these patients, pain originates from trigger points and patients develop severe pain attacks as a result of mechanical stimuli (shaving, brushing teeth, applying makeup) that trigger extraoral or intraoral pain. Trigger points are usually diagnosed as nose, upper lip, teeth, cheek, lower lip (Kheirallah & Ozzo, 2020). In TN, light touch is the most effective stimulus for the development of a pain attack. Painful stimuli and thermal stimuli do not initiate a pain attack. The frequency of pain attacks is variable. Most of them (74%) last from 1 s to 2 min, but in a very few cases they may last up to 10 min (Lambru, Zakrzewska et al., 2021). Patients describe the pain as more intense than any they have ever felt before, like an electric shock. Patients diagnosed with TN have usually undergone root canal treatment in the past due to confusion with toothache. As we mentioned before, patients' pain is usually unilateral, while bilateral pain is seen in multiple sclerosis (Cruccu, Finnerup et al., 2016). Different clinical symptoms can be observed in some patients with TN. These patients do not experience paroxysmal but persistent pain in the sinus region or in the teeth, ranging from minutes to hours. This condition is called pre-TN and it is thought that patients may have TN in the future (Okeson, 2014).

When no clinical features can exclude secondary TN, magnetic resonance imaging (MRI) should be used in the diagnostic process.Also in patients unresponsive to medication, MRI is recommended to exclude a structural anomaly. MRI is also required in patients with bilateral pain or patients with atypical symptoms. In addition, in patients with a dual diagnosis of multiple sclerosis and TN, a MRI scan should be considered to exclude pain due to multiple sclerosis. (Darlow, Brooks et al., 1992). If MRI is contraindicated or difficult to access, trigeminal reflexes should be checked. Trigeminal reflexes are used o identify damage to the trigeminal afferent nerves in individuals experiencing diverse neuropathic pain disorders. (L Bendtsen, Zakrzewska et al., 2019). Three-dimensional fast-in-flow steady-state precession is another of the most important examinations in TN. Mostly neurosurgeons perform MRI and three-dimensional fast-in-flow steady-state precession examinations before microvascular decompression operation to analyse the anatomy of the region (Joanna M Zakrzewska, 2002). MRI shows that 70% to 83% of patients with TN have neurovascular involvement (Darlow, Brooks et al., 1992).

Although TN is generally considered to be an easily identifiable disease, some important differential diagnoses should not be ignored. Below are the differential diagnoses of TN (Lambru, Zakrzewska et al., 2021).

- Dental origin
- Tooth decay
- Pulpitis
- Dental sensitivity

- Periodontal diseases
- Pericoronitis
- Cracked tooth
- Dry socket
- Sinus causes
- Maxillary sinusitis
- Salivary gland causes
- Stone of salivary gland
- Temporomandibular joint causes
- Temporomandibular disorders
- Neuropathic pain
- Glossopharyngeal neuralgia
- Nervus intermedius neuralgia
- Post-herpetic neuralgia
- Post-traumatic trigeminal neuropathy
- Painful trigeminal neuropathies
- Atypical odontalgia
- Burning mouth syndrome
- Trigeminal autonomic cephalalgias
- SUNCT(short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing) / SUNA(short-lasting unilateral neuralgiform headache attacks with cranial autonomic symptoms)
- Paroxysmal hemicrania
- Cluster headache
- Hemicrania continua
- Other
- Persistent idiopathic facial pain
- Primary stabbing headache

1.2. Anatomy of the Trigeminal Nerve

The trigeminal ganglion, found in Meckel's cavity, is both the largest sensory ganglion and the sole sensory ganglion within the cranium. It is also

called the semilunar ganglion and Gasser ganglion. The trigeminal nerve is cranial nerve number 5. It is the largest cranial nerve. Each of its branches has 3 main branches (Singh, 2019). There are three branches within the trigeminal nerve. These branches are ophthalmic (V1), maxillary (V2), mandibular (V3).

V1 (opthalmic nerve) is the smallest branch of the trigeminal nerve and leaves from the fissura orbitalis superior. This nerve branch is pure sensory nerve. It is divided into frontal, lacrimal and nasociliary branches. It also gives fine nerve fibers to the oculomotor, abducens, trochlear nerves. It receives sensations of the orbit and scalp.

V2 (maxillary nerve) branch leaves the foramen rotundum. It has branches from the pterygopalatine ganglion, branches from infraorbital nerve andzygomatic nerve. It is purely sensory nerve. Maxillary nerve receives sensory sensations of the nasal and oral cavities, maxillary teeth and skin over the zygomatic bone.

V3 (mandibular nerve) mandibular branch leaves the skull through the foramen ovale and has sensory and motor fibers. It has motor nerves arising from the forearm, lingual branch, and inferior alveolar branch. Sensory fibers innervate the mandibular teeth, surrounding tissues, tongue, floor of the mouth, skin of the jaw and cheek. Motor fibers innervate the masticatory muscles and mylohyoid muscle (Bathla & Hegde, 2013; Singh, 2019; Weaker, 2014). The innervation area of the trigeminal nerve in the scalp and oral mucosa is shown in Figure 1.

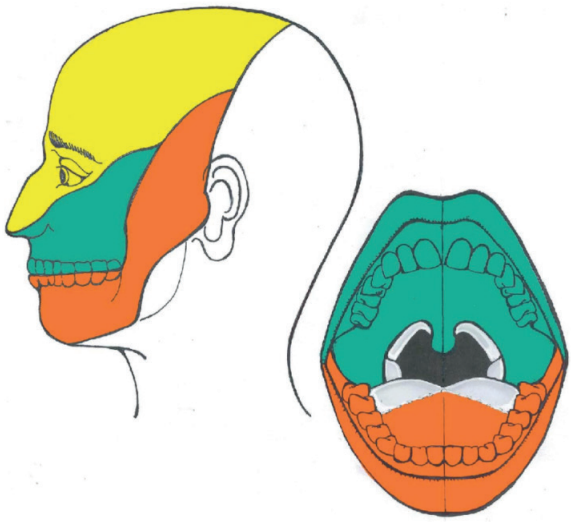

Figure 1: Innervation of the trigeminal nerve on skin and mouth (Yellow: Opthalmic nerve, Green: Maxillary nerve, Orange: Mandibular Nerve) (Cruccu, Finnerup et al., 2016)

1.3.Classification of Trigeminal Neuralgia

Those classifications most frequently utilized for TN include the Association for the Study of Pain and the International Headache Society.

According to the Association for the Study of Pain classification, there are three types of TN; Idiopathic, Classical, Secondary.

1. Idiopathic Trigeminal Neuralgia refers to a pain condition with an undefined cause.

2. Classical Trigeminal Neuralgia is a disease in which the trigeminal nerve is subjected to vascular compression.

3. Secondary Trigeminal Neuralgia is a form of disease secondary to multiple sclerosis or any tumor affecting the nerve (L. Bendtsen, Zakrzewska et al., 2020; Dube, 2019).

According to the classification of the International Headache Society, TN is divided into 2; Classic, Symptomatic.

1.Classic form of trigeminal neuralgia is the more common form of the disease, with painless periods between bouts of pain, severe and sharp pain that causes a sensation of electric shock or stabbing. The classic type is induced by the vascular compression of the trigeminal nerve.

2. The symptomatic form of trigeminal neuralgia is characterized by continuous aching, throbbing and burning pain. Symptomatic form is caused by intracranial tumors and multiple sclerosis (L. Bendtsen, Zakrzewska et al., 2020; Dube, 2019).

In terms of treatment modality, distinguishing classical TN from the symptomatic form is importance. The symptomatic form of the disease is secondary to an underlying disease and the underlying cause needs to be treated (Krafft, 2008).

2.Treatment Methods

2.1.Traditional Treatment Methods

There are various treatment options for TN. Firstly, medical treatment is initiated and surgical treatment is used in patients who do not respond (Figure 1).

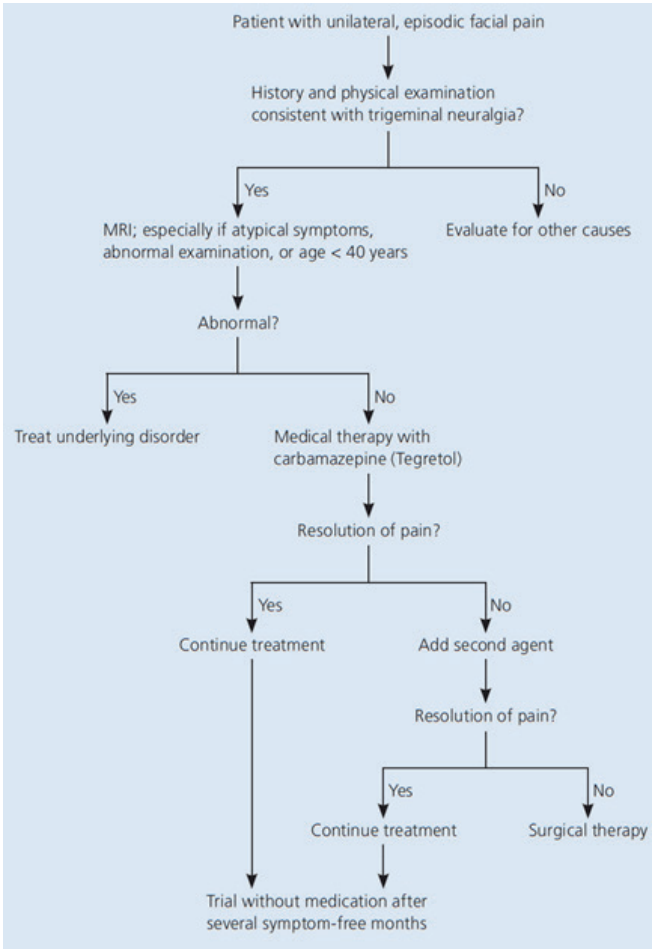

Figure 2: Trigeminal neuralgia diagnosis and treatment algorithm (Krafft, 2008).

2.1.1.Pharmacologic Treatments

The first step of treatment in TN is usually medical therapy. In the newly diagnosed patient, start with 100 mg carbamazepine daily and increase by 100 mg till the pain is relieved. The maximum daily dose is recommended as 1200 mg (Al-Quliti, 2015; Giulia Di Stefano & Truini, 2017). A 56% reduction in maximum pain with the use of carbamazepine 400-800 mg daily for 2 weeks has been reported in the literature (Campbell, Graham et al., 1966; G Di Stefano, Truini et al., 2018). Since periods of remission will occur with the use of medication, dosage should be adjusted according to pain intensity and side effects. (L. Bendtsen, Zakrzewska et al., 2020). In addition, in the Rasmussen and Riishedenin study, placebo and carbamazepine were used in 55 patients and carbamazepine showed good effect in 46 of these patients (G Di Stefano, Tru-

ini et al., 2018; Rasmussen & Riishede, 1970). Oxcarbamazepine is a drug with similar efficacy but more side effects than carbamazepine. However, its better tolerability increases its availability (Al-Quliti, 2015; G Di Stefano, Truini et al., 2018). The second-line medication of choice are baclofen, lamotrigine, gabapentin, phenytoin and pregabalin. These drugs can be used alone or in combination with carbamazepine and oxcarbazepine (Khadilkar & Patil, 2021). Fewer side effects are observed with lamotrigine in comparison to carbamazepine and oxcarbazepine. However, skin rashes may occur as the dose of lamotrigine increases. Therefore, it is recommended to increase the dose slowly. Rarely, it may cause Stevens-Johnson syndrome. Since slow dose titration is required, It is not recommended for treatment of acute patients requiring rapid pain control. Gabapentin and pregabalin give successful results in addition to treatment. However, they should be used with caution as they are addictive drugs. Baclofen can also be an adjunct to treatment, especially in patients with multiple sclerosis. In patients in the acute phase, intravenous administration of fosfenitoin and lidocaine can be administered under monitoring. However, caution should be exercised during administration. These injections should be administered in high dependency unit (Lambru, Zakrzewska et al., 2021).

2.1.2.Chemodenervation

Peripheral injections have been used for a long time. Neurolysis of the peripheral branch where the pain is felt by injections is a treatment option. Agents such as glycerol, phenol, boiled water, chloroform are used in these injections. Peripheral alcohol injection is frequently preferred in patients with severe systemic diseases in whom advanced surgeries cannot be performed (Tiwari, Agrawal et al., 2019). A pain-free relief period of 6-16 months is observed after peripheral alcohol injection (Shah, Khan et al., 2011). Complications such as avascular necrosis, skin necrosis, trismus, osteomyelitis, facial paresis can be seen in patients after alcohol injection.. Phenol can be used alone or in combination with glycerol. Glycerol is a transparent, thick liquid that can also be used alone as a neurolytic agent (Xu, Xie et al., 2021). It has been reported in the literature that glycerol injection is a simpler and more tolerable procedure compared to other surgical procedures (Erdem & Alkan, 2001). In addition, ultrasound-guided injection can be applied to increase the success of these injections (Takechi, Konishi et al., 2015). The second option for chemodenervation is to inject the ganglion or nerve root with a neurolytic agent such as glycerol (Maarbjerg, Di Stefano et al., 2017). Complications such as corneal numbness, dysesthesia, masseter weakness have been reported in percutaneous glycerol gangliolysis (Küçükkurt, Tükel et al., 2019).

2.1.3.Local Anesthetic Injections

Local anesthetics are used for diagnose and treatment of TN (Vlassakov, Narang et al., 2011). Peripheral local anaesthesia administered in high

concentrations has a neurolytic agent-like effect (Isozaki, Ito et al., 2023). In acute patients, local anaesthetic injections or lidocaine spray in the trigger areas may relieve the pain for a while (Lambru, Zakrzewska et al., 2021). Local anesthetic injections are preferred to avoid the complications of injection of agents such as alcohol and phenol. Local anesthetics are thought to have a therapeutic effect in TN by blocking voltage-gated sodium channels (Dergin, Gocmen et al., 2012). Local anesthetics used for the treatment of TN are tetracaine and bupivacaine (Peters & Nurmikko, 2002). Local anesthetics were first used for therapeutic purposes by Dr. Adler. In this study, Adler injected bupivacaine into the ganglion to treat major trigeminalgia. Patients were reported to be free of paroxysmal pain for several months to several years after injection and were more easily treated with carbamazepine (Adler, 1975). A combination of two anesthetics is also used. Injection of highly concentrated tetracaine crystals dissolved in bupivacaine has also been reported to be successful in treatment (Takechi, Konishi et al., 2015). It was also reported that the disease was painless at 9-month follow-up after repeated local anesthetic injections every month for 1 year (Naja, Al-Tannir et al., 2006). Complications of this procedure include dysesthesia, chemical neuritis, facial palsy and oral ulceration (Isozaki, Ito et al., 2023).

2.1.4.Surgical Interventions

Surgical treatments are used in patients with TN if medical treatment fails or if severe side effects of medications are observed. Medical treatments also fail in 30% of cases. Peripheral neurectomy is performed by surgically cutting the peripheral end of the nerve where pain is felt in TN. Local anesthetia is used during the procedure. (Nagy & Mahmoud, 2021). Although most surgical procedures in the treatment of TN fall within the field of neurosurgeons, periperipheral neurectomy is often performed by oral and maxillofacial surgeons (Yuvaraj, Krishnan et al., 2019). After neurectomy, patients may experience a 1-2 year period of relief followed by recurrence. To prevent recurrence in peripheral neurectomy, it is recommended to place materials such as bone wax, stainless steel screws, titanium screws in the foramen after neurectomy. The interposed barrier material prevents nerve regeneration. No recurrence is reported up to 4 years in patients with stainless steel screws (Nagy & Mahmoud, 2021). Peripheral neurectomy is more acceptable by patients because it is simpler and less costly than other surgical interventions. This intervention is also preferred for tolerability in extremely elderly people with systemic diseases (Agrawal & Kambalimath, 2011).

The term “cryotherapy” means using cold to treat. The application of cold causes a reversible pain relief in the nerve. Liquid nitrogen, nitrous oxide, carbon dioxide are used as cryotherapy agents. These agents are applied by spraying, direct application or through a cryoprobe (Bindra, 2019). In the treatment of TN, cryotherapy is a method with similar results to other treatments but

with fewer complications. Therefore, it is preferred (Menon & Muthusekhar, 2020). Selective percutaneous radiofrequency thermocoagulation is also a preferred surgical method because it is reproducible, cost-effective, has few complications and is not as invasive as cryotherapy and neurectomy (Agrawal & Kambalimath, 2011; Wan, Zhang et al., 2017).

Microvascular decompression is the surgical method with the best results in patients in whom the procedure can be performed. This method is based on the rationale that TN is caused by chronic compression of the vessels, which compresses the trigeminal nerve. The arteries most commonly reported to cause TN are the superior cerebellar artery, anterior and posterior inferior cerebellar arteries. General anesthesia is administered for the procedure. During the operation, the trigeminal nerve root is accessed and the vessel is repositioned and stabilized (Borkar, Agrawal et al., 2019). There are complications such as facial nerve palsy, hearing loss, middle ear effusion (M. H. Lee, Jee et al., 2016).

There are also some percutaneous invasive approaches. Percutaneous Radiofrequency Thermocoagulation is performed in the gasser ganglion under fluoroscopic control. Heat of 60-80 degrees Celsius is applied to the nerve for 60-90 seconds. Percutaneous Retrogasserian Glycerol Rhizolysis is a procedure often applied to elderly patients. In the procedure, glycerol is injected into the gasser ganglion. The compression of the balloon placed in the Percutaneous Balloon Compression of the Trigeminal Nerve procedure damages the nerve and prevents it from delivering pain signals. It has been reported that patients have recurrence of pain within 3 years after the procedure (Hupp, Tucker et al., 2013; Miloro, Ghali et al., 2011; Umamaheshwara Rao & Joshi, 2019). Gamma knife radiosurgery is an accepted type of surgery for the treatment of TN. The complication with the highest occurrence is facial hypoesthesia (Barzaghi, Albano et al., 2021). Gamma knife radiosurgery has been reported to provide 75% pain relief in the first year, 50-60% after 5 years and 30-40% after 10 years. It is preferred because it is less invasive than other surgical methods. (S. Lee & Lee, 2022). In a different source, pain-free periods after microvascular decompression and gamma knife surgery were compared. The rate of 1-2 year pain-free period after surgical interventions was reported as 68%-88% in microvascular decompression and 24-71% in gamma knife surgery. The percentage of patients with 4-5 years pain-free period was reported as 61%-88% in microvascular decompression and 33%-56% in gamma knife surgery (L Bendtsen, Zakrzewska et al., 2019).

2.2.Current Treatment Methods and Innovations

2.2.1.Current Pharmaceutical Developments: Vixotrigine and Eslicarbazepine

A novel sodium channel blocker (Nav1.7 selective) for the treatment of TN is under development. Vixotrigine was discovered in 2006 and was first

used to treat depression and bipolar disorder. Vixotrigine (BIIB074) treats seizures by blocking high-frequency impulses during seizures. It was found to be successful compared to placebo in a phase 2 trial. No serious side effects were seen(G Di Stefano, Truini et al., 2018; Obermann, 2019). Eslicarbazepine is an extended-release formula of oxcarbazepine. The drug inhibits Cav3.2 calcium channels with higher affinity than carbamazepine. The efficacy and safety of eslicarbazepine have not yet been established (Kwon & Min, 2023).

2.2.2.Botox Injection

Botulinum toxin substance P, an exotoxin released by Clostridium botulinum, inhibits the release of mediators that cause inflammation and pain, such as CGRP and glutamate. It has been added to the European Academy of Neurology guideline as a therapeutic (L. Bendtsen, Zakrzewska et al., 2020). Botox is used in patients with cronic migraine and gives successful results. It has been reported in the literature that botulinum toxin type-A application reduces symptoms in TN. The application is made in the area of pain subcutaneously or over the gingival mucosa. In addition, placebo, 25U and 75U injections were compared in a study and no difference was found between 25U and 75U injections, but it was found to be effective compared to placebo. Wu et al. stated in their study that success with Botox injections was more pronounced in patients over the age of 50. The side effects of this injection are often asymmetry and bruising that resolves within a few days. (G Di Stefano, Truini et al., 2018; Kwon & Min, 2023; Obermann, 2019; Wu, Lian et al., 2019).

2.2.3.Pain pump

The pain pump is based on the principle of pain relief through the continuity of the injection. In the literature, Umino et al. first presented a treatment method by placing a catheter to anesthetize the mandibular nerve and applying regular anesthesia in 2002 (Umino, Kohase et al., 2002). Later in 2002, Dergin et al. connected a pain pump to the relevant peripheral nerve and administered 1 ml of bupivacaine HCL per hour for 60 hours. In the 9-month follow-up of the patients, a significant difference was found compared to the baseline (Dergin, Gocmen et al., 2012).

3.Conclusion

TN is a disease that significantly reduces the quality of life of patients. Patients have difficulty in performing daily tasks, even eating and drinking. Dentists play a major role in the diagnosis of this disease. A detailed knowledge of the disease will help to avoid unnecessary dental treatments. In case of unidentified pain, referral should be made to an oral & maxillofacial surgeon or neurologist. There are many alternative methods in the treatment process. Alternative methods should be investigated in treatments that do not get a response.

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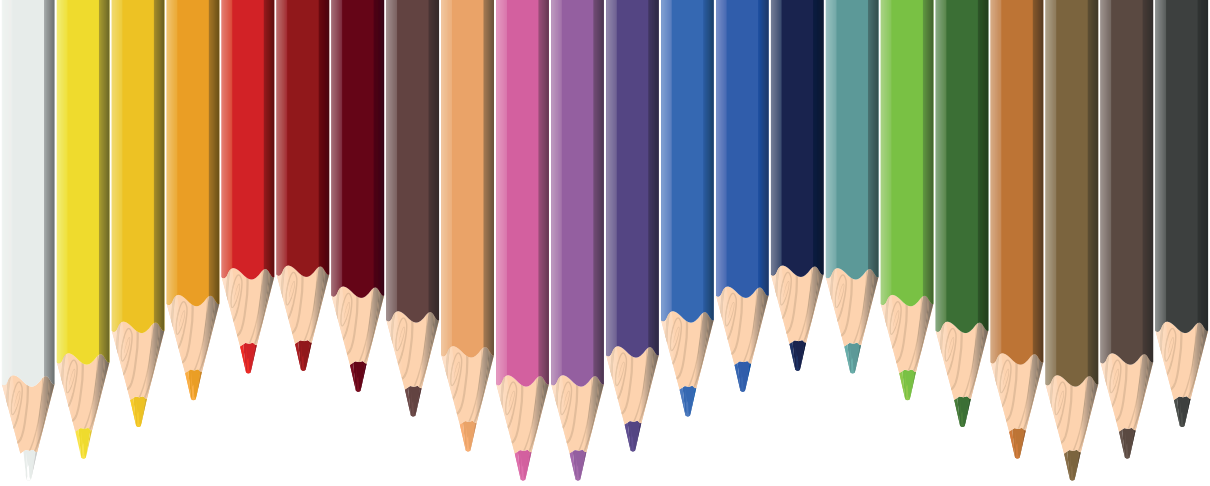

Chapter 2

ALTERNATIVE FOOD SOURCES CELLULAR MEAT AND ANALOGS IN THE PERSPECTIVE OF SUSTAINABLE NUTRITION

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Introduction

The increasing world population brings forth numerous challenges, with nutrition being a primary concern. Scientists are actively engaged in research to address issues related to meeting the energy and nutritional needs of the growing population, fostering the development of healthy generations, and improving the treatment of diseases transmitted through food. Studies are also conducted on proteins, crucial components in our nutrition and integral building blocks of the human body (Auclair and Burgos,2021; Päivärinta et al.,2020). Meat, a protein source derived from animal origins and considered a high-quality protein, has been a preferred food since ancient times. Beyond serving as a significant energy source from ancient hunter-gatherer eras to the present, meat is widely consumed by people due to its high-quality protein content, flavorful attributes, and association with a perception of strength (Latvala et al.,2012). Per capita meat consumption has more than doubled between 1961 and 2007, exhibiting a faster growth rate in developing countries compared to developed ones (Kumar et al.,2017).

The global market for meat analogues is currently dominated by some European countries, principally Germany, Italy, Belgium, Norway, and the Netherlands, which are at the forefront of innovation in the sector of meat protein alternatives (Costa Catala et al.,2023). The rapid increase in population, particularly in both developed and developing nations, is anticipated to lead to a substantial rise in urbanization, industrialization, and dietary demands. This trend is projected to significantly elevate the demand for meat consumption, potentially increasing by as much as 72% by the year 2030 (Kumar et al.,2017; Stinfeld et al.2006). In their study, Steinfeld et al. (2006) projected that the global production of animal-based food, which was 229 billion kilograms for a population of 6.0 billion in the year 2000, would double by 2050, reaching a total of 465 billion kilograms for a projected population of 9.1 billion. Furthermore, meat production has shown a growth of 5-13% in the past decade, approaching its maximum production. Alongside this significant increase, consumers ethical and environmental concerns regarding meat production and consumption have also begun to escalate (Post,2012). As a solution to these challenges, scientists have initiated the development of new alternatives such as lab-grown meats, plant-based meat analogs, and edible insects (Nowacka et al.,2023). Achieving broad acceptance and implementation of these novel alternatives requires a high degree of societal coordination for the potential integration of the technological, organizational, and institutional innovations necessary (Van der Weele et al.,2021).

Researchers are particularly focusing on the investigation of lab-grown meats produced from specific cells, aiming to appeal to the broader society (Post,2012;Van der Weele et al.,2021). Synthetic meats produced in cellular environments can be defined as cultured meats that are derived from cells

proliferating in bioreactors within a controlled culture environment, exhibiting no compromise in animal protein production .These synthetic meats, thus produced, hold the potential to provide a sustainable solution to the increasing demand for animal proteins in society, addressing both environmental and nutritional challenges (Faustman et al.,2020). In a study comparing the nutritional profiles of meat products and plant-based analogues, about half of all products used legumes as the main ingredient. Besides the soy was most frequently used in the form of soy, tofu or soybean flour. Some meat analogues used other legumes besides soybeans, such as 18% pea protein and 9% chickpea protein (Costa Catala et al.,2023). Similar to meat analogues, fish analogues are made from plant-based proteins such as soy, pea, wheat or algae. These products often contain natural flavors and other ingredients to replicate the taste and texture of fish. Some common types of fish analogs include plant-based fish fillets, as well as fish sticks and crab cakes, which can vary depending on the countries where they are available. Fish analogs are often enriched with omega-3 fatty acids, essential nutrients found in oily fish such as salmon, which can be difficult to obtain in a plant-based diet (Nowacka et al.,2023).

However, due to the lack of robust scientific arguments regarding ethics and the production method employed, there is no consensus on the health and nutritional qualities, as well as the potential low environmental impact of "cultured meat" for human consumption (Baybars,Ventura and Weinrich,2023). Additionally, numerous aspects related to the market, regulations, ethics, and consumer perception after meat production are expected to be addressed. The perspective on this novel product is highly influenced by various factors, including safety perception, sensory attributes, and, simultaneously, numerous factors related to environmental and nutritional issues, especially the price (Faustman et al.,2020). Hence, research conducted by public research institutions and accredited departments of universities indicates that the production of "cultured meat" does not confer significant advantages over traditional meat in terms of being more economical, nutritious, environmentally friendly, ethical, or socially beneficial. These findings trigger a cultural bias against cultured meat in society (Ellies-Oury,Chriki and Hocquette,2022). Therefore, diversifying existing plant-based and animal protein sources, consuming other meat alternatives, and reducing food losses and waste seem to be more effective short-term solutions for balanced nutrition compared to cellular meat.

Cell-Cultured Meat

The historical development of the concept of producing meat from cell culture systems dates back approximately to the 1930s (Faustman et al.,2020).

Cell-based meat, also referred to by various names in the literature, such as artificial meat, cell-cultured meat, cellular meat, clean meat, cultured meat, engineered meat, factory-grown meat, synthetic meat, in vitro meat, and lab-grown meat, has a history rooted in different nomenclatures (Kumar et al.,2017). In its simplest form, cell-based meat is defined as muscle grown without the involvement of an animal and its physiological processes (Post,2012;Kumar et al.,2017).

There are debates in the literature regarding the appropriate terminology for cell-based meat, with different authorities providing distinct definitions. For instance, publications from centers in the United States define meat as the parts of animals consumed as food, including skeletal and heart muscle, as well as organs and various meats (Boler and Woerner,2017; Seman et al.,2018). On the other hand, the United States Department of Agriculture (USDA) defines meat products as any product made entirely or partially from any meat or any part of the carcass (USDA,2019; Ong,Choudhury and Naing,2020).

The Food and Drug Administration (FDA) defines meat as a part of the skeletal muscle of any cattle, sheep, swine, or goat (FDA,2023). It has been suggested that cell-based meat, if derived from an animal cell, undergoes regulatory oversight for food safety, and provides nutritional and sensory characteristics comparable to traditional meat, could be considered as "meat" (Boler and Woerner,2017). For usage, both the FDA and USDA prefer the terms "cell-cultured product" or "cell-cultured food product" (Stephens, Sexton and Driesten,2019; USDA,2019). Unlike plant-based analogs or other meat alternatives, cell-cultured meat is obtained from muscle cells and is much closer to the skeletal muscles of animal species (Van der Weele et al.,2019). However, the resulting cultured muscle tissue is not technically considered meat (Hocquette,2019).

Therefore, it is not definitively known whether cell-based meat can provide essential components such as key minerals, creatine, carnosine, and B and D vitamins at the same level as traditional meat. There are numerous crucial points that need to be addressed in the functional engineering of meat, including all these aspects (Kumar et al,2017). The earliest patent related to the tissue engineering of cellular meat in the United States was granted in 2004 (National Center for Biotechnology Information,2023). However, it wasn't until August 2013 that researchers were able to present conclusive evidence for the production of cell-based meat when they introduced the first cell-cultured hamburger to the public (Faustman et al.,2020). As of 2023, over 35 companies worldwide have been engaged in efforts to produce cell-based meat. The primary goal of these companies is to produce these cell-cultured meats and analogs in a cost-effective and scalable manner that can compete with traditional meat (Cell Based Tech,2023).

Processes in the Production of Cell-Cultured Meat

In recent years, efforts related to the production and development of cell-based meat have been extensively documented and systematized through the endeavors of scientists. General cell culture and related requirements to cultivate a meat-like product have been thoroughly reviewed in various studies (Boler and Woerner,2017;Nowacka et al.,2023). The initial materials for the production of cell-based meats are myotubes (immature muscle cells) and myoblasts (satellite cells) that are challenging to replicate in vitro but can easily differentiate into myofibrils under suitable conditions. To facilitate the in vitro replication of skeletal muscle satellite cells, cells are attached to a stationary substrate, such as a scaffold or microbeads, which can be coated with proteins like collagen to mimic the original tissue. This coated scaffold must be edible, biodegradable during the culture process, or made from a material that can be reused to preserve resources (Stephens,Sexton and Driessen,2019). Satellite cells are cultivated in a regulated environment containing antibiotics, antifungal agents, or specific chemicals to prevent potential contamination, in a nutrient-rich medium. The protein content and quality in these cultured muscle cells primarily consist of contractile proteins. However, recent advancements in tissue engineering technologies enable the expression of other proteins crucial for the texture, color, and taste of food products cultured through cell culture. For instance, myoglobin protein is partially responsible for the pink or red color of meat and is naturally present in unprocessed meat. The presence of this protein in cellular meat could be advantageous both in terms of color and taste. Transcriptional regulation of myoglobin has been investigated by Kanatous and Mammen. According to their research, it was found that stimulating myoglobin synthesis before harvesting muscle cells could enhance the flavor of cell-cultured food products (Kanatous and Mammen,2010). Similarly, in studies resembling these findings, bioengineering allows for the removal of galactose-α-1,3-galactose (α-gal), a carbohydrate present in traditional meat, in cell-cultured meats (Kuhn,2020;Kuehn,2018). The elimination of this substance could mitigate the risks associated with red meat allergy in humans resulting from tick bites. Another focal point for producers, consumers, and scientists in cellular meat is the texture and flavor of the meat. However, there is limited research in the literature assessing the safety of substances used to enhance the texture and flavor of meat (Kuhn,2020). In some meat analogues, pigment extracts from red beets, red cabbage, red berries, red peppers and carrots are added to provide a natural meat color (Sha and Xiong,2020). Another concern in the cellular meat domain is potential biological hazards. While the subject is debated, plant-based ingredients are generally considered safer than meat, particularly concerning biological hazards (Fu et al.,2021).

A primary consideration in this context is the impact of intensive processing on product quality. Cellular meats contain significant amounts of protein, thus posing a risk of the formation of toxic substances such as heterocyclic aromatic amines (HAAs), N-nitrosamines, or polycyclic aromatic hydrocarbons (PAHs) that could be detrimental to health, similar to meat (He et al.,2020; Sha and Xiong,2020). On the other hand, there is also a risk of losing valuable nutrients and health-promoting components in plant-based products during processing (Choudhury et al.,2020). Factors influencing the safety of cellular meats include the presence of pathogenic bacteria from raw materials, anti-nutrient protease inhibitors, components like phytic acid, pesticide residues, heavy metal contamination, and the allergenic potential of specific plant proteins (Kanatous and Mammen,2010;Sha and Xiong,2020).

The storage conditions and shelf life of cellular meats are factors that influence their consumption and consumer preferences. Effective techniques need to be developed to extend the shelf life of products and ensure health safety. Additionally, the microbiological stability of the product is a crucial aspect affecting the safety of meat analogs. Various food additives are used to achieve this, but as consumers increasingly seek "clean-label" products, the use of these additives is undesirable. One method to preserve the quality of cellular meats, especially in terms of consumer preference, is the use of natural antioxidants. Natural antioxidants attract consumers' attention due to their consumption preferences. In addition to the use of natural antioxidants, modern preservation methods for food products, especially for meat, can be an interesting alternative to traditional preservatives. However, preservation processes, especially those involving high temperatures, can pose a risk of undesirable by-products in cellular meats. Therefore, special attention should be given to thermal methods that use low temperatures. However, the applicability of preservation methods for cellular meats needs validation. Attention should also be paid to consumer attitudes towards such product preservation techniques, and consumer education on this matter is crucial (Ur Rahman et al.,2018).

Cell-Cultured Meat's Role in Sustainable Nutrition

Global red meat consumption, according to the data from the Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization of the United Nations (FAO), reached an average of 20.1 kg per person in 2019. While the global average for beef consumption stands at 6.4 kg, Turkey exceeds the world average with a per capita consumption of 12.99 kg of beef (Tarım ve Orman Bakanlığı,2018). The worldwide meat consumption increased from 61 g/day per person in 1961 to 80 g/day per person in 2011 (Sans and Combris,2015). Concerns arise regarding the growing meat production, as it necessitates more land, water, and energy to raise a greater number of animals. This increase would contribute to a higher

carbon footprint for livestock and elevate global greenhouse gas emissions (Hocquette,2016; Poore and Nemecek,2018).

Additional concerns for the anticipated increase in traditional meat production stem from issues related to ethically raising animals for food, animal welfare, and perceptions that traditional meat may be harmful to human nutrition (Stephens et al.,2018;Post,2012). A study conducted in 2019 found that consumers' positive perceptions of cell-cultured meat were associated with reduced waste, decreased environmental impacts of farm meats, and improved animal welfare (Wilks and Phillips,2017). Determining consumer preferences for cell-cultured meat and different types of meat alternatives is also crucial. In the majority of studies in this regard, plant-based meat analogs have been found to be more acceptable to people compared to products like insects or cellular meat (Onwezen et al.,2021;Zhang et al.,2021).

Among plant-based meat alternatives more familiar to consumers, products like "tofu" made from soy, "yuba," and "seitan" produced from wheat protein are commonly used (Costa-Catalaet al.,2023). Consumer attitudes toward insect protein consumption have remained relatively low, likely associated with dietary neophobia (Wilky and Phillips,2017). Results from a study by Slade (2018) on consumer dietary preferences indicated that when given a choice between equally tasting beef, plant-based, and cell-cultured meat patties, consumers were likely to choose beef patties. Only 21% of participants expressed a preference for a plant-based patty, and 11% indicated a preference for a patty made from "cultured meat." A study involving consumers in Belgium revealed differences between those expressing a positive attitude toward plant-based meat analogs and those preferring cell-cultured meat. According to the study, plant-based analogs were found to be significantly more preferable for women and vegetarian individuals. In contrast, cell-cultured meat was identified as a more attractive option for men and individuals who prefer meat in their diets (Bryant and Sanctorum,2021).

Acceptability of Cellular Cultured Meat in Dietary Patterns and Sociocultural Contexts

The environment we are in, our country of residence, culture, and habits play a crucial role in the acceptance of new or unconventional food. Cellular cultured meat has numerous positive impacts for the meat production industry, such as high efficiency, energy savings, cleanliness, and robust controllability. Therefore, cellular cultured meat is gradually gaining significant importance in the future of food entrepreneurship (Wang,Liu and Zhou;2021). In the bioethical dimension of cellular cultured meat, there are many positive values for humanity's future development. A study (Siegrist, Sutterlin and Hartmann,2018) has concluded that cellular cultured meat has a significant impact on consumers' acceptance, consumption, and purchase decisions. An-

other study found that consumers' attitudes toward cell-based meat changed, especially when the food product was presented from a technological perspective, showing a more negative trend. The introduction of cellular cultured meat requires careful consideration of consumers' perceptions, particularly concerning genetically modified organisms (GMOs) and sociocultural perspectives (Bryant and Dillard,2019).

Furthermore, in the literature, the connection between animals and meat has been explored in different countries, examining concepts such as consumers' perceptions of naturalness, sustainability, religious perspectives, and affordability (Mohorčich and Reese,2019). Consumers have reported that the most significant determinants of their hesitations in preferring cell-based meat are food, hygiene sensitivity, and food neophobia (Wilks and Phillips,2017). In a study investigating consumer reactions to the concept of cell-based meat in Belgium, Portugal, and the United Kingdom in 2015, consumers expressed that the potential global benefits of cell-based meats replacing traditional meats were crucial. However, they often did not hold a positive view, citing concerns about the taste, health effects, and numerous unknowns associated with cellular meat (Verbeke et al.,2015). Similar results regarding the unnaturalness of cellular meat, along with potential environmental benefits, were reported in surveys with consumers residing in different countries (Baybars,Ventura and Weinrich,2023;Wilks and Phillips,2017; Bryant and Sanctorum,2021).

In a study conducted in Turkey, consumers' evaluations of cell cultured meat were examined. Turkish consumers' attitudes towards the consumption of cell cultured meat were found to be negative. Even if participants considered cell-cultured meat as a viable alternative to conventional meat, they did not consider it natural, healthy, tasty or safe. It was also found that Turkish consumers showed no interest or intention to try cell cultured meat on a regular basis (Baybars,Ventura and Weinrich, 2023).

A comprehensive analysis of 38 studies in a study revealed that consumers, in general, were not aware of the environmental challenges associated with traditional meat supply and appeared reluctant to reduce meat consumption, even in the context of the concept of sustainable protein consumption (Hartmann and Siegrist,2017). Similarly, a systematic review of 14 studies on the acceptance of cell-cultured meat by consumers in at least eight different countries found that the perceived greatest benefit of cellular meat compared to traditional meat was related to the welfare of animals (Bryant and Barnett,2018). In a study examining potential consumers of cell-cultured meat in the United States, approximately one-third of the participants were willing to consume the new product that would replace traditional meat. However, most participants indicated a preference for consuming traditional meat derived from animals such as fish, poultry, pork, and beef, over cell-based meat op-

tions from the same animals (Wilks and Phillips,2017).

Religious Suitability of Cellular Meat Consumption

In terms of religion, Islamic and Orthodox Jewish communities adhere to specific dietary rules. Studies have assessed the extent to which cell-based meat aligns with these rules and is considered acceptable for consumption by consumers (Chriki and Hocquette,2020). Hamdan et al. (2018) emphasized two crucial conditions for cell-based meat to be considered halal (permissible) and suitable for consumption from an Islamic perspective. Firstly, the stem cells required for the production of the product must be obtained from an animal that is slaughtered in accordance with Islamic law and is considered halal. Secondly, it was specified that there should be no blood or serum in the production process of cell-based meat. The feasibility of particularly the second condition is expected to be challenging given the current procedural practices. Similarly, concerns exist about whether cell-based meats can be deemed "Kosher," meaning permissible for consumption by Jews. A study on this matter indicated that the source of the cells and the culturing method would determine the suitability of cell-based meat for the Kosher definition (Kenigsberg and Zivotofsky,2020). Considering all these study results, more research is needed to reach a conclusion on the status of cell-based meat and/or meat analogs regarding whether they are halal or Kosher.

Reliability, Regulation, and Positive Aspects of Cellular Meat: Safety and Oversight of Cellular Meat Production

Safety is of paramount importance for new food substances/products, and safety assessment applies to the processes used to produce these substances. Cultured meat produced via cell culture is a innovative food product. It is classified as a novel food item. In light of this, a fitting appellation must be determined (Verbeke et al.,2015). Therefore, certifications demonstrating the step-by-step monitoring of all processes are required for safety. However, the regulation of safety certificates for the inclusion of chemicals necessary for tissue engineering remains unclear (Farhoomand et al.,2022; Ong et al.,2020). In 2019, the USDA and FDA reached an agreement on the responsibilities of agencies involved in regulating cell-based meat. According to this agreement, the FDA will oversee the collection and development of cells to be harvested in the production and inspection of cellular meat. The USDA, on the other hand, will be responsible for the regulation of the production and labeling of food products derived from cells. The labeling of cell-cultured meat will fall under the USDA's jurisdiction, and conditions for defining cellular meat as organic or natural will be established. Additionally, cell-based meat will not be considered a genetically modified substance (USDA,2019). The International Agency for Research on Cancer (IARC), affiliated with the World Health Organization (WHO), officially reported red meat and processed meat as sub-

stances with a high probability of being carcinogenic to humans (Bouvard et al.,2015). Moreover, the use of antibiotics in the production of cellular meat is a bioethical issue that needs examination (Hocquette,2016). The growth and proliferation of the production industry increase the tendency for animals to be centrally raised. Animals in close proximity are prone to bacterial production and infection. The animal breeding industry extensively uses antibiotics for reproductive efficiency. The transition between humans and animals in the use of antibiotics in the livestock and feed industries leads to problems such as the use of poor-quality and banned antibiotic products, causing serious harm to the human body and the natural ecology (Wang,Liu and Zhou,2021). The use of cell-cultured meat technology can effectively reduce antibiotic use. It not only maintains the balance between consumption demand and supply but also helps optimize the ecological environment, improve food safety, and enhance dietary health.

Conclusion

Throughout history, meat has been a significant source of nutrition for the human body. However, in the present day, driven by changes in sustainability, food supply, and adequacy, efforts are being made to develop new meat alternatives. One such alternative is cultured meat. Cultured meat can be defined as a novel food produced in a laboratory setting, developed with various technological innovations. Consumers generally view this new product with skepticism. Many questions regarding safety testing, inspections, nutritional values, dietary suitability, religious acceptability, and standardization processes in production remain unclear. According to study results, the use of cultured meat and meat analogs is particularly viable for vegetarian individuals and those environmentally conscious. While these alternatives have both positive and negative aspects, further research is essential to form definitive opinions about the future of cellular meat and meat analogs among food products.

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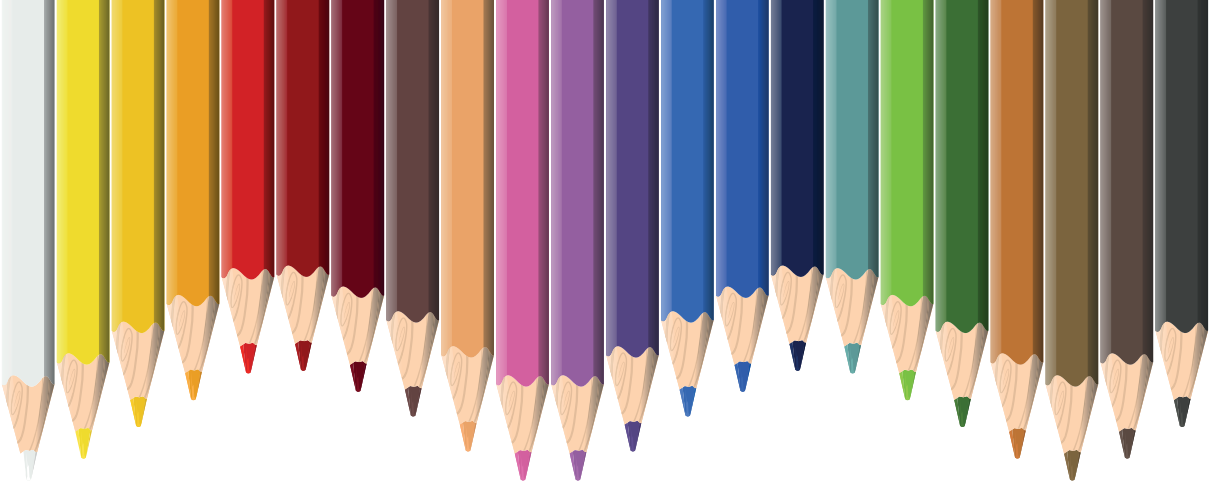

Chapter 3

EXPLORING THE POTENTIAL CONTRIBUTION OF IL-37 IN ATOPIC DERMATITIS

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Exploring the Potential Contribution of IL-37 in Atopic Dermatitis

Introduction

Interleukin 37 (IL-37) stands out as a recently identified anti-inflammatory member within the IL-1 cytokine family [1]. The IL-1 cytokine family is categorized into three subfamilies, namely IL-1 (IL-1α, IL-1β, IL-33, and IL-1Ra), IL-18 (IL-18 and IL-37), and IL-36 (IL-36α, IL-36β, IL-36γ, IL-36Ra, and IL-38) [1].

Initially discovered through in silico analysis in 2000, IL-37 was formerly denoted as IL-1F7 [2]. Unlike its counterparts in the IL-1 cytokine family, no homologous gene for IL-37 has been identified in mice, necessitating the use of transgenic mice for research purposes [2]. In healthy individuals, IL-37 serum concentrations remain notably low (100 pg/mL) [2]. However, its production can be stimulated by pro-inflammatory cues, serving as a self-protective mechanism against excessive inflammation and tissue damage, thereby limiting both innate and acquired immunity [3]. Consequently, elevated levels of IL-37 have been observed in patients grappling with inflammatory and autoimmune disorders [4]. Notably, IL-37-transgenic (-tg) mice have demonstrated protection against diverse animal model diseases, spanning colitis, acute myocardial infarction, idiopathic pulmonary fibrosis (IPF), obesity-induced inflammation, allergic airway inflammation, and surgical operation-induced spinal cord injury, among others [5–8]. Moreover, IL-37 exhibits promising potential in oncology due to its inhibitory effects on cancer initiation and progression [9]. Despite its recognized role in maintaining immune-microbial homeostasis, the specific involvement of IL-37 in the skin microbiome in atopic dermatitis (AD) remains a subject of limited exploration [2].

However, the apparent involvement of IL-37 in AD pathogenesis prompts inquiry into the extent of its role, as outlined in Table 1. AD, characterized as a chronic and inflammatory dermatosis with pruritic manifestations, affects approximately 20% of children and 3–7% of adults worldwide, with prevalence varying across geographical locations [10]. The condition poses a significant burden on the quality of life for both affected individuals and their parents [10].

Table 1. A succinct summary of key investigations resulting from a PubMed database search using the keywords “atopic dermatitis AND IL-37”

Study	Subject or Model	Most Important Findings
China 2020; Hou et al. [11]	CRISPR/Cas9 human IL-37b knock-in mice	1. IL-37b inhibited in vitro production of pro-inflammatory cytokines IL-6, TNF-α, CXCL8, CCL2, and CCL5. 2. IL-37 enhanced autophagy (LC3 conversion) and reduced p62. 3. IL-37b boosted Foxp3+ regulatory T cells (Treg) and IL-10, reducing eosinophil infiltration. 4. IL-37b restored disrupted gut microbiota diversity via autophagy modulation.
China 2021; Hou et al. [12]	CRISPR/Cas9 human IL-37b knock-in mice or mice with direct treatment with human IL-37b antibody	1. IL-37b reduced TSLP expression, TSLP receptor, and CD203c on basophils. 2. IL-37 reduced IL-4 release. 3. Direct IL-37b antibody treatment alleviated AD symptoms (ear swelling and itching).
United States 2019; Guttman-Yassky et al. [13]	51 children (less than 5 years); RNA extracted from tape strips; quantitative RT-PCR	1. IL-37 significantly downregulated in skin barrier of children with AD.
Japan 2022; Tsuji et al. [14]	Normal human epidermal keratinocytes	1. Tapinarof and Galactomyces ferment filtrate blocked IL-37 upregulation induced by AHR suppression. 2. IL-37 increased IL-33 expression. Tapinarof and Galactomyces ferment filtrate inhibited IL-37 expression.
United States 2021; Zhou et al. [15]	Skin and blood samples from moderate-to-severe AD patients	1. Significant decrease in IL1F7 transcripts in AD patient samples, correlating with decreased transcript levels for skin barrier function genes. 2. Th2 cytokines reduced epidermal IL-37 levels in skin models.
Norway 2020; Lossius et al. [16]	16 adult patients treated with nb-UVB therapy; blood samples from 20 healthy controls	1. After nb-UVB treatment, pro-inflammatory IL-36 decreased, while anti-inflammatory IL-37 increased.
China 2021; Hou et al. [17]	Blood samples from 30 patients with AD and 30 healthy controls	1. Levels of IL-37 and its receptor IL18R significantly reduced in AD patients. 2. Negative correlation between IL-37 and involucrin; IL-37 suppressed involucrin expression in vitro epidermal cell models.
Denmark 2022; Hu et al. [18]	30 patients with AD; 393 skin samples from multiple regions and time points	1. IL-37 expression decreased from healthy controls to non-lesional to lesional tissues.

In light of recent advancements in molecularly targeted therapeutic strategies for patients, global research endeavors are currently focused on identifying additional molecular targets. Although there is a limited number of reviews exploring the involvement of IL-37 in Atopic Dermatitis (AD) [19–22], these reviews consistently characterize IL-37 as an anti-inflammatory agent alleviating inflammation in AD [19–22]. Furthermore, there is unanimous agreement among these reviews that more research is essential to comprehensively determine the therapeutic potential of IL-37. It is noteworthy that in asthma, IL-37 demonstrates the ability to reduce allergic inflammation not only by targeting the Th2 cytokine axis but also all Th1/Th2/Th17 cytokine axes [21]. Additionally, IL-37 exhibits a protective effect by modulating atherosclerotic mechanisms, potentially offering significant advantages in addressing cardiovascular comorbidities in AD [21].

These findings collectively suggest that the anti-inflammatory nature of IL-37 is a newly recognized and intriguing player in the expansive landscape of cytokines involved in the pathogenesis of AD, necessitating further focus and understanding.

2. Background

2.1. Brief Overview of IL-37

2.1.1. Production and Processing

The IL-37 gene, located on chromosome 2q12–13 in close proximity to the regulatory areas of the IL-1α and IL-1β genes, is potentially crucial for IL-37's anti-inflammatory functions. When pro-inflammatory stimuli induce transcription of the IL-1 genes, IL-37 is also activated [23]. Five distinct isoforms of IL-37 (a–e) are generated by alternative splicing of IL-37 mRNA [24]. These isoforms are expressed in distinct tissues, with IL-37b being the longest and most researched isoform [26]. It encodes functional proteins fundamental for the proper extracellular function of IL-37 [24].

2.1.2. Release

IL-37 is not constitutively expressed in cells from healthy subjects but exhibits elevated expression in response to pro-inflammatory stimuli, functioning as a negative feedback system to suppress excessive inflammation [23]. IL-37 is expressed in various cells, possibly contributing to the preservation of immunological homeostasis [24]. While IL-37 transcript levels are generally low in resting human cells, they significantly increase when stimulated with lipopolysaccharide (LPS) or other exogenous stimuli [25].

2.1.3. Mechanism of Action

IL-37 serves as a dual-function cytokine, exerting powerful anti-inflammatory effects intracellularly or extracellularly. The specific conditions or

factors governing the selection of one mechanism over another remain unclear [24]. The specific receptor for IL-37 is yet to be identified. IL-37 exhibits binding affinity towards IL-18Rα, facilitating the recruitment of IL-1R8 to establish a receptor complex conveying an anti-inflammatory signal [2,30]. Interestingly, the extracellular presence of IL-18 binding protein (IL-18BP) has been observed to impede the binding of IL-37 to IL-18Rα [32]. However, IL-18BP also exhibits greater affinity for IL-18 compared to IL-18Rα, inhibiting the binding of IL-18 to its receptor [25,32]. The weak inhibitory effect of high doses of IL-37 on the production of inflammatory cytokines may be attributed to the spontaneous formation of homodimers of IL-37 at high concentrations, perceived as an auto-regulatory mechanism limiting excessive immunosuppression [24].

2.2. IL-18 Cytokine and its Relevance to IL-37

A concise description of the IL-18 cytokine is imperative due to its significance within the context of IL-37 [33]. The binding of Pathogen Associated Molecular Patterns (PAMPs) to Toll-like receptors (TLRs) initiates the NF-κB pathway, leading to the transcription of the precursor form of IL-18 [33]. Synthesized by various cell types, including hematopoietic and non-hematopoietic cells like monocytes, endothelial cells, osteoblasts, and keratinocytes, IL-18 serves as a potent, pro-inflammatory agent influencing both innate and adaptive immune responses. It has been widely used as a biomarker in studies assessing inflammasome activity [33].

To inhibit inflammation, IL-37 suppresses pathways such as mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK), NF-κB, and various transcription factors [2]. Additionally, IL-37 activates signal transducer and activator of transcription (STAT)3, protein phosphatase and tensin homolog (PTEN), and 5'AMP-activated protein kinase (AMPK) [2]. Lacking a nuclear localization sequence, IL-37 likely reaches the nucleus bound to Smad3, enhancing Smad3's anti-inflammatory activity rather than through direct DNA binding [34]. In response to pro-inflammatory stimuli, the IL-37 precursor concentration rises intracellularly, caspase-1 cleaves the precursor, and the C-terminal domain of IL-37 binds to Smad3. This complex, upon phosphorylation, relocates to the nucleus, participating in the regulation of gene expression [2].

2.3. IL-37 and Immune Cells

2.3.1. Monocytes and Macrophages

IL-37 production is induced in monocytes and macrophages by Toll-like receptor (TLR) ligands, such as LPS and Pam3CysSerLys4 (Pam3CSK4), as well as transforming growth factor (TGF)-β1 [35]. IL-37 inhibits inflammatory pathways, including the reduction of pro-inflammatory cytokines and

chemokines, thereby influencing macrophage proliferation, apoptosis, and transmigration [38]. IL-37 also modulates macrophage polarization, inhibiting M1 polarization and enhancing M2 macrophages and their anti-inflammatory cytokine secretion [39].

2.3.2. Dendritic Cells (DCs)

DCs secrete IL-37 in response to LPS stimulation and contribute to maintaining an anti-inflammatory state. IL-37 induction in DCs modulates DC maturation unfavorably, inducing tolerogenic DCs that impede T effector (Teff) cell activation and promote regulatory T (Treg) cell development [40].

2.3.3. Pathogenesis of Atopic Dermatitis (AD)

The multifactorial etiology of AD involves genetic and environmental factors, immune system abnormalities, defects in the epidermal barrier, and microbial imbalances. Genetic factors include epidermal barrier genes, immune mechanism genes, DNA methylation genes, vitamin D metabolism genes, and genes encoding alarmins produced by keratinocytes. Environmental factors, such as lifestyle changes, air pollution, obesity, antibiotic use, and smoking, contribute to AD development through epigenetic mechanisms [42,43].

AD, primarily a Th2-mediated dermatosis, involves an overproduction of Th2 cells leading to elevated cytokines (IL-4, IL-5, IL-13), IgE production, and eosinophils. Th1, Th22, and Th17 responses also play a role in acute and chronic phases, with Tregs contributing to immune suppression [42].

2.4. IL-37 and Immune Response in AD

IL-37 levels are observed to be decreased in the serum and skin lesions of AD patients. Downregulation of IL-37 in allergic patients may lead to ineffective immune suppression and dysregulation in response to allergen stimulation. Th2 immune response has the capacity to inhibit IL-37 production, suggesting a potential deficiency in IL-37 production in individuals with AD and other allergic inflammatory disorders [17,28].

2.5. IL-37 in Allergic Models and Immune Cells

2.5.1. Allergic Asthma and Rhinitis Models

In an experimental allergic asthma mouse model, local application of recombinant IL-37 demonstrated a reduction in Th2-mediated allergic airway inflammation by lowering IL-4, IL-5, and IL-13 secretion [50]. Similarly, in a murine allergic rhinitis model, IL-37 administration resulted in decreased eosinophils in the nasal mucosa, restored mucosal thickness, reduced nasal rubbing and sneezing frequency, and lowered levels of IgE, IgG1, IgG2a, IL-4, IL-13, IL-17a, and CCL11 [51]. IL-37 also inhibited inflammatory cell recruitment and Th2 activation in a murine invasive pulmonary aspergillosis model [52].

2.5.2. Mechanisms in Allergic Models

IL-37 demonstrated diverse effects on Th2 cells in different models. It decreased Th2-mediated inflammation in the allergic asthma model, potentially by downregulating GATA3 expression through the IL-4/STAT6 signaling pathway [53]. However, in an ovalbumin (OVA) model of house dust mite-induced asthma, IL-37 treatment did not significantly affect Th2 cell differentiation, recruitment, or activation [54]. Th2 cytokines (IL-4 and GM-CSF) were found to suppress constitutive IL-37 expression, while Th1/Th17 cytokines (IFN-γ and IL-17) induced IL-37 production in CD4+ T cells [56].

2.6. Regulatory T Cells (Tregs)

IL-37 played a role in regulatory T cell (Treg) function. It maintained low expression in freshly isolated human CD4+CD25+ Tregs and its silencing led to decreased suppressive activity [58]. Stimulation with IL-37 enhanced Treg suppressive activity, upregulated CTLA-4 and FOXP3, and increased TGF-1 levels [59]. Silencing IL-37 led to reduced TGF-β and IL-10 levels, indicating IL-37's contribution to Treg activity and immune suppression [58,59].

2.6.1. Eosinophils

IL-37 demonstrated inhibitory effects on eosinophils, reducing their infiltration in skin-like lesions and decreasing CCL11 expression [11,51,54]. IL-37 also promoted autophagy in eosinophil-mediated allergic inflammation, as evidenced by decreased autophagy inhibitor 3-methyladenine (3-MA) and increased AMP levels [11].

2.6.2. Basophils and Mast Cells

IL-37 inhibited the expression of the TSLP receptor on basophils, reducing basophil infiltration in AD models [12]. Additionally, IL-37 suppressed NF-κB activation and P38 MAPK phosphorylation in mast cells, indicating a potential role in reducing mast cell inflammation [69].

2.6.3. B Cells

In a murine AR model, IL-37 administration resulted in reduced IL-4 and IL-13 levels, subsequently lowering IgE production [51]. However, some studies reported a lack of statistically significant association between IL-37 and IgE levels specifically in AD [17,28,73].

These findings collectively suggest that IL-37 exerts immunomodulatory effects in allergic models by influencing various immune cell types and pathways.

2.7. IL-37 and Skin Barrier Disruption

2.7.1. Skin Barrier in Atopic Dermatitis (AD)

In atopic dermatitis (AD), the expression of key proteins, including filaggrin (FLG), loricrin (LOR), involucrin (IVL), and FLG2, is downregulated, leading to an abnormal corneocyte lipid envelope and impaired epidermal barrier function [74]. This disruption results in increased water loss, higher absorption of pollutants and allergens, and elevated susceptibility to microbial infections [75]. The skin's pH levels, serine proteinase activity, and cytokine levels further contribute to the inactivation of enzymes involved in ceramide synthesis, altering the physical, chemical, and antimicrobial properties of the skin [75].

2.7.2. IL-37 and Epidermal Barrier

IL-37 protein expression is decreased in the epidermis of AD patients, with the most significant reduction observed in chronic AD skin lesions [13,15,17]. IL-37 production is reported in mature and differentiated epidermal keratinocytes [15,77]. Studies show mixed results regarding the correlation between IL-37 and key epidermal proteins. While one study found a positive correlation with FLG, FLG2, and IVL [15], another reported a negative association between IL-37 and IVL expression [17].

In vitro studies using a 3D human skin model demonstrated that IL-4, IL-13, and IL-31 decreased IL-37, while IL-37 increased FLG and FLG2, indicating a potential impact on specific epidermal differentiation complexes (EDCs) [15]. IL-37 was found to inhibit the expression of keratinocyte-derived IL-33, a cytokine associated with AD pathogenesis, presumably by inhibiting MAPK and STAT1 activation in keratinocytes [14,78]. IL-37-tg mice showed reduced epidermal thickness and scratching frequency [11].

2.7.3. IL-37 as a Therapeutic Target in AD

IL-37 is considered a potential therapeutic target in AD due to its strong anti-inflammatory properties. The increasing availability of biological drugs and Janus kinase inhibitors has led to a search for therapies that precisely target immune pathways involved in AD pathogenesis. IL-37, known as a 'peacemaker,' may offer a novel approach to modulate inflammation [79]. However, the tight control of IL-37 function is crucial to prevent excessive silencing of immunity. While IL-37 has shown success in animal models of allergic diseases, further research is needed to determine its efficacy in humans, especially in chronic conditions like AD [6,50,51]. The potential use of IL-37 extends beyond AD, with promising applications in various autoimmune, neurological, cardiovascular, and neoplastic diseases [80–83].

Conclusions

IL-37 demonstrates a noteworthy anti-inflammatory effect, playing a crucial role in modulating inflammatory responses, particularly in the context of inflammation. Its involvement in the pathogenesis of atopic dermatitis (AD) through various immunological mechanisms is evident, though the full extent of its role remains uncertain and warrants further investigation.

To unlock the potential therapeutic benefits of IL-37 in the future, additional research is imperative. Conducting in-depth studies to elucidate the pathways triggered or inhibited by IL-37 is crucial. This deeper understanding will contribute to the development of safer and more precise therapeutic strategies. By unraveling the intricate mechanisms through which IL-37 operates, researchers can explore its therapeutic potential not only in AD but also in a broader spectrum of inflammatory and immune-related conditions.

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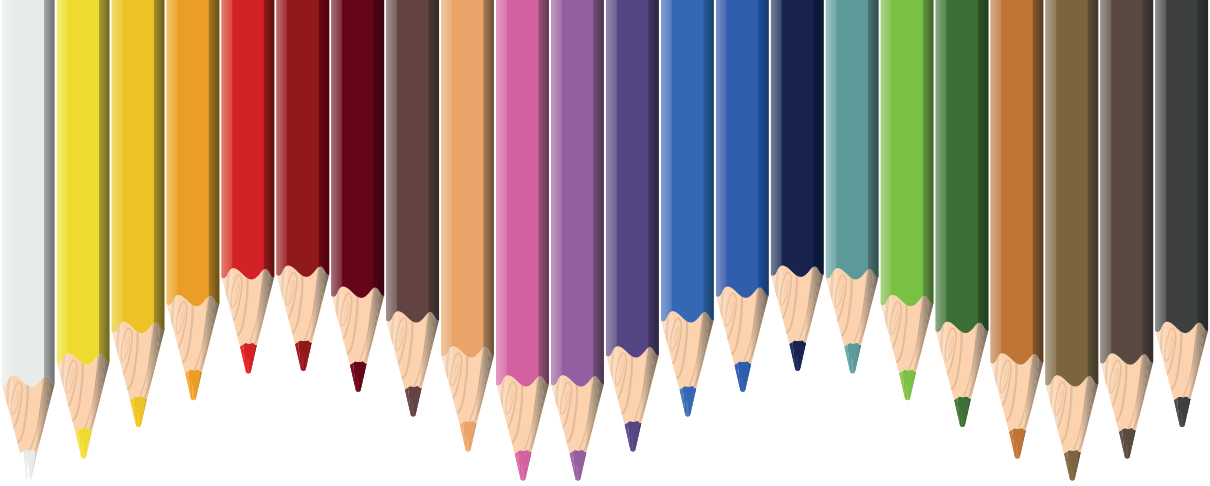

Chapter 4

INVESTIGATION OF SELF-EXAMINATION METHOD KNOWLEDGE FOR EARLY DIAGNOSIS AMONG UNIVERSITY STUDENTS

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INTRODUCTION

Early detection and treatment of health issues are crucial in today's world. However, it should be noted that many diseases do not manifest symptoms in their early stages. Thus, performing self-examinations on a regular basis is a vital tool in the timely detection of potential health problems (Consolaro & Morais, 2021; Kim & Lee, 2019). Self-examination methods allow individuals to monitor their health and provide opportunities for early diagnosis (Ayres et all., 2021; Kılıç & Öz, 2021). It is worth noting that this approach is particularly relevant for conditions such as breast, testicular and skin cancers. Additionally, these techniques assist people in familiarising themselves with their bodies and promote prompt commencement of treatment in the event of any anomalies. Being aware of and engaging in the practice of self-examination methods, particularly in the initial identification of critical illnesses such as cancer, is essential in maintaining a healthy lifestyle (Caner, Dündar & Kırcı, 2021; Yang & Chang, 2020).

When examining the demographic structure of our country, it is crucial to elevate the awareness level among the younger population. In this regard, university students need to be knowledgeable about early detection methods to preserve their health and identify significant health issues (Kim & Park, 2021; Kostovska & Pemovska, 2019; Özdemir & Erdem, 2020). Self-examination methods, particularly for cancer types prevalent among young adults such as breast and testicular cancer, serve as a vital component in early detection and treatment (Hernandez & Martinez, 2022; Hsiao et all., 2018). Therefore, it is recommended that university students acquire knowledge about self-examination methods and practice them regularly.

When analysing the demographic makeup of our nation, it is imperative to raise awareness among the younger generation. In this context, it is imperative for university students to possess information regarding early detection techniques to safeguard their physical well-being and identify health issues that require immediate attention. The practice of self-examination methods, notably for common malignancies among young adults, like breast and testicular cancer, is a crucial aspect of early detection and subsequent treatment. It is therefore advisable for university students to ascertain knowledge regarding self-examination methods and regularly carry them out (Hsiao et all., 2018; Kim & Park, 2021).

When examining national and international literature on self-examination techniques for early detection, it was discovered that studies concentrate on specific facets of early diagnosis in university students (Çelik, Yıldırım & Türk, 2019; Karaaslan & Özkan, 2020; Sharma & Shankar, 2020). Nonetheless, no extensive inquiry particularly comprehensively scrutinizes self-examination techniques. Thus, the objective of this investigation is to assess university students' awareness of self-examination methods for early detection.

METHOD

Study Design and Sample: This descriptive study was conducted at a vocational school of a foundation university, with a study population that comprised of 1,150 students. Due to societal awareness surrounding self-examination methods for early detection, the objective was to reach the entire population without utilizing a sample selection process. Out of those approached to participate, 864 students completed the form comprehensively, providing a participation rate of 75.1%.

Data Collection Process: The study data was collected using Google Forms. It took an average of 10 minutes to complete the form. Each participant was asked to fill out the form once.

Data Collection Tools: The data collection tool used in this research was a 26-question survey designed by the researcher. The survey included sociodemographic items such as gender, age, marital status, family structure, and income status, as well as questions pertaining to self-examination habits, cancer diagnosis history, regular check-ups, cancer screening tests, and preferences on self-examination methods. The questionnaire was formulated based on literature reviews. Expert opinion was taken before the application (Şişman et all., 2022; Yılmaz, Nilüfer & Aykota, 2020; Yüksekol et all., 2022).

Ethical Considerations: The study obtained ethical approval from the relevant ethics committee with approval number E-53938333-050-17977, to ensure its ethical compliance. Written permission was also obtained from the Directorate of Health Services Vocational School for the implementation of the study. Before participation, the students were provided with information about the purpose and objectives of the study, and their written consent was obtained through Google Forms. All ethical guidelines and privacy principles were strictly adhered to throughout the study.

Limitations of the Study: The data is limited to students from the institution where the study was conducted. Due to the online nature of the study, only students with internet access were reached. Therefore, the results may not be representative of all students.

Data Analysis: For the statistical analysis of the data, SPSS (Statistical Package for the Social Sciences) 22.0 software package was used, employing descriptive statistics, Chi-square test, and Multiple Regression analysis.

RESULTS

Among the participants, 72.5% were female and 27.5% were male. It was found that 97% of the participants were single and 18% were on continuous medication. Regarding smoking habits, 29% of females and 44% of males reported being smokers. Additionally, 23% of females and 37% of males stated that they consume alcohol. In terms of exercise, 49% of females and 65% of males engage in regular physical activity. Furthermore, 9% of females and 18% of males undergo regular check-ups. Among the female participants, 20% reported having a first-degree relative with a cancer diagnosis, while the

corresponding percentage for males was 17% (Table 1).

Regarding cancer screening tests, it was found that 64% of females and 47% of males know these tests. Among those who know cancer screening tests, 37% acquired this knowledge through university education. Only 9% of the participants were recommended to undergo cancer screening tests. The percentage of students who underwent cancer screening tests was 3% for females and 2% for males. When asked about the reasons for not undergoing cancer screening tests, 27% of females mentioned a lack of time, while 38% of males stated a lack of concern. In terms of the primary institution where cancer screenings are conducted in our country, 78% of females and 82% of males mentioned KETEM (Table 2).

When examining the application of self-examination methods, 44% of females and 54% of males stated that they sometimes do not perform a self-examination. As for the reasons for practicing self-examination, 68% of females and 71% of males mentioned that it is for preventive purposes. In terms of the types of cancers for which self-examination is applied, 94% of females and 63% of males reported knowledge about self-breast examination, 8% of females and 37% of males mentioned self-testicular examination, 18% of females and 43% of males stated self-skin examination, and 6% of females and 13% of males indicated self-vulva examination. Regarding the source of information about self-examination methods, 33% of females and 35% of males mentioned that they acquired knowledge through university education (Table 3).

The multiple regression analysis revealed a significant relationship between the knowledge level of self-examination methods and the gender variable. It was found that gender significantly influenced the knowledge level of self-examination methods for breast and testicular examinations ($p<0.05$) (Table 4).

DISCUSSION

This study, which aims to examine the knowledge levels of university students regarding self-examination methods for early diagnosis, demonstrates the originality of the research by taking a comprehensive approach to examine self-examination methods and reaching a large sample group.

When examining the distribution of healthy lifestyle behaviors among students in our study, it was observed that both female and male students had notable rates of tobacco and alcohol consumption. Studies have found that the risk of developing cancer is significantly higher in smokers and alcohol users compared to non-users. Therefore, early detection of health problems caused by these harmful habits and providing appropriate treatment and support to individuals are important (Larsson et all., 2020; Scherübl et all., 2021). According to the findings, almost half of the students reported engaging in regular exercise. This suggests that a significant proportion of the student population incorporates physical activity into their lifestyles. Another study

investigating the relationship between physical activity and 26 different types of cancer emphasized the association between regular physical activity and a decrease in cancer risk (Moore et all., 2020). A study examining the relationship between physical activity and cancer risk using UK Biobank data revealed that low levels of physical activity were associated with an increased risk of various cancer types (Boyle & Vallance, 2018). It was observed that only a small number of students underwent regular check-ups, drawing attention to a low rate of regular health screenings among the student population. The rate of regular check-ups can vary from community to community and from region to region. These rates differ depending on various factors such as the healthcare system, health awareness, accessibility, cultural factors, and economic status.

Both women and men participants have first-degree relatives diagnosed with cancer. In another study, it was found that approximately 20% of women and 17% of men had first-degree relatives diagnosed with cancer (Johnson, Smith & Davis, 2021). The results indicate the need to increase the implementation of cancer screening methods among this group. It was found that 64% of women and 47% of men know cancer screening tests. Among them, 37% acquired knowledge about cancer screening tests through university education. In a study conducted by Çilengiroğlu et al. (2022), it was stated that 51.6% of nursing students had knowledge about cancer, and the source of information was the courses they took at the university (Çilengiroğlu et al., 2022).

When examining the self-examination method application status among students, it was found that nearly half of the participants did not practice it regularly. In a study conducted with students, it was determined that almost 47% of them did not regularly apply the self-examination method (Tanık & Naz, 2022). These findings highlight the lack of knowledge regarding health issues among young individuals and emphasize the importance of educational interventions in the method.

In our study, it was found that both women and men had different levels of knowledge regarding different self-examination methods. Altınel and Avcı (2013) found that 3.3% of the participants knew how to perform testicular self-examination (Altınel &Avcı, 2013). Asgar Pour and Çam (2014) determined that 30.7% knew testicular self-examination (Asgar Pour & Çam, 2014). Türkmen (2017), in a study on students' knowledge of breast self-examination, found that 34.6% of the participants knew how to perform it (Türkmen, 2017). Tuna, Soran, and Karaaslan (2022) found that 30.4% of the participants had prior knowledge about breast self-examination (Tuna, Soran & Karaaslan, 2022). Karaman (2020) found that 28.9% of the participants knew vulva self-examination (Karaman, 2020). Altun (2019) determined that 45.2% of the students knew skin self-examination (Altun, 2019). These results demonstrate the students' knowledge levels regarding serious conditions like

cancer, while also highlighting the need for improvement.

There is a significant relationship between the knowledge level of self-examination methods and the variable of gender. This is because certain self-examination methods are recommended or more widely known based on gender. For example, breast self-examination is more commonly recommended for women in breast cancer screening, while testicular self-examination may be more emphasized for men in testicular cancer screening. Therefore, it is believed that gender influences the knowledge level of these self-examination methods. However, further research is needed to determine the nature of this relationship.

Declaration of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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TABLES

Table 1. *The Distribution of Some Descriptive Characteristics of the Students Participating in the Research*

Gender	**N**		**Percent**	
Female	626		72,5	
Male	238		27,5	
Marital Status	**N**		**Percent**	
Single	838		97,0	
Married	26		3,0	
Drug Use	**Yes**		**No**	
Drug Use	**N**		**Percent**	
Female	82	13	**N**	**Percent**
Male	15	5	544	87
Smoking	**Yes**	**No**	223	95
Smoking	**N**		**Percent**	
Female	182	29	**N**	**Percent**
Male	104	44	444	71
Alcohol Use	**Yes**	**No**	134	56
Alcohol Use	**N**		**Percent**	
Female	146	23	**N**	**Percent**
Male	87	37	480	77
To exercise	**Yes**	**No**	151	63
To exercise	**N**		**Percent**	
Female	304	49	**N**	**Percent**
Male	154	65	322	51
Check-up	**Yes**	**No**	84	35
Check-up	**N**		**Percent**	
Female	54	9	**N**	**Percent**
Male	44	18	572	91
Have any of your first-degree relatives been diagnosed with cancer?	**Yes**	**No**	194	82
Have any of your first-degree relatives been diagnosed with cancer?	**N**		**Percent**	
Female	124	20	**N**	**Percent**
Male	40	17	500	80

Table 2. Knowledge Levels of the Students Participating in the Study of Cancer Screening Tests

Do you know about cancer screening tests? answers to the question (n=864)								
	Yes				**No**			
	N		**Percent**		**N**		**Percent**	
Female	398		%64		228		%36	
Male	112		%47		126		%53	
Where did you get information about cancer screening tests?								
	Health Person		**Friend / Neighbor**		**Media (TV, Radio, Internet)**		**University Education**	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**
Female	166	%27	22	%3	208	%33	230	%37
Male	61	%26	18	%8	70	%29	89	%37
Have you ever had a cancer screening test?								
	Yes				**No**			
	N		**Percent**		**N**		**Percent**	
Female	21		%3		605		%97	
Male	5		%2		233		%98	
Have you been offered a cancer screening test by a physician or other healthcare professional?								
	Yes				**No**			
	N		**Percent**		**N**		**Percent**	
Female	57		%9		569		%91	
Male	22		%9		216		%91	
In which health institutions/organizations are primary cancer screenings performed in our country? answers to the question (n=864)								
	KETEM		**Community Health Center**		**Family Health Center**		**Other**	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**
Female	490	%78	96	%15	31	%5	9	%2
Male	195	%82	20	%8	17	%7	6	%3

If you have never had a cancer screening test in your life, please indicate why?																		
	I don't care		I am scared		I can't find time		I'm not in the age group		I didn't need		No symptoms		I didn't think		I had it done		I don't Know Where It Was Made	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**
Female	156	%25	63	%10	169	%27	148	%24	57	%9	18	%3	6	%1	8	%1	1	0
Male	91	%38	15	%6	69	%29	43	%18	8	%4	8	%4	0	0	4	%1	0	0

How often do you apply to the health institution?answers to the question (n=864)								
	Once a week		Once a month		Several times a year		Once a year	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**	**N**	**Percent**
Female	4	%1	160	%26	370	%59	92	%14
Male	2	%1	35	%15	126	%53	75	%31

Table 3. Knowledge Levels and Frequency of Applications of Students Participating in the Study on Self-Examination Methods

Do you practice a self-examination method? Answers to the question (n=864)								
	Yes		No		Bazen			
	N	Percent	N	Percent	N	Percent		
Female	188	%30	165	%26	273	%44		
Male	40	%17	129	%54	69	%29		
If you are using the self-examination method, what is the reason?								
	To take precautions		Because it has to be done		Because I have cancer		Because I'm afraid of getting cancer	
	N	Percent	N	Percent	N	Percent	N	Percent
Female	424	%68	140	%22	2	0	60	%10
Male	168	%71	46	%19	0	0	24	%10

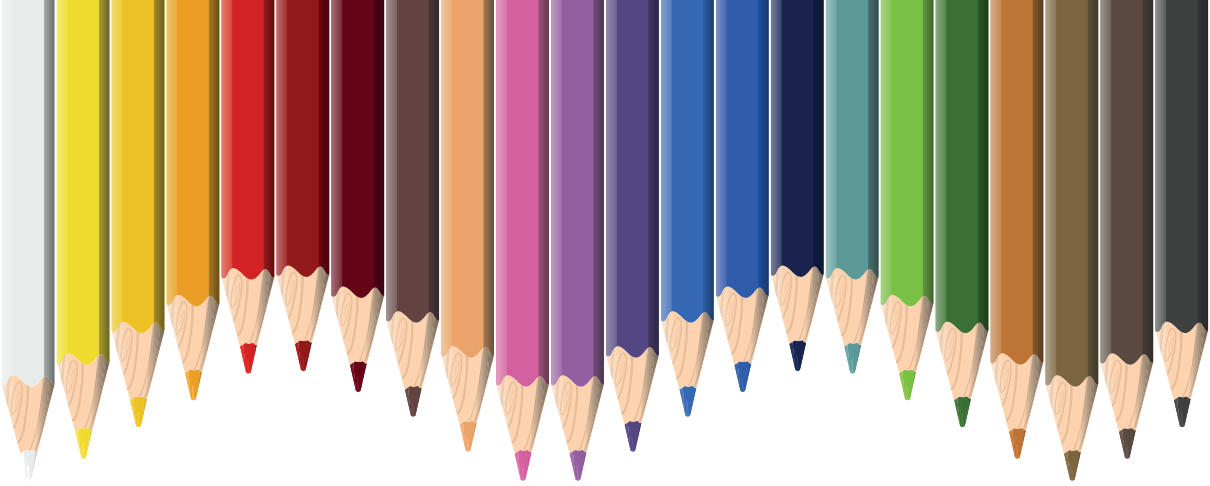
If you do not use the self-examination method, what is the reason?														
	'Cause I don't know what to do		Because I don't see myself at risk for cancer		Because I think I'm healthy		Because I think it's an inconvenient procedure		Because I'm afraid / afraid of the result		Because there is no cancer in my family		Because I do not believe that early diagnosis will affect	
	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent
Female	118	%19	126	%20	221	%35	7	%1	54	%9	83	%13	17	%3
Male	46	%19	54	%23	79	%33	6	%3	11	%5	26	%11	16	%6

Which types of cancer do you know about the self-examination method?				
Breast Self-Examination	I know		I don't know	
	N	Percent	N	Percent
Female	588	%94	38	%6
Male	151	%63	87	%37
Self Testicular Examination	I know		I don't know	
Female	54	%8	572	%92
Male	87	%37	151	%63
Self Skin Examination	I know		I don't know	
Female	115	%18	511	%82
Male	103	%43	135	%57
Vulva Self Examination	I know		I don't know	
Female	39	%6	587	%94
Male	31	%13	207	%87

Where did you get the self-examination method information?								
	Health Person		Friend / Neighbor		Media (TV, Radio, Internet)		University Education	
	N	Percent	N	Percent	N	Percent	N	Percent
Female	183	%29	40	%6	201	%32	202	%33
Male	67	%28	21	%9	66	%28	84	%35

Tablo 4. *The Effect of Gender on Self-Examination Method Knowledge*

	Standard Beta	T	p
Breast Self Examination	-,328	-9,941	,000
Testicular Self Examination	,295	9,089	,000
Skin Self Examination	,061	1,707	,088
Vulva Self Examination	-,034	-,986	,324
R=0.488 R^2 =0.238 Adjusted R^2 = 0.235 p= 0.000			



Chapter 5

RHEUMATOID ARTHRITIS AND CANCER RISK: EPIDEMIOLOGICAL INSIGHTS AND CLINICAL IMPLICATIONS

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Rheumatoid Arthritis and Cancer Risk: Epidemiological Insights and Clinical Implications

Section 1: Rheumatoid Arthritis Overview

- Epidemiology and risk factors.

- Genetic of RA

- Pathophysiology of RA, including the role of immune system.

Section 2: Cancer Overview

- Epidemiology of cancer in the general population.

- The Pathophysiology of Cancer

Section 3: Rheumatoid Arthritis and Cancer Risk

- Types of cancers most commonly associated with RA.

- Potential reasons for increased or decreased cancer risk in RA patients.

Section 4: Shared Pathways

- Examination of the immune system dysregulation in both RA and cancer.

- Chronic inflammation in RA and its potential contribution to carcinogenesis.

- Genetic factors that might predispose individuals to both RA and certain cancers.

Section 5: Impact of RA Treatment on Cancer Risk

- Overview of RA treatments, including DMARDs, biologics, and corticosteroids.

- Discussion of how these treatments might influence cancer risk.

1. Rheumatoid Arthritis Overview

Rheumatoid arthritis (RA) is a chronic and complex autoimmune disease primarily affecting the joints but capable of systemic involvement. Its hallmark features include symmetrical joint inflammation, which often progresses from smaller to larger joints and may extend to impact skin, eyes, heart, kidneys, and lungs (1). The disease is characterized by the destruction of bone and cartilage and the weakening of tendons and ligaments, leading to joint deformities and bone erosion, often accompanied by severe pain. The typical age of onset of the disease is usually between 35 and 60 years of age, with periods of remission and exacerbation (2).

Common Symptoms

Morning Stiffness: Affected joints display stiffness for more than 30 minutes after waking.

Systemic Symptoms: Fatigue, fever, and weight loss in patients.

-Joint Involvement: Tenderness, swelling, warmth in the joints, and rheumatoid nodules under the skin.

1.1 Epidemiology and Risk Factors

Rheumatoid arthritis (RA) has a global prevalence of approximately 1%, but this rate varies geographically and among different ethnic groups. In Western countries, the prevalence is typically higher, ranging from 0.5% to 1.0% in white individuals (3). Interestingly, some populations exhibit notably different rates; for instance, Native American populations have reported a 5–6% prevalence (4). Epidemiological research shows that Rheumatoid Arthritis (RA) is more prevalent in women, who are two to three times more likely than men to develop the condition. RA typically manifests between the ages of 35 and 60, although it can occur at any age, including in children, where it is known as Juvenile RA (JRA). The disease's course is marked by alternating phases of remission and flare-ups, which complicates its management and treatment strategies (5).

The Age-Standardized Rate (ASR) of a particular condition showed significant variations globally, ranging from 3.47 (with an Uncertainty Interval [UI] of 2.96 to 4.1) to 30.03 (UI: 26.97 to 33.31) per 100,000 individuals. Ireland had the highest ASR at 30.03 (UI: 26.97 to 33.31 per 100,000), with Finland closely following at 27.89 (UI: 25.5 to 30.76 per 100,000). Other notable countries included Kazakhstan at 25.5 (UI: 23.45 to 28.01 per 100,000), Mexico at 25.43 (UI: 22.94 to 28.03 per 100,000), and Honduras at 25.06 (UI: 22.49 to 27.68 per 100,000). Equatorial Guinea experienced the most substantial increase in ASR, with an Estimated Annual Percentage Change (EAPC) of 1.78% (Confidence Interval [CI]: 1.60% to 1.96%). Bhutan, Peru, Turkey, and

Bangladesh also reported significant increases in their ASRs. On the other hand, countries like Italy, Kenya, and the United Kingdom observed a decline in their ASRs over the period from 1990 to 2019 (6).

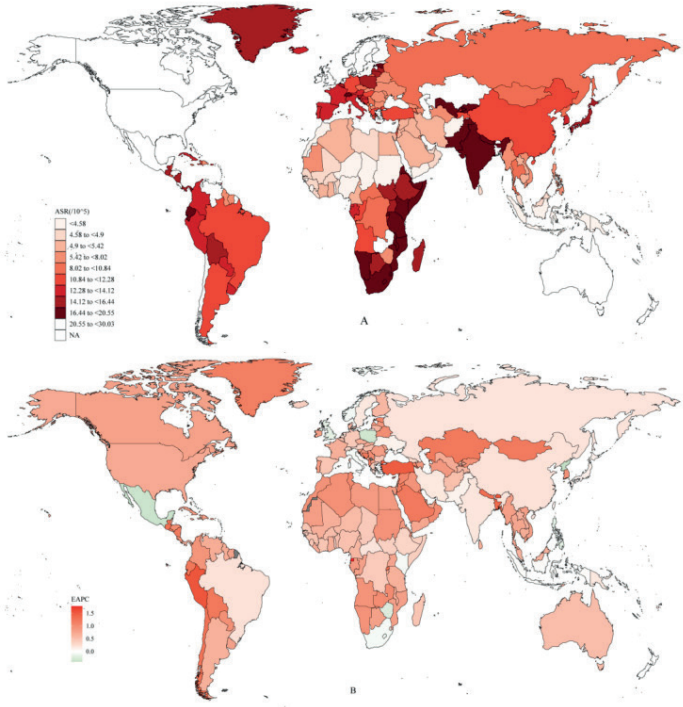

Figure 1. *The study encompassing the global disease burden of rheumatoid arthritis across both sexes in 204 countries and territories revealed significant findings. A. The Age-Standardized Incidence Rate (ASR) of rheumatoid arthritis in 2019. B) The Estimated Annual Percent Change (EAPC) of rheumatoid arthritis for the same year (6).*

These findings are crucial as they provide a comprehensive overview of the global impact of rheumatoid arthritis, reflecting both current incidence rates and trends over time. The use of ASR and EAPC as metrics offers a standardized method to assess and compare the burden of rheumatoid arthritis across diverse populations and geographical locations.

Risk factors for RA include a combination of genetic and environmental elements. The strongest genetic risk is associated with certain HLA-DRB1 alleles, particularly those containing the shared epitope sequence (7,8). First-degree relatives of individuals with RA have a significantly increased risk, indicating a hereditary component. Environmental factors, such as smoking and exposure to certain types of dust or fibers, have also been implicated in the development of RA. Some infectious agents are also thought to trigger the onset in genetically susceptible individuals. The interaction between these

genetic and environmental factors is complex and not fully understood, but they collectively contribute to the risk and severity of RA (9). Some studies showed a concordance rate of 15% in monozygotic twins and 5% in dizygotic twins in the UK (10, 11).

First-degree relatives of RA patients have a 2-5 times higher risk. The HLA-DRB1 gene's shared epitope sequence is particularly significant. Moreover, non-MHC genes like PTPN22, CTLA4, and PADI4 have been linked to RA, albeit with a modest individual contribution compared to the HLA region. Genome-wide association studies have uncovered various genetic factors and locations linked to Rheumatoid Arthritis, including genes like TNIP2, WISP1, and TNFRSF11A (12-14).

1.2 Genetic Rheumatoid Arthritis

RA, a complex autoimmune disease, exhibits a significant genetic underpinning, as evidenced by the identification of more than 150 genetic loci associated with its development. These genetic markers, in conjunction with environmental factors, contribute to an elevated risk of RA. Notably, the genetic basis of RA is not attributed to a singular gene; rather, it involves multiple genes, predominantly within the human leukocyte antigen (HLA) system and others that function in immune system regulation (7).

Among the critical genetic markers for RA, STAT4, TRAF1/C5, and PTPN22 are particularly noteworthy. STAT4 plays a pivotal role in regulating and activating the immune system, and mutations in this gene are also observed in other autoimmune conditions, including lupus (15). The TRAF1/C5 gene complex is instrumental in driving chronic inflammation (16). Furthermore, in Caucasian populations, the PTPN22 gene is instrumental in encoding specific immune cells, playing a significant role in influencing the progression and expression of RA. This gene is considered one of the primary genetic links to RA risk (17).

Research on single nucleotide polymorphisms (SNPs) in ISG15 expression has shown significant links to the altered expression of key type 1 interferon response genes, including IFI27, IFI35, IFI44, IFIT1, IFIT3, IFIT5, and OAS1. The study also found that the trans-expression quantitative trait locus (transEQTL) of ISG15 is associated with the infiltration of synovial B cells, as evidenced by CD20 histology. This supports earlier findings that connect the type 1 interferon gene expression signature with synovial B cell infiltration. Additionally, research by Cooles and colleagues identified a blood interferon gene signature as a useful prognostic biomarker for patients with early-stage Rheumatoid Arthritis undergoing treatment with methotrexate-based disease-modifying antirheumatic drugs (DMARDs), (20).

In summary, while RA genetic markers can illuminate aspects of the

disease, it is crucial to note that not all individuals with these genetic markers develop RA. Conversely, not all RA patients possess these markers. This highlights the complex interplay of genetic and environmental factors in the pathogenesis of RA.

Rank	Gene Name	Locus(Chromosomal Location)	Notes
1	HLA-DRB1	6p21.3	Strongest genetic association with RA, part of the HLA complex.
2	PTPN22	1p13.2	Linked to immune system regulation, it is significant in Caucasian populations.
3	STAT4	2q32.2-q32.3	Regulates the immune system; mutations are also observed in other autoimmune conditions like lupus.
4	TRAF1/C5	9q33.2	Major role in chronic inflammation.
5	TNFAIP3	6q23	Involved in the NF-kB signaling pathway.
6	CD28	2q33.2	Critical for T-cell activation and survival.
7	CTLA4	2q33.2	Negative regulator of T-cell activation.
8	IRF5	7q32.1	Role in regulating the immune response.
9	BLK	8p23-p22	Involved in B-cell receptor signaling.
10	REL	2p13-p12	Part of the NF-kB complex is involved in immune response.
11	CCR6	6q27	It is important for the migration of immune cells.
12	IL2RA	10p15.1	Involved in T-cell activation and proliferation.
13	IL23R	1p31.3	Role in autoimmune inflammation.
14	CD40	20q12-q13.2	Important in B-cell proliferation.
15	AFF3	2q11.2	Role in lymphoid cell development.
16	GATA3	10p14	Regulates T-cell development.
17	PRKCQ	10p15	Involved in T-cell activation.
18	FCRL3	1q21.1	Role in B-cell regulation.
19	CDK6	7q21-q22	Involved in cell cycle regulation.
20	IRF8	16q24.1	Regulates immune cell development.
21	TNFRSF14	1p36	Part of the TNF receptor superfamily.
22	IL6ST	5q11.2	Involved in cytokine signaling.
23	TAGAP	6q25.3	Role in T-cell activation.
24	TYK2	19p13.2	Involved in cytokine signaling.
25	IL10	1q31-q32	Anti-inflammatory cytokine.

Figure 1. *Top RA-related genes. This table provides a snapshot of genes commonly associated with RA. Each gene's specific role and significance in RA pathogenesis can vary, and ongoing research continues to uncover new insights into how these genes contribute to the disease.*

1.3 Pathophysiology of Rheumatoid Arthritis and the Role of the Immune System

Rheumatoid arthritis represents a multifaceted disease with a significant genetic component, modulated by environmental factors. Its heterogeneity is reflected in the variable clinical presentation and the range of pathogenetic mechanisms. Understanding these aspects is crucial for developing targeted therapies and managing the disease effectively.

Rheumatoid arthritis (RA) is a chronic autoimmune condition primarily characterized by inflammation and subsequent damage to joint structures, but it also has the potential to affect multiple systemic organs. The etiology of RA is complex and not entirely elucidated, involving an intricate interplay of genetic predispositions, environmental triggers, and immune system dysregulation (7). Genetically, certain alleles, such as HLA-DR4, are significantly correlated with an elevated risk of RA, predisposing the immune system to react against the body's tissues. Environmental contributors, including smoking and certain microbial infections, are thought to act as catalysts in genetically susceptible individuals, either initiating or perpetuating the autoimmune response (21). Central to the pathophysiology of RA is the immune system's compromised ability to differentiate between self and non-self antigens, leading to an attack on host tissues, notably the synovial membrane in joints. Autoantibodies, particularly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) are prominent in RA, targeting joint components and exacerbating inflammation and damage (22). The immune response is further characterized by the activation of T cells, B cells, and other immune cells within the synovial tissue, which release a cascade of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and various interleukins, intensifying the inflammatory process. This immune activation results in synovial hyperplasia, characterized by an inflamed and thickened joint lining and an accumulation of inflammatory cells (23). This proliferative and invasive tissue erodes cartilage and bone, leading to the deformities and functional impairments typical of RA. Beyond the joints, RA's systemic inflammation can adversely affect other organs, including the skin, lungs, heart, and eyes. The chronic nature of inflammation and joint damage in RA is a major contributor to persistent pain and fatigue, markedly diminishing the quality of life for affected individuals (1).

2. Cancer Overview

Cancer, a broad term encompassing a multitude of diseases, is characterized by the abnormal and uncontrolled growth of cells that can invade and destroy normal body tissue. Cancers are classified based on their origin and the type of cells they arise from. The main categories include carcinoma, from epithelial cells; sarcoma, from connective tissues; leukemia,

from blood-forming tissues; lymphoma and myeloma, from immune system cells; and central nervous system cancers, from brain and spinal cord cells (24).

2.1 Epidemiology of Cancer

Globally, the most frequently occurring cancers vary but typically include breast, lung, prostate, colorectal, and skin cancers. Lung cancer ranks as a leading cause of cancer-related deaths globally, with breast cancer being prominent among women and prostate cancer among men (26). The occurrence of different types of cancer is influenced by a combination of genetic factors, environmental conditions, and lifestyle choices. The incidence, which refers to the rate of new cases, and the prevalence, indicating the total existing cases, are shaped by factors like genetic predispositions, environmental factors, and lifestyle habits. Breast, lung, colorectal, prostate, and stomach cancers rank as the most prevalent worldwide, showing distinct gender variations, such as breast cancer being more common in women and prostate cancer in men. Lung and colorectal cancers are common in both genders (26).

Geographical differences in cancer incidence are notable. Developed countries tend to have higher rates of breast and prostate cancers, attributed to lifestyle factors and possibly more robust cancer detection methods. In contrast, developing countries often report higher incidences of cervical cancer, linked to limited access to healthcare and preventive measures. Additionally, the prevalence of particular cancers in specific regions can be attributed to unique environmental or genetic influences. For example, liver cancer has a higher incidence in East Asia and Sub-Saharan Africa, mainly due to the widespread presence of risk factors such as hepatitis B and C infections (27).

Epidemiological trends in cancer also reveal age as a significant factor, with the majority of cancers being diagnosed in older individuals, reflecting the culmination of lifetime exposures and genetic risk factors. The influence of lifestyle factors such as smoking, diet, physical inactivity, and obesity cannot be overstated, as they contribute significantly to cancer risk. In recent years, there has been a growing recognition of the role of infectious agents in causing certain cancers, exemplified by the links between human papillomavirus (HPV) and cervical cancer and Helicobacter pylori infection with gastric cancer (28).

2.2 The Pathophysiology of Cancer

The pathophysiology of cancer involves complex interactions between cancer cells and the body's immune system. Normally, the immune system detects and eliminates abnormal cells, including precancerous or cancerous

cells. However, cancer cells can develop mechanisms to evade immune detection by expressing proteins that suppress immune responses or inducing a local immunosuppressive environment. This immune evasion is a key factor in the development and progression of cancer (24). Furthermore, chronic inflammation, which can result from persistent infections, autoimmune diseases, or other long-term inflammatory stimuli, is known to increase the risk of several types of cancer by creating an environment conducive to cellular transformation and tumorigenesis. Understanding these immunological aspects is crucial for developing targeted cancer therapies, including immunotherapies that enhance the immune system's ability to fight cancer (29).

3. Rheumatoid Arthritis and Cancer Risk

Recent epidemiological studies have revealed an increased occurrence and frequency of various comorbidities in individuals with rheumatoid arthritis (RA), especially cardiovascular diseases, certain types of cancers, infections, and osteoporosis. A significant study conducted in France found an elevated risk for various cancers in RA patients. These include lung cancer (with a Standardized Incidence Ratio [SIR] of 1.41, 95% Confidence Interval [CI] [1.36–1.46]), bladder cancer (SIR 2.38, 95% CI [2.25–2.51]), cervical cancer (SIR 1.80, 95% CI [1.62–2.01]), prostate cancer (SIR 1.08, 95% CI [1.04–1.13]), and melanoma (SIR 1.37, 95% CI [1.29–1.46]). Additionally, hematological malignancies like diffuse large B cell lymphoma (SIR 1.79, 95% CI [1.63–1.96]), follicular lymphoma (SIR 2.16, 95% CI [1.94–2.40]), Hodgkin's lymphoma (SIR 2.73, 95% CI [2.31–3.23]), and multiple myeloma (SIR 1.42, 95% CI [1.27–1.60]) were more common in RA patients. Contrarily, the incidence of pancreatic cancer was lower in the RA group (SIR 0.90, 95% CI [0.83–0.97]), as were certain cancers specific to women, such as breast and endometrial cancers (SIR 0.91, 95% CI [0.88–0.94] and SIR 0.77, 95% CI [0.71–0.84], respectively) (30).

These observations are particularly significant in understanding the pathophysiology of RA, as the disease is often accompanied by chronic inflammation, which is hypothesized to contribute to the increased risk of lymphomas. This is attributed to the heightened activity of lymphocytes, specifically B cells and T cells, which are known to become malignant in lymphomas. The chronic inflammatory stimulation in RA may make these cells more prone to malignancy. Additionally, Chatzidionysiou et al., underscored that seropositive RA is a risk factor for lung cancer, extending beyond the risks posed by smoking, though residual confounding factors such as airway exposures cannot be entirely ruled out. Data from the National Health and Nutrition Examination Survey (NHANES) 2011-2014, which included 11,262 individuals (826 diagnosed with cancer), revealed that 8.96% of the individuals with cancer had an RA diagnosis, compared to only 3.56%

in the non-cancer group. This statistically significant association (p-value: < 0.0001) underscores the complex interrelation between RA and cancer, suggesting an underlying mechanism that extends beyond the direct effects of chronic inflammation (32).

Key types of cancer associated with RA include:

Lymphoma: Individuals with Rheumatoid Arthritis (RA) are approximately twice as likely to develop lymphoma, a type of cancer originating in the blood, compared to the general population. This elevated risk is thought to be related to the continuous inflammatory stimulation of the immune system seen in RA. Lymphocytes, particularly B cells and T cells, play a central role in RA-related inflammation and are the same cells that turn cancerous in lymphomas. The increased activity of these lymphocytes in RA patients amplifies their chances of becoming malignant (33).

Lung Cancer: RA patients face an elevated risk of developing lung cancer. Although smoking is a recognized risk factor for both RA and lung cancer, studies suggest that RA patients who smoke have a roughly 40% higher chance of developing lung cancer compared to smokers who do not have RA. This indicates that besides smoking, chronic inflammation associated with RA also significantly contributes to this increased risk (34).

Skin Cancer: Treatment of Rheumatoid Arthritis (RA) with Methotrexate and biologic drugs has been linked to a modest increase in the risk of two types of skin cancer: basal BCC and SCC. Research indicates that using biologics can elevate the risk of SCC by 30%. However, it's important to emphasize that both BCC and SCC are highly treatable, and the overall increase in risk associated with these treatments is relatively minor (35).

These findings emphasize the importance of regular cancer screenings and vigilant monitoring for signs of malignancy in individuals with RA. It also highlights the complexity of RA as a disease that extends beyond joint inflammation, impacting various systems in the body and increasing susceptibility to serious conditions like cancer.

3.1 Potential reasons for increased or decreased cancer risk in RA patients.

Individuals with RA have an enhanced risk of developing certain types of cancer, which can be attributed to several factors, including chronic inflammation, RA medications, etc. Chronic inflammation, a hallmark of RA, is a primary link between RA and cancer. Inflammatory stimulation of the immune system, particularly involving lymphocytes like B cells and T cells, increases the risk of cancers such as lymphoma (33). These cells, central to RA-related inflammation, are also the ones that become cancerous in lymphomas.

The other factor is RA Medications. Certain medications used to treat RA can affect the immune system and potentially increase cancer risk. Drugs like cyclophosphamide and azathioprine, though less commonly used for RA, have shown an association with increased cancer risk (36). Moreover, Methotrexate, a widely used RA medication, has been linked to an increased risk of lymphoma, especially in patients with the Epstein-Barr virus (37). The use of Methotrexate and biologic drugs in RA treatment has been linked to a slight increase in the risk of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). For example, taking a biologic medication can raise the risk of SCC by 30%, though this risk is relatively lower (38).

Moreover, smoking, a recognized risk factor for Rheumatoid Arthritis (RA), significantly escalates the risk of lung cancer. Studies show that RA patients who smoke face an approximately 40% increased risk of developing lung cancer compared to non-RA smokers, indicating that chronic inflammation plays a crucial role in this increased risk (31, 34). These factors highlight the complex interplay between RA, its treatment, and cancer risk, underscoring the importance of regular monitoring and cancer screening for RA patients.

3.2 Shared Pathways

The relationship between immune system dysregulation in rheumatoid arthritis (RA) and cancer is multifaceted and complex. In RA, prolonged immune dysregulation and the resulting inflammatory response can lead to an increased risk of developing certain types of cancer. Specifically, RA has been associated with a heightened risk of non-Hodgkin's lymphoma, and there is evidence supporting a relationship with lung cancer (31, 34).

3.3 Examination of the immune system dysregulation in both RA and cancer

A key pathway in this context is the IL-6 pathway, which is crucial in immune regulation and dysregulation in various rheumatic diseases. Elevated levels of IL-6 have been implicated in both RA and cancer, leading to new therapeutic approaches targeting this pathway for treating rheumatic diseases and, interestingly, COVID-19, especially in older individuals. The treatment of RA itself can further complicate this relationship. RA treatments often suppress the immune system, placing patients at a higher risk of infections, such as the common cold, flu, and COVID-19 (39). Similarly, cancer treatments like chemotherapy and targeted therapy can weaken the immune system, making cancer patients more susceptible to illnesses (40). Furthermore, the drugs used in RA treatment, such as Methotrexate and rituximab, have a complex connection with cancer (37). Dysregulation of the immune system is a critical factor in both autoimmunity, as seen in RA, and cancer. This connection underscores the increased baseline risk RA patients have for

developing non-Hodgkin lymphoma compared to the general population (33). This interplay highlights the need for careful management and monitoring of patients with RA, especially those undergoing treatment for cancer, due to the compounded risks and interactions between their treatment regimens and their immune systems.

The connection between chronic inflammation in RA and its possible role in cancer development is a subject of considerable research and interest. In RA, persistent inflammation is a central feature. The disease's pathology is marked by swollen, painful, and deformed joints, indicating the pervasive impact of chronic inflammation in its progression. This ongoing inflammation is driven by a state of perpetual activation of immune mediators such as cytokines, chemokines, and free radicals, which can cause tissue damage and increase the risk of carcinogenesis. Chronic inflammation in RA and other systemic inflammatory rheumatic diseases has been linked to an increased risk of malignancy, particularly lymphoproliferative disorders (41). This is believed to be due to the chronic inflammatory state creating a microenvironment that can promote the development of cancer. However, there is ongoing debate about the role of immunosuppressive therapy in contributing to carcinogenesis in these patients.

Furthermore, the molecular mechanisms linking chronic inflammation to cancer development are complex. Pro-inflammatory mediators play a crucial role in inflammation-driven carcinogenesis, and there is evidence of the involvement of molecular mechanisms like IL-1 signaling in tumors. Also, stress proteins have dual roles in this context, acting as danger signals in developing anti-cancer immunity and having anti-apoptotic functions (29).

3.4 Genetic factors that might predispose individuals to both RA and certain cancers

In a broader context, chronic inflammation is known to cause various pathologies, including cardiovascular diseases, diabetes, Alzheimer's, autoimmune diseases, and cancer. This suggests that the systemic effects of chronic inflammation extend far beyond the joints affected in RA, impacting multiple body systems and increasing the risk of a range of diseases, including cancer.

This research underscores the importance of understanding and managing chronic inflammation in RA, not only to address the joint damage and pain associated with the disease but also to mitigate the increased risk of cancer associated with this chronic inflammatory state.

Research has shown a significant occurrence of neoplasms in people with rheumatoid arthritis (RA). A study employing Mendelian randomization (MR) investigated the potential causal link between RA and two types of

neoplasms: benign neoplasms of bone and articular cartilage (BNBAC) and malignant neoplasms of bone and articular cartilage (MNBAC). The findings indicated a notable genetic link between RA and MNBAC, but not BNBAC. Moreover, the study found no genetic evidence suggesting that either BNBAC or MNBAC could causally lead to RA (43).

RA, characterized as a chronic autoimmune inflammatory disorder, has a significant genetic basis, with its heritability estimated at around 60%. This condition is marked by an impaired immune system and is associated with a heightened risk of various cancers. This increased susceptibility may be due to genetic predispositions and interactions between genes and environmental factors. Furthermore, the administration of immunosuppressive and antirheumatic drugs in individuals with rheumatoid arthritis is linked to an elevated risk of tumor development. (44).

In addition, estrogen metabolites, which play a role in the development of both RA and tumors, might be a key factor in the connection between RA and certain cancers. These metabolites have the potential to cause DNA alterations, which can provoke immune reactions, resulting in increased levels of antibodies related to both tumors and RA. Understanding the relationship between RA and cancer requires considering variations in estrogen levels across different age groups. RA patients often show a higher occurrence of various cancers, such as lung, skin, and breast cancers. This increased cancer incidence could be linked to the medications used to treat RA or to the inflammatory nature of the disease itself.

4. Impact of RA Treatment on Cancer Risk

4.1 Overview of RA treatments, including DMARDs, biologics, and corticosteroids.

Methotrexate is classified into DMARDS and often the first line of treatment. It reduces inflammation and slows disease progression. Methotrexate is often combined with other DMARDs for a more aggressive treatment approach in moderate to severe RA (37). Hydroxychloroquine is used in mild-term of RA (45). Sulfasalazine is effective in controlling inflammation and joint damage (46). Leflunomide is an another option for reducing symptoms and slowing the progression of RA (47).

Biologics target specific parts of the immune system that drive inflammation. They are usually prescribed when traditional DMARDs are insufficient (48). TNF inhibitors include etanercept, infliximab, and adalimumab. They block tumor necrosis factor, a pro-inflammatory substance. IL-6 Inhibitors, such as tocilizumab, block the interleukin-6 pathway. Abatacept is an example of T-cell activation inhibitors that interferes with T-cell activation (49). Rituximab targets B cells in the immune system which

aims B-cell depletion therapy. A newer class of oral biologics that block Janus kinase pathways, important in the immune response (50).

Corticosteroids are potent anti-inflammatory drugs often used for short-term management of acute RA flares or while waiting for DMARDs to take effect. Prednisone is the most commonly used corticosteroid in RA (51). It is usually prescribed for short durations due to their side-effect profile, which can include osteoporosis, weight gain, and increased infection risk.

The choice of treatment often depends on the severity and progression of RA. Moreover, age, overall health, comorbidities, and preferences play a role. Regular monitoring of side effects and efficacy is essential, especially when using biologics or corticosteroids.

4.2 How might these treatments influence cancer risk?

DMARDS

Concerns have been raised regarding the possible carcinogenic effects of Methotrexate, a common treatment for Rheumatoid Arthritis (RA). However, research generally indicates that Methotrexate does not significantly elevate the overall risk of cancer. There is, nonetheless, a potential for a slightly increased risk of certain skin cancers. Regarding other RA medications like hydroxychloroquine, sulfasalazine, and leflunomide, current evidence does not strongly suggest any substantial increase in the risk of developing cancer (52).

Biologics

-TNF Inhibitors: Studies on TNF inhibitors (e.g., etanercept, infliximab, adalimumab) have shown mixed results. While some studies suggest a potential increased risk of certain cancers, such as skin cancers and lymphoma, others have not found a significant increase in overall cancer risk.

- IL-6 Inhibitors, T-Cell, and B-Cell Inhibitors: Data on other biologics, like IL-6 inhibitors (tocilizumab), T-cell activation inhibitors (abatacept), and B-cell depletion therapy (rituximab), are still emerging. These drugs modify specific immune pathways, which theoretically could impact cancer risk, but conclusive evidence is lacking.

- JAK Inhibitors: Being relatively new, the long-term cancer risks associated with JAK inhibitors are still under investigation (53,54).

Corticosteroids

Generally, short-term use of corticosteroids is not associated with a significant increase in cancer risk. Prolonged use of corticosteroids may have implications for cancer risk due to their impact on the immune system and general health, but specific associations are not well-defined.

RA, as an autoimmune disease with chronic inflammation, may inherently carry an increased risk of certain cancers independent of medication. When prescribing RA treatments, clinicians weigh the benefits of controlling inflammation and preventing joint damage against potential risks, including cancer. Individual patient factors, such as sex, family history of cancer, smoking status, and age may also influence the risk of cancer. Patients on certain RA medications may require regular monitoring and screening for cancer, depending on their individual risk factors and the medications they are taking (55,56).

Recent epidemiological studies have deepened our understanding of the comorbidity profile in Rheumatoid Arthritis (RA), with an evident increase in the incidence and prevalence of various conditions, notably cardiovascular diseases, infections, osteoporosis, and specific cancers. A landmark study in France has particularly highlighted an augmented risk of several cancers in RA patients, including lung, bladder, cervical, prostate, and melanoma, along with a higher prevalence of hematological malignancies such as diffuse large B cell lymphoma, follicular lymphoma, Hodgkin's lymphoma, and multiple myeloma. Conversely, a decreased incidence of pancreatic, breast, and endometrial cancers was observed. These trends, underpinned by chronic inflammation and heightened lymphocyte activity inherent in RA, suggest a complex pathophysiological link between RA and cancer. Notably, seropositive RA emerges as a distinct risk factor for lung cancer, extending beyond the conventional risks associated with smoking. Data from the National Health and Nutrition Examination Survey (NHANES) 2011-2014 reinforce this association, indicating an underlying mechanism beyond direct inflammatory effects. This abstract underlines the importance of regular cancer screenings. It highlights the need for comprehensive patient monitoring, acknowledging the extended impact of RA on various body systems and its potential to increase susceptibility to serious conditions like cancer. This research not only contributes to the growing body of literature on RA comorbidities but also underscores the necessity for integrative care approaches in managing RA patients.

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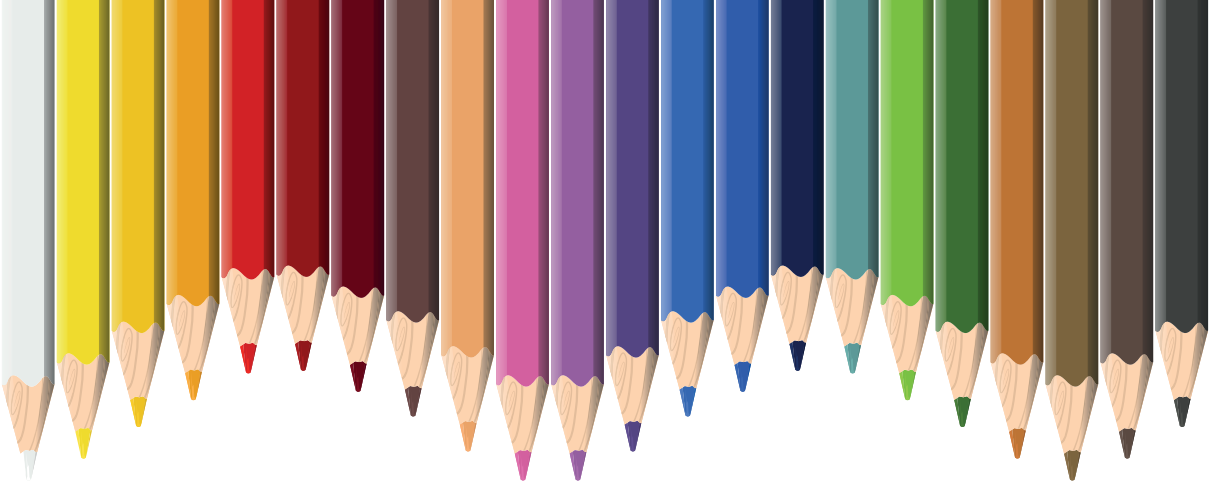

Chapter 6

ROTATOR CUFF LESİONS

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INTRODUCTION

The shoulder joint is the body part with a complex anatomy and the highest joint range of motion (Donnelly, Ashwin, MacFarlane, & Waseem, 2013). It is highly vulnerable to damage because it is such a mobile joint. Shoulder pain, which affects over 70% of the general population, is the second most frequent kind of pain after low back pain (Luime et al., 2004). The rate of rotator cuff lesion among individuals with shoulder pain is more than 70% (Chang, Lee, & Lo, 2016; Mitchell, Adebajo, Hay, & Carr, 2005). Studies have shown that the frequency of rotator cuff lesions increases over the age of 40, and that it exceeds 50% over the age of 60 (Milgrom, Schaffler, Gilbert, & Van Holsbeeck, 1995; Sher, Uribe, Posada, Murphy, & Zlatkin, 1995). Rotator cuff lesions begins as acute tendinitis in the tendons of the rotator cuff muscles, continues with partial rupture, and results in full-thickness rupture (Neer, 1993). In general, rotator cuff lesions is divided into 3 groups: subacromial impingement syndrome, calcific tendinitis and rotator cuff injuries (partial or total) (Öztürk, 2004). Its symptoms are pain, limitation of movement, muscular weakness and functional disabilities (Cofield, 1985).

Genetic factors (Harvie et al., 2004), hormonal changes (Magnusson et al., 2007), habits such as smoking and alcohol (Mallon, Misamore, Snead, & Denton, 2004), education level (Dunn et al., 2014), biochemical and sensorymotor cortex changes (J. Lewis, 2014), psychological factors (J. Lewis, 2016) and biomechanical changes (J. Lewis, 2016; McCreesh & Lewis, 2013) are possible causes of rotator cuff lesions. As a result of biomechanical changes, movements and forces acting on the shoulder change, placing excessive load on the soft tissues. Overloaded tissues cause a series of neuromuscular changes such as muscular weakness and decreased endurance, which paves the way for shoulder pathologies (Bowman, Hart, McGuire, Palmieri, & Ingersoll, 2006; Escamilla et al., 2007; Ludewig & Cook, 2000; Sood, Nussbaum, & Hager, 2007). Acute muscle fatigue disrupts the normal synergistic activities of the muscles in the shoulder area by creating muscle strength imbalance in the short term. Acute fatigue of the muscles responsible for scapulothoracic stabilization, such as the serratus anterior and lower trapezius, which are a component of muscular synergies and force couple, can cause subacromial impingement syndrome and glenohumeral instability (Bosch, De Looze, & Van Dieen, 2007; Szucs, Navalgund, & Borstad, 2009).

ROTATOR CUFF MUSCLES

The rotator cuff muscles are involved in shoulder motions and glenohumeral stability. Any weakness or dysfunction in these muscles can limit mobility as well as compromise stability (Cael, 2011; Houglum & Bertoti, 2011).

1. M. Supraspinatus

M. Supraspinatus extends from the scapula's supraspinatus fossa to the humerus's greater tubercle. It has the nerve suprascapularis (C5-C6) (Drake, Vogl, & Mitchell, 2014). This muscle's body is covered by the trapezius muscles, and its tendon is covered by the deltoid muscles. It is essential in the first 15 degrees of glenohumeral joint abduction, together with the middle section of the deltoid. It may, however, accomplish the whole abduction action without the assistance of the deltoid muscle (Houglum & Bertoti, 2011; Peat, 1986).

2. M. Infraspinatus

M. Infraspinatus extends from the scapula's infraspinous fossa to the humerus's greater tubercle. The suprascapularis (C5-C6) nerve innervates the infraspinatus muscle, which provides external rotation, adduction, extension, and horizontal adduction of the shoulder (Drake et al., 2014). It works with the teres minor to move the humeral head posteriorly in the glenoid fossa (Cael, 2011).

3. M. Teres Minor

M. Teres minor connects to the larger tubercle of the humerus from the upper section of the lateral margin of the scapula. N.axillaris (C5-C6) is its nerve. It produces shoulder external rotation, adduction, extension, and horizontal adduction (Drake et al., 2014). The clavicular region of the pectoralis major, in conjunction with the latissimus dorsi, aids in the lowering of the elevated arm (Cael, 2011; Peat, 1986).

4. M. Subscapularis

M. Subscapularis extends from the scapula's fossa subscapularis to the humerus's lesser tubercle. N.subscapularis (C5-C6) is its nerve (Drake et al., 2014). Although it may conduct flexion, extension, adduction, or abduction depending on the arm position, its primary purpose is to rotate the arm internally. When the arm is raised, the subscapularis muscle helps in extension. When the arm is in internal rotation, it aids adduction, and when the arm is in external rotation, it aids abduction (Hughes, Johnson, Skow, An, & O'Driscoll, 1999).

ROTATOR CUFF LESION TYPES

Rotator cuff lesions is one of the most common problems in the shoulder (Van der Windt, Koes, de Jong, & Bouter, 1995; Vecchio, Kavanagh, Hazleman, & King, 1995). In general, it is divided into three groups: subacromial impingement syndrome, calcific tendinitis and rotator cuff injuries (partial or total) (Öztürk, 2004).

1- Subacromial impingement syndrome

In the subacromial impingement syndrome, rotator cuff tendons are compressed between the subacromial bursa and the long head of the biceps tendon, the coraco-acromial arch, and the humeral head (Neer, 1993). The relationship between these structures in certain shoulder positions leads to subacromial impingement syndrome, which is one of the general causes of shoulder pain (Codsi & Howe, 2015; Mullaney & Nicholas, 2014; Valadie, Jobe, Pink, Ekman, & Jobe, 2000). The rate of shoulder problems in the general population varies between 7% and 27% (Luime et al., 2004). Subacromial impingement syndrome appears to be between approximately 2.4% and 14% of the general population (Littlewood, May, & Walters, 2013).

Subacromial impingement syndrome can be basically classified by several mechanisms:

a. Intrinsic and extrinsic *(Factor & Dale, 2014)*

Intrinsic (Non-outlet) subacromial impingement

It occurs due to minor or major trauma to the tendons as a result of degenerative conditions. It is a condition characterized by complete or partial tears in tendons. These factors, which consist of intrinsic factors, directly include changes in the vascularity of the subacromial space and rotator cuff, degeneration, and anatomical or bone anomalies.

Extrinsic (Outlet) subacromial impingement

Tendon inflammation or degeneration develops as a result of mechanical compression applied to the tendon from the outside. Extrinsic factors include muscle imbalance and motor control problems of the rotator cuff and scapular muscles, functional arc of movement, postural changes and training errors, and occupational or environmental risks. Extrinsic subacromial impingement usually occurs as a result of a combination of many problems rather than a single problem (Donatelli, 2011).

b. Primary and Secondary *(Cools, Witvrouw, Declercq, Vanderstraeten, & Cambier, 2004)*

Primary subacromial impingement

It is usually seen in middle-aged patients. It occurs as a result of mechanical compression of the structures in the subacromial space.

Secondary subacromial impingement

It occurs as a result of shoulder instability or weakness of the muscles around the scapulapacic joint, which causes posterior capsule tension and functional shoulder instability.

c. Three phases subacromial impingement syndrome *(Neer, 1993)*

Stage I *(Edema and hemorrhage phase)*

It is caused by mechanical irritation caused by compression of the tendon during overhead activities. In general, in people under the age of 25, there is reversible edema and bleeding in the tendon, mostly in the area close to the area where the tendon attaches to the greater tubercle of the humerus. It can be treated with physiotherapy methods (Neer, 1972, 1993).

Stage II *(Fibrosis and tendonitis phase)*

In people between the ages of 25 and 40, findings of fibrosis and thickening in the subacromial-subdeltoid bursa and tendinosis in the tendon occur due to repeated microtraumas. Histopathologically, during this period, there is more degeneration in the tendon than inflammation. Although the function of the shoulder is sufficient in non-strenuous activities, the patient becomes symptomatic with excessive and strenuous use (Neer, 1972, 1993).

Stage 3 *(Bone spurs and tendon rupture)*

Progressive degeneration in the supraspinatus tendon causes first a partial and then a full-thickness tear. Patients are generally over 40 years of age and have new bone formation on the lower surface of the acromion and biceps tendon injuries (Neer, 1972, 1993).

Etiology

There are 6 main anatomical structures underlying the subacromial impingement syndrome in the shoulder. These structures are as follows (Donatelli, 2011):

1. Acromion
2. Coracoacromial ligament
3. Glenoid labrum
4. Rotator cuff muscles
5. Biceps long head tendon
6. Subacromial bursa

Symptoms

The most common symptoms of subacromial impingement syndrome are; pain, crepitus in the tendon, muscle weakness, loss of movement in the shoulder joint, painful arc varying between 60°/70°–120° in shoulder elevation, excessive scapular mobility, functional loss, and insufficiency in movements (Fongemie, Buss, & Rolnick, 1998; J. S. Lewis, Green, & Wright, 2005; Michener, McClure, & Karduna, 2003).

Possible causes of subacromial impingement syndrome are different for extrinsic and intrinsic subacromial impingement. These reasons can be listed as follows:

Causes of Extrinsic Subacromial Impingement Syndrome

a. Subacromial spurs

b. Type 2 and 3 acromions

c. Osteoarthritic spurs of the acromioclavicular joint, including subacromial spurs

d. Thickened/calcified coracoacromial ligament

Causes of Intrinsic Subacromial Impingement Syndrome

a. Rotator cuff weakness which may cause upward displacement of the humerus

b. Secondary subacromial compression due to instability

c. Acromial defects (os acromiale)

d. Anterior/posterior capsular tension (adhesive capsulitis)

e. Thickening in the subacromial bursa (Fongemie et al., 1998)

2- Calcific tendinitis

Calcific tendinitis of the shoulder is a condition characterized by reactive calcification affecting the rotator cuff tendons. Its incidence varies between 3% and 20%. It occurs especially in young and active people (BOSWORTH, 1941). Supraspinatus is the most commonly affected tendon. Clinically, three stages are observed: first pre-calcification stage, second calcific stage, third post-calcification stage. The calcific phase is divided into three phases: formation, rest and resorptive phase. Patients mostly consult a physician in the resorptive phase, which is accompanied by severe pain (KÖMÜRCÜ & KILIÇ, 2007). Pain and limitation of movement are the main complaints of patients. It is a sudden and severe pain and occurs with high-angle restriction in active and passive shoulder movements (Aina, Cardinal, Bureau, Aubin, & Brassard, 2001). Because the condition usually resolves on its own, treatment is mainly conservative; drugs, physical therapy, and iontopheresis can all be used. In cases where these methods fail, surgical treatment can be preferred (KÖMÜRCÜ & KILIÇ, 2007)

3- Rotator cuff injuries

The rotator cuff is a complex tissue that wraps the humeral head from front to back and is made up of the supscapularis, supraspinatus, infraspinatus, and teres minor muscles. The tendons of these four muscles cannot be

considered separately from each other, and therefore the pathological change in the rotator cuff may affect a single tendon or more than one tendon. Rotator cuff injuries consists of a series of abnormal changes in a broad framework. This abnormality can progress from inflammation to full-thickness tears in one or more tendons of the cuff (Baltacı, 2015; Matava, Purcell, & Rudzki, 2005).

Rotator cuff injuries can be seen in 65% to 70% of individuals with shoulder pain (Nazarian et al., 2013). While these lesions are generally seen with degenerative changes in the elderly, they occur due to secondary causes due to overuse or trauma in young people (Brox, Staff, Ljunggren, & Brevik, 1993; Hawkins & Abrams, 1987).

As a result of rotator cuff injury, a series of changes occur in the tendon, starting with tendinopathy, continuing as a partial tear, and ending with a full-thickness tear. In magnetic resonance imaging examination, along with these changes in the rotator cuff, secondary findings of other bones and soft tissues of the shoulder joint can also be evaluated (Arkun, 2014).

1. Tendinopathy

Regardless of the cause other than acute trauma, the first pathological change that changes the normal structure in rotator cuff is tendinopathy. This change is most seen in the supraspinatus tendon. Tendinopathy is an indicator of bleeding, edema and mucoid degeneration that occurs within the tendon as a result of tendon degeneration. While tendon diameter increases in the early period, tendon thickness decreases focally or diffusely in prolonged damage. It may be difficult to distinguish late tendinopathy from a small partial tear (McMonagle & Vinson, 2012; Opsha et al., 2008). Additionally, calcific tendonitis may occur within the tendon along with tendinopathy in the area where the supraspinatus tendon attaches to the greater tubercle of the humerus (Stäbler, 2004).

2. Partial tear

In a partial tear, the disruption in tendon continuity does not extend through the entire tendon. Partial tears are grouped into three groups: intrasubstance (inside the tendon), articular face, and bursal face (Matava et al., 2005; McMonagle & Vinson, 2012). Cadaver studies regarding the incidence of partial tears show that intratendon tears are more common than other types of tears, and some of the partial tears may be asymptomatic (Matava et al., 2005). Vinson et al. (Vinson, Helms, & Higgins, 2007) in their study on 200 individuals, reported that the rate of intra-tendinous tears was 24.5%, articular face tears were 12.9%, and bursal face tears were 2.9%.

3. Full-thickness tear

If the discontinuity in the tendon fibers continues from the joint face to the bursal face, Rotator cuff full-thickness tears occur. Suprascapularis and infraspinatus tears are most commonly seen isolated in the supraspinatus tendon (78%), and isolated tears are not seen in the teres minor (Arkun, 2014; Stäbler, 2004). The size of the full-thickness tear is important in surgical planning. According to anteroposterior extension, DeOrio and Cofield full thickness tears;

Small: <1cm,

Medium: 1-3 cm,

Large: 3-5 cm and

Massive: >5 cm. Grouped into four groups (McMonagle & Vinson, 2012).

Different theories have been put forward on the etiology of rotator cuff injuries. Although it is a theory that microtraumatic tendinopathies resulting from overuse of rotator cuff muscles progress to full-thickness tears, another etiological factor is that rotator cuff injuries occurs in addition to glenohumeral joint instability and subacromial impingement syndrome (Baltacı, 2015; Bigliani, Ticker, Flatow, Soslowsky, & Mow, 1991).

Rotator cuff injuries can be classified in many ways. One of these classifications is the classification according to pathophysiology and is divided into four groups (Baltacı, 2015; Donatelli, 2011).

1. Primary compressive pathologies

Primary compressive pathologies occur as a result of compression of the rotator cuff tendons between the humeral head and the anterior part of the acromion and the coracoacromial ligament, coracoid or acromioclavicular joint (Neer, 1972, 1993). In individuals with rotator cuff problems, the subacromial space decreases due to many reasons. Acromion type is considered to be one of them (Zuckerman, Kummer, Cuomo, & Greller, 1997). Type III acromion was found in 70% of patients with full-thickness rotator cuff tears, while type I acromion was found in only 3% of the remaining patients. The second factor is that the subacromial bursa swells and thickens due to inflammation, narrowing the subacromial space. Other reasons are the thickening of the coracoacromial ligament and finally the thought that excessive anterior tilt and internal rotation of the scapula narrows the subacromial space (J. S. Lewis et al., 2005).

2. Secondary compressive pathologies

Impingement or compressive pathologies may occur secondary to glenohumeral joint instability. During overhead activities such as throwing, ante-

rior instability occurs due to excessive strain on the capsular ligaments and labrum, which are the static stabilizers of the glenohumeral joint, and subsequent displacement of the humeral head increases. The biceps tendon and rotator cuff are compressed as a result of this increase. Secondary compression occurs as a result (Blevins, 1997; Jobe, Kvitne, & Giangarra, 1989).

3. Overloads

Pain caused by tendon overload during the throwing phases of overhead sports activities and repetitive extrinsic factors cause strain on the posterior rotator cuff muscles (Blevins, 1997). In addition, in excessive use of the rotator cuff muscles, the different shear forces applied to the five layers of the tendon make the tendon vulnerable to stress and intra-tendinous tears occur. Intratendon tears can turn into full-thickness rotator cuff tears over time (Matava et al., 2005).

4. Macrotraumatic tendon injuries

Macrotraumatic tendon injuries occur when more force is applied than a healthy tendon can bear. This tear, which can occur with a single trauma, only occurs with the rupture of the greater tubercle (Baltacı, 2015). However, according to Cofield, the risk of a tear in the tendon with a single trauma is very low. In order for such a tear to occur, at least 30% of the injured tendon must be damaged beforehand (Cofield, 1985).

Symptoms include discomfort, muscular weakening, loss of flexibility in the shoulder joint, a painful arc varying between 60°/70°-120° in shoulder elevation, excessive scapular mobility, functional loss, and movement deficiencies, similar to subacromial impingement syndrome (Fongemie et al., 1998; J. S. Lewis et al., 2005; Michener et al., 2003).

Spontaneous tear of the rotator cuff in a normal healthy individual is rare (Neer, 1993). Partial tear occurs following trauma in any age group, and in young adults it usually occurs after excessive shoulder movements or after a fall. Acute complete tear may develop following a fall on a stretched arm, a hyperabduction injury, or a fall on the shoulder (Brox et al., 1993; Hawkins & Abrams, 1987).

EVALUATION OF ROTATOR CUFF LESIONS

Physical evaluation in rotator cuff lesions includes anamnesis, inspection, palpation, joint range of motion, muscle strength and function evaluations (Poustie, 2010). When taking anamnesis, great attention should be paid to the age range. During observation, attention should be paid to asymmetry, atrophy and full abduction/extension during scapulohumeral rhythm.

Pain and crepitations should be taken into account when evaluating joint range of motion and strength (Başkurt, 2007 ; Koester, George, & Kuhn, 2005; Poustie, 2010).

It is stated that scapular evaluation in shoulder evaluation increases the success in the treatment of upper extremity and shoulder girdle injuries and the treatment plan should be planned in accordance with these evaluations (Başkurt, 2007 ; Odom, Taylor, Hurd, & Denegar, 2001). The examination of scapular muscular endurance should be included in the evaluation of rotator cuff lesions for this purpose (Day, Bush, Nitz, & Uhl, 2015; Edmondston et al., 2008; McQuade, Dawson, & Smidt, 1998).

Various end-state measures have been developed to be used in shoulder evaluation to determine the patient's quality of life and functionality in daily life. These measures examine the shoulder from different perspectives (physical, emotional, social, pain and function). Some of the criteria developed for this purpose are Nottingham Health Profile, Short Form 36, Western Ontario Rotator Cuff Index, Disabilities of the Arm, Shoulder and Hand Scale, Western Ontario Shoulder Instability Index, Simple Shoulder Test (SST), Upper Extremity Function Scale, American Shoulder and Elbow Surgeons Evaluation Form, Shoulder Pain and Disability Index, and Constant & Murley Functional Shoulder Scoring.

TREATMENT APPROACHES FOR ROTATOR CUFF LESIONS

Many methods such as conservative treatment, medication, and surgical treatment are used in the treatment of rotator cuff lesions (Faber, Kuiper, Burdorf, Miedema, & Verhaar, 2006; İKİZ, 2008; Kuhn, 2009).

1. Conservative treatment

Physiotherapy applications applied within the scope of conservative treatment are very useful in the short-term treatment of rotator cuff lesions in terms of significantly reducing the symptoms (Donatelli, 2011). In general, physiotherapy applications can be divided into three categories: acute, subacute and chronic (Borstad et al., 2007; Bullock, Foster, & Wright, 2005; Donatelli, 2011; Faber et al., 2006; Fongemie et al., 1998).

Acute period

- Resting in 45° abduction position
- Ice application
- Electrotherapy (Transcutaneous Electrical Nerve Stimulation-TENS etc.)
- Ultrasound (intermittent, low dose)
- Scapula stabilization exercises

• Massage and mobilization

Subacute period

• Ultrasound

• Massage and glenohumeral joint mobilization

• Capsule and muscle stretching exercises

• Postural training of cervical and thoracic vertebrae

• Isotonic (fixed weight) exercises instead of variable weight exercises (such as rubber band)

• Exercises with relatively light weights

• Transverse friction massage

• Taping techniques

Chronic period

• Resistant exercises

• Activity modifications

2. Medication

One of the treatments to be used in rotator cuff lesions is medical treatment, especially the use of analgesics. Non-steroidal anti-inflammatory drugs to be used in medical treatment should be used with caution as they may cause side effects in the elderly. In addition, glucocorticoid injection can also be performed (Fongemie et al., 1998; Steenbrink et al., 2006).

3. Surgical treatment

In cases where the desired results are not obtained from conservative treatment and drug therapy, surgical treatment may be required. Surgery is generally performed for full-thickness rotator cuff injuries or chronic problems in active people. The most minor surgical procedure is arthroscopy and shaving small defects in the cuff (Fongemie et al., 1998; Michener, McClure, & Sennett, 2002).

CONCLUSION

Rotator cuff lesions are the result of trauma or chronic degeneration. It is a common musculoskeletal disease that can occur as a result of pain, whose incidence increases with age and significantly affects functionality and social life. There are various treatment options ranging from conservative and systemic pain modalities to surgery.

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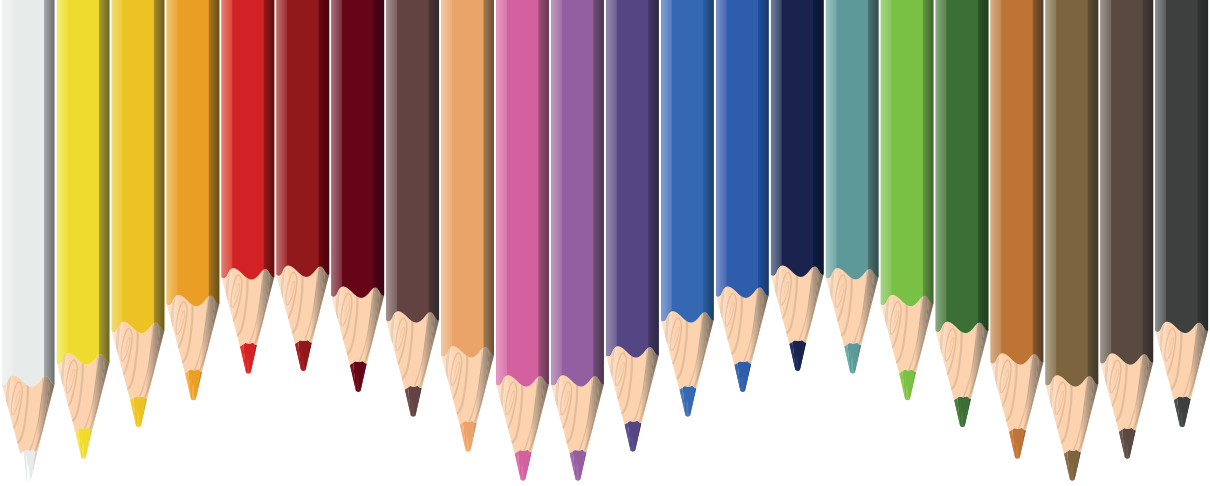

Chapter 7

IMMUNOTHERAPY AND EPIGENETIC TREATMENT IN TRIPLE NEGATIVE BREAST CANCER THERAPY

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IMMUNOTHERAPY AND EPIGENETIC TREATMENT IN TRIPLE NEGATIVE BREAST CANCER THERAPY

Breast Cancer (BC)

BC, the most prevalent cancer in women, is the second place cause of female mortality, with only lung cancer surpassing it in terms of death rates. The incidence of BC has been increasing, leading to late-stage diagnoses, which adversely impact the success rates of treatment. BC is a localized disease, and although surgical treatment is the primary choice, it is often complemented by options such as radiotherapy, chemotherapy, endocrine and targeted therapies (WHO, 2020; T.C Sağlık Bakanlığı, 2020).

Breast tumors, characterized by a diverse and varied structure, are categorized into four primary groups founded on existence of hormone receptors (HR, particularly estrogen receptor;ER and progesterone receptors;PR) and human epidermal growth factor 2 (HER2/neu), considering whether there is amplification or not. Treatment strategies are subsequently customized according to these specific subtypes (Wacks and Winer, 2019; Kohler et al.,2015).

Triple Negative Breast Cancer (TNBC)

TNBC constitutes 15-20% of BC cases (Michel et al., 2020) and is a diverse form of BC defined by the absence of HR, as well as no expression of the HER2/neu. It is defined by a high metastatic rate, poor prognosis, and the highest resistance to treatment among BC types (Nath et al., 2022) and is classified into six molecular subtypes (Yin et al.,2020). Cases diagnosed with metastatic TNBC, on average survival rate is approximately 18 months.

In TNBC treatment, chemotherapy is the most important treatment option. In addition, various different treatment approaches are available. Around 20% of TNBC patients exhibit positive response to conventional therapy. However, due to the heterogeneity of TNBC tumor cells and their high mutation burden, resistance can develop in treatment, ultimately leading to the development of metastases and making the disease fatal (Vagia vd., 2020). BC is acknowledged for its lower immunogenicity, stemming from a limited presence of genetic mutations in tumors and a scarcity of lymphocytes infiltrating the cancerous tissue. Despite these challenges, TNBC demonstrates a more positive response to cancer immunotherapy when contrasted with other forms of BC (Zhang et al, 2022; Schmid et al., 2020). The effectiveness of numerous new therapeutic agents, including targeted treatments such as PARP inhibitors and immune checkpoint inhibitors (ICi), is being evaluated through preclinical and clinical studies. Therefore, it is important to discover new treatment methods with the aim of achieving complete remission in the treatment of the disease.

Cancer Immunity Cycle

In the cancer immune cycle, antigen-presenting cells (APCs) capture, process, and present specific neoantigens created by tumor cells to T cells via the MHC I/II pathway. The binding of presented neoantigens to the T cell receptor upon antigen-presenting cells generates the necessary first signal for T cell induction. However, for the complete effect of T cells, a second co-stimulatory signal is required. The co-stimulatory signal occurs as a result of the interaction between the CD80/86 proteins on the APC and the CD28 receptor on the T cell (Figure 1).

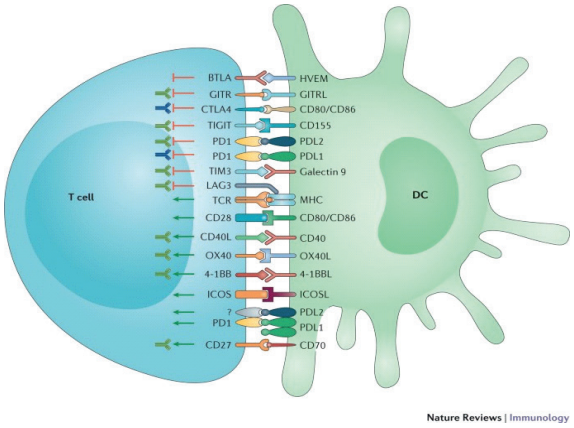

Figure 1. Interactions between antigen-presenting cells (APCs) and immune checkpoint (IC) molecules (Wykes and Lewin, 2018).

Through these interactions, T cells are activated and become functional, undergoing autocrine clonal expansion with producing of the proliferative cytokines. The proliferating effector T cells pass away to the tumor site, recognize cancer cells, and induce their apoptosis through the action of perforin and granzyme enzymes. Dead tumor cells release new neoantigens to re-activate immune cells, which are then captured, processed, and presented again to T cells, ensuring the continuation of the cancer immunity cycle (Figure 2) (Chen and Flies, 2013; Cogdill et al., 2017).

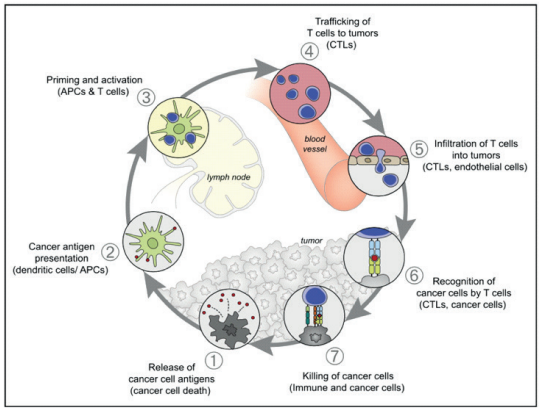

Figure 2. Cancer Immune Cycle (Chen and Mellmann, 2013)

There are various mechanisms that influence the expansion in the quantity of malignant cells in the body, and one of them involves the activation of IC. These mechanisms block immune-mediated cellular damage, allowing malignant cells to evade immune surveillance (Chen and Flies, 2013). In chronic infections and neoplastic processes, T cells can survive when exposed to chronically weak antigenic stimuli, but this can lead to T cell exhaustion. ICs, known as negative regulators of T cell activation, regulate the immune response and prevent immunohyperactivity. Cancer cells can hijack these points to evade the body's immune response. Programmed Death Ligand-1 (PD-L1) is expressed in various cancer cells and TILs. PD-L1 conjugate to the Programmed Death-1 (PD-1) receptor on T cells by this way reduces T cell signaling and inhibiting the cytotoxic T cells function. ICis like PD-1 and PD-L1 inhibitors reactivate impaired anti-tumor immune responses, restore T cell function, increase tumor cytolysis through the release of granzyme and perforin, and decrease metastasis (Figure 3) (Waldman et al., 2020; Patel and Kurzrock, 2015).

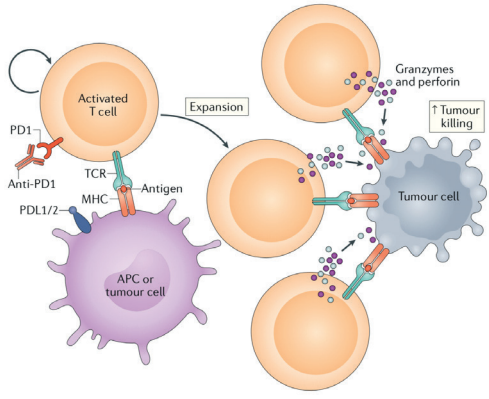

Figure 3. PD1/PD-L1 Signal Pathway (Waldman et al. 2020)

Immune Checkpoint Inhibition (ICi) In TNBC Treatment

In the field of immunotherapy, ICi is a precise method aimed at hindering particular proteins—known as ICs. The goal is to fortify the inherent immune response against cancer cells. ICs function as supervisors, overseeing and maintaining the delicate balance of the immune system. They have a vital part in preventing immune system from inadvertently targeting healthy cells. By interrupting these checkpoints, this strategy seeks to unleash and reinforce the body's intrinsic defenses against cancer cells.

TNBC patients, characterized by high PD-L1 expression, an abundance of tumor mutations in TNBC cells, and the presence of high tumor infiltrating lymphocyte (TIL) levels observed within the tumor microenvironment, have the potential to create a more immunogenic microenvironment in comparison with other types of BC. These characteristics suggest the potential to enhance the effectiveness of immunotherapy in the treatment of TNBC (Kwapisz D., 2021). Several clinical trials have investigated the use of ICi, particularly targeting the PD-1/PD-L1, in BC. In this context, the ICi atezolizumab is used, which is a monoclonal antibody that conjugate to PD-L1 expressed in tumor cells and TILs. It blocks interactions with both PD-1 and CD80 receptors. By inhibiting PD-1, it restores T cell activation, preventing cancer cells from evading immune surveillance and inducing anti-tumor immune responses. Mechanisms leading to the loss of expression of neoantigens in cancer cells, defects in antigen presentation, and changes in the tumor microenvironment affecting immune cell composition and cytokine profiles due to defects in oncogenic signaling pathways, oncogenes, and tumor suppressor genes contribute to resistance to ICi therapy. To determine which patients will benefit from treatment, the use of biomarkers is necessary. Currently, in ICI therapy, PD-L1 expression and tumor mutation burden are used for this purpose. Tumor mutation burden refers to the total number of mutations present in tumor cells. It reflects the neoantigen load presented by tumors to immune cells, and therefore, it has been shown that immunotherapy can be more effective in tumors with a high mutation burden (O'Meara and Tolaney, 2021). Clinical studies have demonstrated that TNBC cells, having a higher tumor mutation burden compared to other types of BC and exhibiting a higher level of PD-L1 expression, respond better to immunotherapy. These findings suggest that TNBC patients may experience an improved response to immunotherapy and an extended progression-free survival compared to other BC types. (Schmid et al., 2020; Loibl et al., 2019). Taxane group drugs, such as paclitaxel, have been acknowledged by the FDA since the 1990s for the treatment of breast, ovarian, lung, prostate, and gastric cancers. The response rate of paclitaxel in the treatment of BC is around 50% in first-line chemotherapy but decreases to 20-30% in second and third-line chemotherapies. Drug resistance to paclitaxel is associated with overexpression of P-glycoprotein, multidrug resis-

tance genes (MDR), ATP-binding cassette transporters, point mutations in the β-tubulin gene, changes in the structure due to abnormal expression of microtubule-associated proteins, and activation of anti-apoptotic pathways. (Yusuf et al, 2003; Jiang et al., 2014; Shimomjura et al., 2012). Due to the highly lipophilic nature of paclitaxel, it is formulated with a solvent such as Cremophor EL (polyoxyethylated castor oil) and ethanol to facilitate its passage into tumor cells. Therefore, pre-treatment with steroids is necessary to prevent the occurrence of hypersensitivity reactions in patients (Schettini et al., 2016). Nab-paclitaxel (Abraxane) is a formulation consisting of albumin-bound paclitaxel nanoparticles in a colloidal suspension. It does not require any solvent for passage into tumor cells; instead, the albumin within it binds to the gp60/caveolin-1 receptor, facilitating entry into tumor cells. As it does not have a solvent-containing formulation, it does not necessitate pre-treatment with steroids, and due to its more linear pharmacokinetics compared to paclitaxel, it can be better tolerated at higher doses (Schettini et al., 2016). Nab-paclitaxel enhances antigen presentation by increasing the maturation of antigen-presenting cells and cytokine expression. Additionally, it blocks myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells, assists in the differentiation of T cells into effector T cells, promotes the infiltration of cytotoxic T cells into the tumor microenvironment, and function in the programmed cell death of tumor cells. With these characteristics, nab-paclitaxel has important roles in assisting the induction of immune response in the cancer immune cycle (Chen et al., 2021). Various ICi approaches for the treatment of BC are currently in clinical trials. As indicated by the findings of the Phase III IMpassion130 clinical trial, which focused on TNBC cases with PD-L1 expression (≥ 1%), the combination of the PD-L1 inhibitor atezolizumab with the taxane group antineoplastic agent nab-paclitaxel received the first immunotherapy approval for BC by the FDA and the European Commission in 2019. The approval was granted based on progression-free survival outcomes from the mentioned trial (Schmid vd., 2020; Keenan and Tolaney, 2020). In contrast, the results of the Impassion 131 clinical trial, which involved a randomized group of 651 TNBC patients, demonstrated that the combination of paclitaxel with atezolizumab did not improve progression-free survival compared to paclitaxel alone in the PD-L1 positive patient group. This finding contradicts the results of the Impassion 130 study (Miles et al., 2021). The inconsistency between the results could potentially base to the significant use of steroids in paclitaxel applications, which may lead to immunosuppression in the tumor microenvironment. Despite that, further research is needed to clarify the reasons for these conflicting results.

Epigenetic Therapy and HDAC Inhibition In Cancer Treatment

Epigenetics is defined as the scientific field that studies non-genetic phenotypic variations occurring without changes in the DNA sequence, by

modulating which genes are expressed, when, and where. Epigenetic mechanisms is crucial in the communication between tumor cells and immune cells. These mechanisms involve processes such as DNA methylation, modifications to histones after protein synthesis, the regulation of chromatin structure and noncoding RNAs. There are H2A, H2B, H3, and H4 histone proteins in DNA organization and form histone octamers, with each histone consisting of two copies. Histone acetylation is an epigenetic modification that is a reversible and dynamic process, allowing DNA to open up and transcription to occur. It is catalyzed by enzymes such as histone acetyltransferases (HATs), which activate gene transcription, and histone deacetylases (HDACs), which inhibit it (Figure 4). Overexpression of HDACs and decreased expression of HATs can disrupt this balance. Abnormalities in chromatin structure and gene transcription can contribute to tumor formation. Therefore, disruptions in the delicate balance of histone acetylation and deacetylation, leading to chromatin and transcriptional abnormalities, can play a role in the development of tumors (Orr ve Hamilton, 2007).

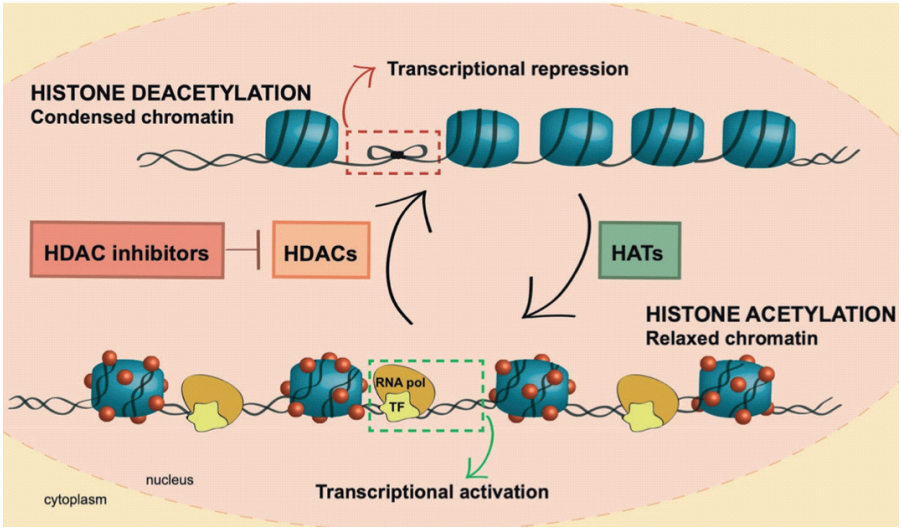

Figure 4. HDAC inhibition in the reorganization of chromatin (San-Jose Eneriz et al., 2019)

HDAC inhibitors are considered promising candidates for combined anticancer therapies. When applied to various cancer cells, HDAC inhibitors exhibit antiproliferative effects by causing cell cycle arrest, premature cell aging, and apoptosis. They play significant roles in epigenetic regulation and are considered cancer-preventive agents (Kim and Bae, 2011).

Deacetylation of histones not only suppresses gene transcription but also leads to the rearrangement of chromatin structure. This process can induce DNA damage by reducing the expression of proteins involved in the oxidative stress mechanism and/or the repair of oxidative damage. Additionally, his-

tone deacetylation can regulate epithelial-mesenchymal transition, increase the expression of proapoptotic genes, decrease the expression of anti-apoptotic genes, and impact both intrinsic and extrinsic apoptosis mechanisms (Fedele et al., 2017). It has been expressed that histone deacetylation decreases the expression of vascular endothelial growth factor receptor (VEGFR) and inhibits endothelial cell proliferation, invasion, migration, and adhesion. In this context, histone deacetylation may modulate angiogenesis and endothelial cell activity by regulating the vascular endothelial growth factor (VEGF) signaling pathway. Histone deacetylation can control these biological processes by influencing gene expression that regulates cellular responses. (Deroanne et al., 2002). HDAC8 is highly expressed in BC tissues, particularly in TNBC cells. In TNBC, there is a significant relationship between tumor size, lymphatic invasion, tumor grade, perineural invasion, and HDAC8 overexpression. Therefore, it is considered that HDAC8 expression could potentially be used as a tumor marker in the diagnosis of TNBC (Menbari et al., 2020). Also due to the increased invasion and MMP-9 expression observed in MCF-7 BC cells overexpressing HDAC1, 6, or 8, it is believed that HDAC isoforms could be developed as biomarkers for metastasis in BC (Park et al., 2011).

HDAC inhibitors have low toxicity in normal, non-cancerous healthy cells (Blattman et al., 2010; Konsoula et al., 2011; Li et al., 2011). HDAC inhibitors have proven effective when tested alone in early laboratory studies, but their performance has been constrained in more advanced clinical trials. However, combined treatments with different anticancer agents have demonstrated synergistic effects by increasing the sensitivity of tumor cells to the drugs used, making tumor cells more responsive to treatment. This synergy has been observed in both preclinical and clinical studies (Hontecillas-Prieto et al., 2020). It was documented that the combination of the HDACi OBP-801 with the antimicrotubule agent eribulin exhibits a synergistic effect on the inhibition of TNBC cells. This synergistic effect is associated with the suppression of apoptotic inhibitor proteins such as Survivin, as well as the downregulation of the anti-apoptotic protein Bcl-XL and the MAPK signaling pathway (Ono et al., 2018). HDAC inhibition induces the formation of the DISC complex by activating TRAIL ligand and related receptors on the cell surface in the extrinsic apoptotic pathway. This leads to caspase 8 activation and subsequently triggers apoptosis. The cellular FLICE inhibitory protein (c-FLIP) is an important component of DISC (Huang et al., 2018). c-FLIP forms an Apoptosis Inhibitory Complex (AIC) by binding to FADD and the TRAIL receptor in ligand dependent-independent manner. This interaction leads to the inhibition of DISC formation and caspase cascade activation, thereby inhibiting apoptosis. c-FLIP activates cytoprotective and survival signal proteins in the cell, such as Akt, ERK, and NF-kB. Additionally, it can induce chemoresistance by suppressing apoptosis triggered by chemotherapy agents

in malignant cells (Safa A.R, 2012) and plays a major role in chemotherapeutic drug resistance in various cancers, contributing to tumorigenesis and poor prognosis. High levels of c-FLIP expression have been associated with aggressive tumors. Moreover, excessive c-FLIP expression has been shown to induce the Wnt signaling pathway by inactivate the proteasomal degradation of β-catenin, resulting in elevated levels of β-catenin. This, in turn, leads to the induction of cyclin D expression, promoting cancer cell proliferation and the continuation of the cell cycle (Naito et al., 2004). c-FLIP shares structural similarity with caspase-8. Therefore, the inhibition of c-FLIP leads to the inhibition of caspase-8, preventing the induction of apoptosis. Consequently, selective inhibition of c-FLIP is necessary for the induction of apoptosis (Bijangi-Vishehsaraei et al., 2010). It is considered that selectively inhibiting c-FLIP could be an effective method in overcoming drug resistance and contributing to the improvement of poor prognosis, making it a potential strategy in treatment. In addition to their antiproliferative effects, HDAC inhibitors can sensitize cancer cells to genotoxic therapy by altering the DNA damage response (Konsoula et al., 2011; Thurn et al., 2013; Wu et al., 2017). Moreover, when applied to normal human lung fibroblasts, HDAC inhibitors do not sensitize healthy cells to radiotherapy, suggesting their selectivity against cancer cells (Munshi et al., 2005). It was documented that HDAC inhibitors regulate the DNA damage response by attenuating the expression of proteins involved in DNA damage and repair through oxidative stress-induced pathways, playing a role in cancer development. (Munshi et al., 2005; Lee vd., 2010). However, additional research is necessary to clarify the molecular basis of these mechanisms, as well as to understand the potential effects on healty cells, and to determine targets that play a role in the sensitivity mechanisms induced by HDAC inhibitors in cancer cells.

In tumors with weak immunogenicity like TNBC, limited antitumor responses occur with ICIs monotherapy due to the immunosuppressive tumor microenvironment. Combination therapies are considered effective to improve the sensitivity of ICIs and activate immune cells (Zhang et al., 2022). Moreover, tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC) are other factors in the tumor microenvironment that impact immune escape, enhance metastatic spread, and increase resistance to immunotherapies. HDAC inhibition has been shown to decrease the infiltration of MDSCs into tumor tissues, determine an anti-tumor phenotype in tumor-associated macrophages, and assist in the activation of T cells in the tumor area (Li et al., 2021).

The control of gene expression through epigenetic modifications significantly influences the dynamics of tumor immunity. Crucial in this regulation are histone post-translational modifications and DNA methylation, pivotal players in shaping the adaptive immune response. These modifications exert

profound effects on essential processes such as dendritic cell development, T cell priming, and activation. In the context of tumor cells, alterations in histone and DNA modifications have far-reaching consequences. They impact the generation of tumor antigens, silence anti-tumor cytokines, and trigger the expression of the PD-L1 checkpoint, contributing to immune evasion. Recent research has brought to light the involvement of chromatin remodeling as a response to cytotoxic attacks on tumor cells. Additionally, insights into the exhaustion phenotype observed in tumor-infiltrating CD8 T cells underscore the critical role of epigenetic regulation in shaping the complex landscape of tumor immunity (Figure 5)(Cao and Yan, 2020).

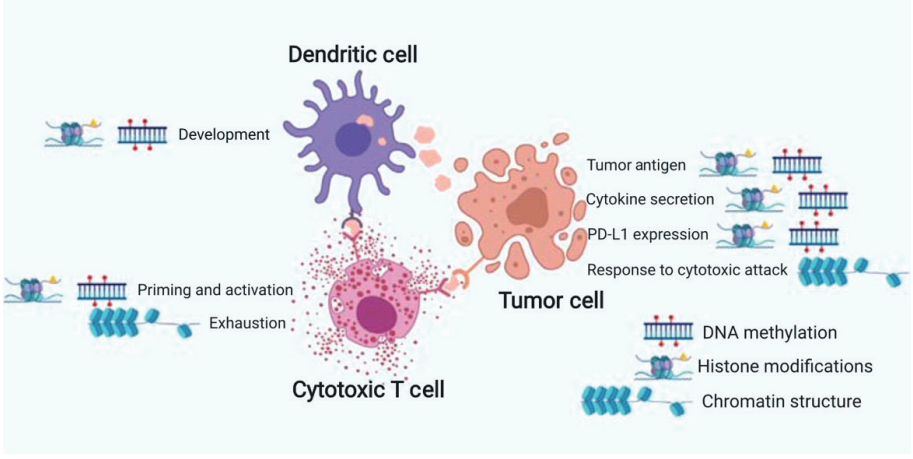

***Figure 5**. Epigenetic Control of Tumor Cell Immunity (Cao and Yan, 2020).*

HDACs have critical roles in the epigenetic regulation of T cell functions. The decrease in antigen presentation and MHC-I expression contributes to becoming ineffective to ICi therapy. HDAC inhibition helps strengthen the anti-cancer immune reaction by increasing MHC-I expression and antigen presentation, contributing to overcoming resistance that may develop against ICI therapy (Borcoman et al., 2021). In a study using the HDAC inhibitor Vorinostat to improve the response to ICi treatment, it was expressed that Vorinostat increased PD-L1 protein and mRNA expression levels in the TNBC cell line MDA-MB-231 and in a murine BC model created with 4T1 cells. When combined with ICIs, it was observed that tumor growth decreased (Terranova-Barberio et al., 2017).

In anaplastic thyroid cancer cells, HDAC inhibitors such as SAHA or Valproic acid induced cell cycle arrest and increased PD-L1 expression. Furthermore, when combined with paclitaxel-cisplatin agents, it was observed that PD-L1 expression was enhanced. Therefore, it is thought that HDAC inhibition, when used alone or in combination with standard chemotherapies, may contribute to the success of immunotherapy (Hegedűs et al., 2020). There are numerous preclinical and clinical studies on various HDAC inhibitors,

but their effectiveness in TNBC treatment is still under investigation.

Conclusion

ICi is increasingly recognized as a promising strategy for tumor treatment, providing significant benefits in various cancer types. However, the effectiveness of ICi in BC remains a controversial topic. Responses to ICi in metastatic BC have been studied in numerous clinical trials, but the results from these studies are still inconclusive. The effectiveness of immunotherapy is significantly influenced by the expression profiles of IC molecules, which differ based on the cancer immune microenvironment. Consequently, improving the sensitivity of ICis and delineating the expression profiles of genes related to IC to understand the molecular pathogenesis are very important. This data is essential for the development of targeted treatment approaches.

HDACs have an impact on cancer initiation and development through the deacetylation of histone proteins. Abnormal expression of HDACs is considered a potential factor in the onset and advancement of BC, suggesting a possible strategy for BC treatment. Therefore, targeting HDACs with specific inhibitors could potentially suppress the proliferation of tumor cells in patients with BC.

Combining HDAC inhibitors with IC blockade based on the idea that HDAC inhibition may sensitize cancer cells to IC inhibition, potentially improving the overall therapeutic response. While there have been numerous preclinical and clinical studies on this topic, research in this area is ongoing.

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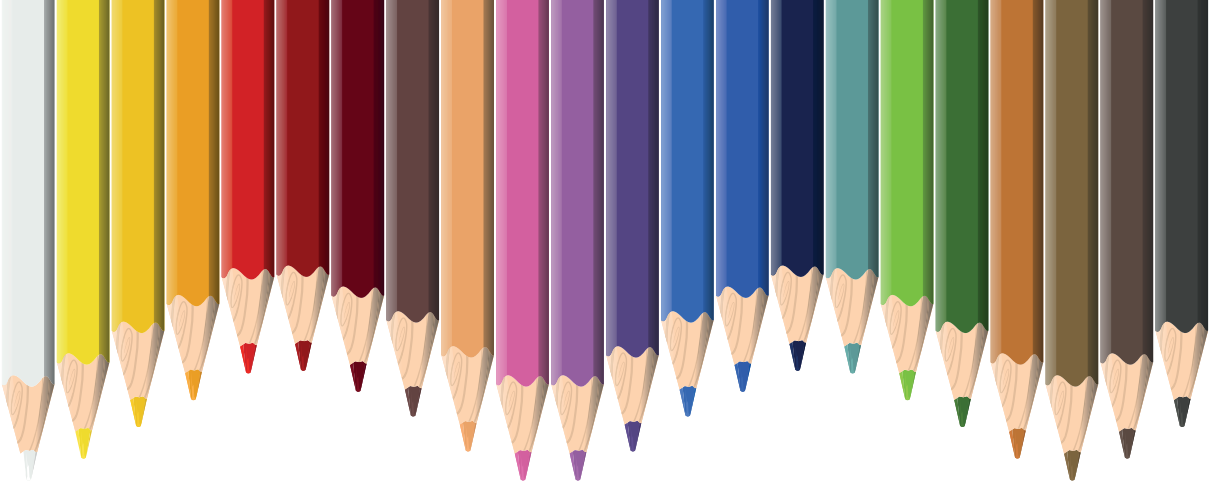

Chapter 8

APPLİED CROSS-CULTURAL PSYCHOLOGY: A THEORETİCAL EXPLORATİON

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Applied Cross-Cultural Psychology: A Theoretical Exploration[1]

Abstract

This study theoretically explores applied cross-cultural psychology, applying cross-cultural psychology approach to various subdivisions of psychology such as peace psychology, political psychology, environmental psychology, health psychology, educational psychology, personality psychology, sport psychology, clinical psychology, forensic psychology, history of psychology, philosophical psychology, economic psychology, critical psychology, social psychology, (industrial) organizational psychology, positive psychology, cognitive psychology, and consumer psychology. Emerging research in each blossoming subfield is reviewed respectively. In some fields, we see a higher number of research in this area such as "cross-cultural social psychology", while in others the research is null or near null (e.g., cross-cultural forensic psychology and cross-cultural philosophical psychology). One distinction that appears in those new research areas as a whole is that between within country cultural comparisons involving races and ethnicities, and across-border comparisons. Both are useful for research interests. The author hopes that this paper contributes to the cross-cultural turn in psychology fields, challenging colonial-era assumptions of universality of psychological knowledge developed in the Global West and WASP only.

Keywords: Applied Cross-Cultural Psychology, Cross-Cultural Peace Psychology, Cross-Cultural Political Psychology, Cross-Cultural Environmental Psychology, and Cross-Cultural Health Psychology.

Introduction

This paper came up from a simple but tedious idea generating a set of questions: What if we apply the cross-cultural turn to different subfields of psychology? Are they equally developed? What are the prospects of development for these subfields? In the upcoming sections we take each subfield, apply the cross-cultural turn and review relevant emerging literature.

Cross-Cultural Peace Psychology

Although cross-cultural psychology is booming (see for example Berry et al., 2022; Gabrenya, & Glazer, 2022; Smith & Bond, 2022), its application to divisions of psychology is limited. For instance, search for a cross-cultural peace psychology returns no result. The term appeared only once in 'The Encyclopedia of Peace Psychology', but without any definition (Hulsizer & Woolf, 2011).

So it is the time to provide a definition of cross-cultural peace psychology. According to Berry (2017), a leading cross-cultural researcher, cross-cultural psychology is "the study of similarities and differences in behavior among individuals who have developed in different cultures" (np). This definition is obviously too behavioristic, excluding mental states. According to Christie (nd) a pioneering peace psychology researcher, peace psychology is an "area of specialization in the study of psychology that seeks to develop theory and practices that prevent violence and conflict and mitigate the effects they have on society. It also seeks to study and develop viable methods of promoting peace" (np).

If we combine these definitions and paraphrase them, we can define 'cross-cultural peace psychology' as the comparative research of cultural similarities and differences of individual affects, behaviors and cognitions that promote or block peace efforts, and prevent or aggravate violence and conflict.

Cross-Cultural Political Psychology

Likewise, work on cross-cultural political psychology is quite rare. The journal 'Political Psychology' had a number of papers on cross-cultural political psychology in 1997 (Feldman, 1997; Renshon, & Duckitt, 1997; Ross, 1997), but other than those, researchers just mentioned the term without explaining it. In special issue introduction (Renshon, & Duckitt, 1997), and in one of the research studies on Japan (Feldman, 1997), the term appeared shallowly and in a conjunctive way: "cultural or cross-cultural political psychology" (p.327, 329, 349). No definition was provided. Barceló (2017) is the only one who mentioned the term in a cross-cultural psychology journal. In his study, he compared political behaviors in 47 countries. Other researchers barely mentioned the term (Khetrapal, & Khera, 2021; Nesbitt-Larking, 2003). Thus, it is time to provide a definition for cross-cultural political psychology: the comparative research of cultural similarities and differences of individual political affects, behaviors and cognitions. If the research is not comparative, it will be cultural psychology, not cross-cultural psychology (see, Shiraev & Levy, 2020).

Cross-Cultural Environmental Psychology

Research on cross-cultural environmental psychology is relatively more numerous (Blades et al. 1998; Huang et al., 2022). The coinage of the term dates back to 1979 (Stea, 1979). Recent typical studies involve cross-cultural comparison of environmental variables such as environmental values, perceived social norms, environmental risks, power distance, individualism, pro-environmental attitudes and behaviors, subjective well-being, pro-environmental behavior, wildlife value orientations, climate change denial, famil-

iarity with climate change (Capstick et al., 2022; Chan et al., 2022; Duff et al., 2022; Jacobs et al., 2022; Jylhä et al., 2021; Lou & Li, 2022; Xia & Li, 2022). In a study, cross-cultural environmental psychology was listed as a keyword, but it was not mentioned even once in full text (Jylhä et al., 2021). Tam & Milfont (2020) briefly define cross-cultural environmental psychology as "a culturally informed understanding of human–environment interactions" (Tam & Milfont, 2020, np). They point out that cross-cultural environmental psychology should involve cross-border collaboration (Tam & Milfont, 2020) which differentiates the emerging field from cultural environmental psychology. Following the definition above, we can define cross-cultural environmental psychology as the comparative research of cultural similarities and differences of individual environmental affects, behaviors and cognitions.

Cross-Cultural Health Psychology

Cross-cultural health psychology is mentioned in Kazarian, & Evans (2001), while Tanaka (2021) lists the term as a keyword of her study. Tanaka (2021) defines it as "psychology that is related to culture and health" (p.2). In an earlier study, Tanaka (2018) mentions the term in the title of her work. However, an earlier mention is in Mullin & Cooper (1998), where they investigate health psychology through Hofstede's 5 cultural dimensions (Hofstede, 2011). Considering Tanaka's other works (e.g., Tanaka & Hyodo (2021) where health behaviors of Japanese and international students are compared), we can clearly state that she is the pioneer in the field of cross-cultural health psychology. On the other hand, Berry (1994, 1998) stands as the first to comment on cross-cultural health psychology (see also Berry & Sam, 1997). Flowers et al. (2006) can be considered as one of the empirical studies in cross-cultural health psychology, as they investigate HIV positive Black African in UK with regard to stigma and identity. Although, Flowers et al. (2006) Tanaka & Hyodo (2021) do not research at cross-border settings, they can still be considered within the field as long as they make cross-cultural comparisons. Following the above definitions, we define cross-cultural health psychology as the comparative research of cultural similarities and differences of individual health affects, behaviors and cognitions.

Cross-Cultural Educational Psychology

Maybe we can state that cross-cultural educational psychology makes more sense when we consider PISA comparisons (see Goldstein, 2004; Jerrim et al., 2018; Kjærnsli, & Lie, 2011; Niemann et al., 2017; Schmidt, & Burroughs, 2015). The major work in cross-cultural educational psychology is an edited volume (Liem, & Bernardo, 2013) which comprises 17 great chapters

on the subject. First two sections are theoretical. Third section is about cross-board comparisons, while the fourth and final section is dedicated to within country comparisons involving ethnoreligious groups in the Philippines, Anglo and Asian children in Australia, Aboriginal/Indigenous students etc. An early work in the field is by Kurachi (1998) which compares foreign students' learning process of Japanese language. In an empirical study comparing 25 countries, Marsh et al. (2006) rightly warn that

Even when cross-cultural educational psychology studies explicitly compare results collected in different countries, such comparisons are typically compromised by the use of ad hoc samples of limited size and scope. Without appropriately matched samples, it may be impossible to distinguish differences due to country from those associated with mismatched samples used to represent different countries (p.346-347).

For King et al. (2018), cross-cultural educational psychology is a keyword for their work; they discuss the relevance of culture for educational psychology. Another book on the subject involves 'the psychology of Asian learners' (King & Bernardo, 2016). The book consists of 40 chapters covering a wide geography spanning Macao to Arab countries. Another book is about 'Asian Education Miracles' (Liem, 2018). Bernardo's other works to be noted as well: In Bernardo (2021) and Bernardo et al. (2021), he investigates Filipino culture and education, while in Bernardo & Li (2020) and Dong et al. (2006), he compares educational psychologies of Macau and Mainland Chinese. Let us note that this international scope also coincides with the field of comparative education (see Post, 2016). Our definition of cross-cultural educational psychology is the comparative research of cultural similarities and differences of individual educational affects, behaviors and cognitions.

Cross-Cultural Personality Psychology

Adams & Hanna (2012) provide a summary of the findings of cross-cultural personality psychology. Catal et al. (2017) compare Korean and Turkish Twitter users' personalities. Van de Vijver, & van Hemert (2008) present the way to assess personality in a cross-cultural manner. According to Church (2000), "Cross-cultural personality psychologists are often interested in identifying cultural universals, testing the generality of personality theories and constructs, and clarifying the role of cultural influences in personality and behavior" (pp.653-654). This view is partially correct, as cross-cultural personality psychology also focuses on differences. Mõttus et al. (2012) is an empirical example of cross-cultural personality psychology. They investigated self-reported conscientiousness in 21 countries (Mõttus et al., 2012). Another comparative study is Khwaja et al. (2019) which involves 5 countries. There are some other comparative studies that are in press. Other than these, the

relevant literature is quite limited. We define cross-cultural personality psychology as the comparative research of cultural similarities and differences of individual personalities.

Cross-Cultural Sport Psychology

This subfield is important, as we have various cross-national encounters in sports including Olympics. Terry (2009) discusses the term in book chapter, providing his professional experiences as a sport psychology in Olympics and more than 50 international sport events. His work is very useful as a starter for discussions and further elaborations. Rawat, & Błachnio, (2022) review 18 studies that cross-culturally comparative in sport psychology area. This shows an outline of a nascent field. Ryba et al. (2013) offer a cross-cultural sport psychology research example. There are other one-country studies (Jowett, & Ntoumanis, 2003; Storm, 2020; Yang, & Jowett, 2010), but they can be subsumed under cultural psychology, but not cross-cultural psychology without comparison. Brian (1995), in a socially critical dissertation mentions the term within-U.S. referring to cultural and ethnicity differences. In another dissertation, Blodgett (2015) studies Aboriginal athletes in Euro-Canadian context. Guest (2007) compares soccer teams in U.S. and Malawi; while Alfermann et al. (2013) make a comparison between swimmers in Germany and Japan. In another thesis (Valbuena, 2015), Filipino and U.S. athletes are compared. Likewise, Ahmad (2014) investigates coach-athlete relationships in Middle Eastern countries. Our definition of cross-cultural sport psychology is the comparative research of cultural similarities and differences of individual sport affects, behaviors and cognitions.

Cross-Cultural Clinical Psychology

Leong et al. (2012) provides a book chapter-length discussion on cross-cultural clinical psychology. Their focus is within-country differences. Arrindell (2003) is a comparative study on phobic anxiety in 11 countries. Bieda et al. (2017) is a comparative study of happiness and positive mental health. Lee & Sue's (2001) book chapter needs to be noted. Tan (2019) mentions the term in her discussion of indigenous psychologies. According Spilka, & Dobson (2015), one of the problems of cross-cultural clinical psychology is whether diagnostics such as DSM-V are appropriate for different cultures. Works like Butcher et al. (1998) which presents international applicability of The Minnesota Multiphasic Personality Inventory (MMPI-2) remind us that many of international psychometric studies can be deemed under cross-cultural clinical psychology. A similar work is Butcher et al. (2003) which discusses uses of MMPI-2 for Asian populations. Knipscheer, & Kleber (2004) discusses the

role of ethnic similarity in therapist-patient relationship which is a useful discussion. Wohl (1976)'s work on intercultural therapy is a precursor to current discussions in cross-cultural clinical psychology.

Cross-Cultural Forensic Psychology

Search for cross-cultural forensic psychology returns nearly null sources. First use of the term dates back to 2003 (Andy, 2003). Woldgabreal (2022) is the most recent to use the term, while in the sense of cross-cultural encounters with domestic minorities. Other than these two, there are no scholarly sources on the subject. More research is necessary on cross-border basis. We can define cross-cultural forensic psychology as the comparative research of cultural similarities and differences of individual forensic affects, behaviors and cognitions.

Other Cross-Cultural Psychologies

Search for "cross-cultural history of psychology" returns no results. This area may develop in the future with the internationalization efforts concerning history of psychology (cf. Brock, 2006). We define cross-cultural history of psychology as the comparative research of cultural similarities and differences of histories of psychology.

"Cross-cultural philosophical psychology" returns no result. This is an area to develop. Our definition of cross-cultural philosophical psychology is the comparative research of cultural similarities and differences of philosophical psychologies.

"Cross-cultural economic psychology" returns only one result (Crothers, & Fletcher, 2015). Although there are very well-known cross-border economic psychology comparisons (e.g., Batrancea et al., 2019; Leiser et al., 1990; Müller-Peters, 1998; Wang et al., 2016), none of them uses the term. Our definition of cross-cultural economic psychology is the comparative research of cultural similarities and differences of individual economic affects, behaviors and cognitions.

The term "cross-cultural critical psychology" returns no result, while "critical cross-cultural psychology" returns 3 results (Moghaddam, & Studer, 1997). Gonzales (2000) had a presentation about the subject, concluding that "The option of critical cross-cultural psychology is proposed. There is an alternative approach that can more realistically encompass the principles of social justice that are required to include excluded groups into the discipline of psychology; the profession and the practice" (p.8). The other one is a thesis (Radbourne, 1999). This is another area to develop. Our definition of critical

cross-cultural psychology is the comparative research of cultural similarities and differences of psychology criticisms or critical psychologies.

Search for cross-cultural social psychology returns the highest number of results, so it requires a separate discussion in another article. A neighboring discipline is much more developed. That is cross-cultural gerontology (see Chi, 2011; Giles, & Dorjee, 2004). The field has its own journal (e.g., Glicksman, & Jawad Aydin, 2009; King, 2008). Another neighboring field is cross-cultural neuroscience with a moderate development (e.g., Bánki, et al., 2019; Cai et al., 2016; Kitayama, & Park, 2010). Cross-cultural social psychology can be defined as the comparative research of cultural similarities and differences of group affects, behaviors and cognitions.

Cross-cultural social psychology is followed by cross-cultural developmental psychology. We can define cross-cultural developmental psychology as the comparative research of cultural similarities and differences of developmental affects, behaviors and cognitions.

Search for cross-cultural (industrial) organizational psychology returns moderate number of results which is not surprising, considering increasing number of research on the multi-national staff of international companies (cf. Bibi et al., 2018; Hosie, 2020; Wen et al., 2018). Cross-cultural (industrial) organizational psychology can be defined as the comparative research of cultural similarities and differences (industrial) organizational affects, behaviors and cognitions.

Search for cross-cultural positive psychology returns moderate results, rather than null. This is surprising considering the short history of positive psychology (cf. Alex Linley, 2006; Froh, 2004; Khademi, & Najafi, 2020). We can define cross-cultural positive psychology as the comparative research of cultural similarities and differences of positive psychologies.

Cross-cultural cognitive psychology returns moderate results, rather than null. This is unexpected as the major assumption of cognitive psychology is search for universals in isolation from cultural influences (Donald, 2000). Cross-cultural cognitive psychology can be defined as the comparative research of cultural similarities and differences in cognitions.

Cross-cultural consumer psychology returns moderate results, possibly because of the fact that more research is done about cross-border advertisements of international companies (cf. Okazaki, & Mueller, 2007; Samiee, & Jeong, 1994; Whitelock, & Chung, 1989). We can define cross-cultural consumer psychology as the comparative research of cultural similarities and differences of consumer affects, behaviors and cognitions.

Conclusion

In this theoretical paper, we delineated the emerging subfields that are influenced by the cross-cultural turn in psychology. Some of the research studies are cross-culturally comparative, but they do not use the emerging terms. Unfortunately, they are excluded from this review most of the time. The good news is: that means we have a higher number of works relevant for these subfields. The bad news is: that means the relevant research is still disorganized. We expect that all subfields will sooner or later be influenced by the cross-cultural turn although some will be slower, while others faster.

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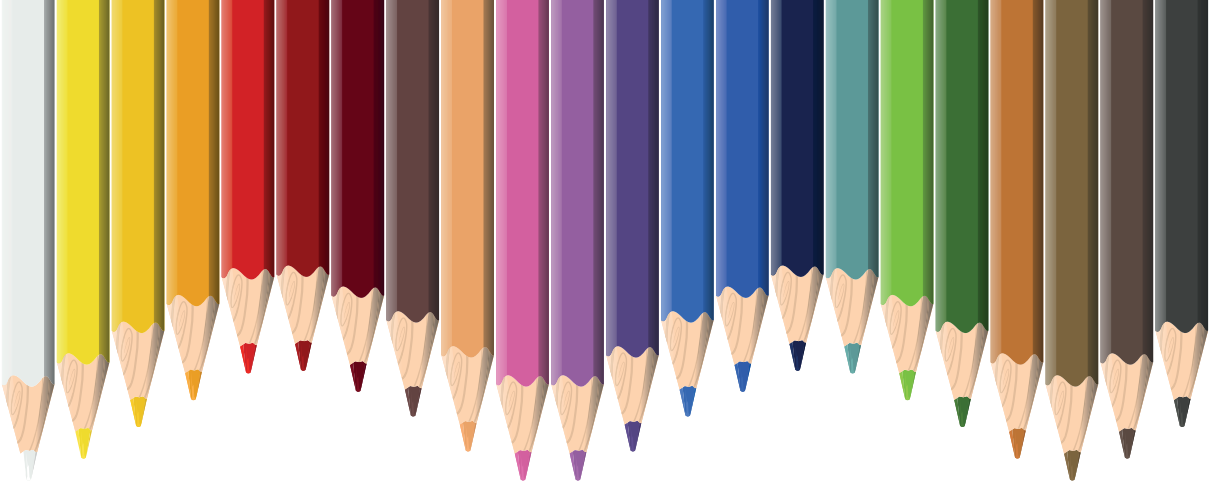

Chapter 9

DEPRESSION AND DNA METHYLATION: EPIGENETIC PERSPECTIVE

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Depression

The updated version of the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) moved depression from the *"Mood Disorders"* category to a new category under *"Depression Disorders"* in 2013. Under this new category, there are subdiagnostic categories such as "disruptive mood dysregulation disorder, premenstrual dysphoric disorder (PMDD), major depressive disorder (MDD), ongoing depressive disorder, substance/medication induced depressive disorder and depressive disorder due to another medical condition." According to DSM-5, for a diagnosis of depressive disorder, significant symptoms must persist for at least two weeks. These symptoms must include at least one of the following: depressed mood, decreased desire or lack of pleasure in almost any activity. Symptoms may also include weight change, sleep disturbances, psychomotor agitation or retardation, persistent lack of energy, feelings of worthlessness or guilt, concentration difficulties, indecisiveness and thoughts of death or suicide. At least five (including the first two symptoms) of these symptoms must be present, causing significant distress to the individual and impairment in social, work, or other important areas. Additionally, care must be taken to ensure that the symptoms are not arisen because of any physiological effects or another medical condition.

According to epidemiological studies, annual prevalence of depression is 6% and its lifetime prevalence is between 15% and 18% (Malhi and Mann, 2018). The disease usually begins between the ages of 30-35 (Kessler et al., 2010). Depression, which affects approximately 300 million people worldwide, is assumed to be the main cause of disease burden in near future (Zhu et al., 2023) It is well known that environmental factors such as chronic stress and socioeconomic level increase the risk of depression. As a serious mental health problem globally, depression is considered as a biopsychosocial disorder and genetic factors are thought to play a role in etiology. A definitive mechanism describing depression pathophysiology has not yet been determined (Malhi and Mann, 2018).

Depression Theories from Biological Perspective

From a pathophysiological perception on depressive disorder, four theories generally stand out including monoamine theory, stress-induced theory, neurotrophic theory and cytokine theory. (Šalamon Arčan et al., 2022).

1. Monoamine Theory

This theory focuses on the roles of monoamine neurotransmitters such as serotonin, dopamine, and norepinephrine in emotional regulation, alertness, and some memory processes. Decreases in neurotransmitter levels cause disruptions in signal transmission between neurons and ultimately trigger symptoms of depression (Šalamon Arčan et al., 2022). However, the limitations of this theory are revealed by factors including large differences in the

course of MDD, antidepressants not being effective in every patient and/or their effects often take weeks (Willner et al., 2013).

2. Diathesis–Stress Theory

Factors such as prenatal stress, early life adversity and chronic stress are among the strongest triggers of depressive disorders (Figure 1). The hypothalamic-pituitary-adrenal (HPA) axis is an important neuroendocrine system for adaptation to environmental changes; and, in this context, plays a central role in depression studies (Knorr et al., 2010; Šalamon Arčan et al., 2022). The stress response begins with the hypothalamus secreting corticotropin-releasing hormone (CRH) for stimulating the pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH in the peripheral blood circulation increases the release of glucocorticoid hormones (cortisol) from the adrenal cortex. The binding of cortisol to glucocorticoid receptors (GRs) in the brain enables it to function as a key regulator of the stress response. Cortisol has a suppressive effect on the HPA axis. Disorganization of this negative cycle has been associated with depressive disorders (Šalamon Arčan et al., 2022; Shadrina et al., 2018). Despite all this knowledge, it has not been possible to develop effective clinical treatments so far. Potential treatments, such as GR antagonists, have not produced expected results (Malhi and Mann, 2018).

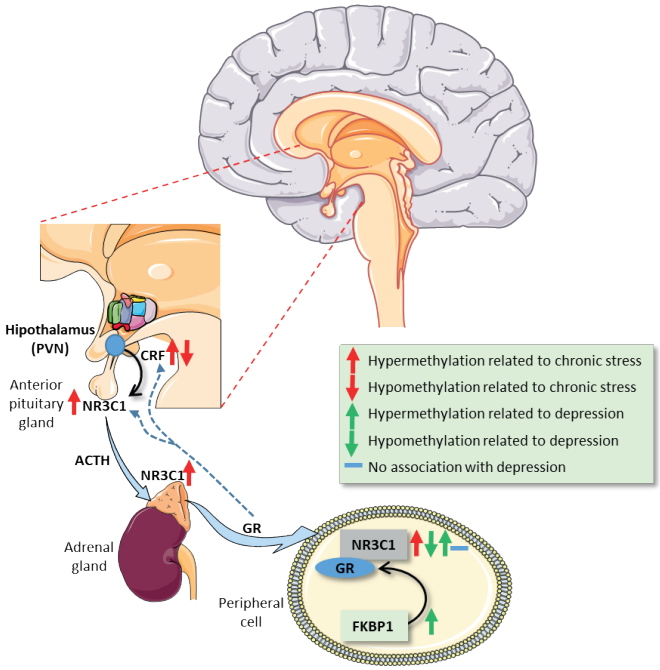

Figure 1. *Effects of DNAm on HPA axis. (Adapted from Bakusic et al. 2017). ACTH: adrenocorticotropic hormone, CRF: corticotropin-releasing factor, GR: glucocorticoid receptor, PVN: paraventricular nucleus. (Partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.*

3. Neurotrophic Theory

One of the most important discoveries of this century demonstrated that neurons can be produced in the adult brain through pluripotent stem cells. This process is called neurogenesis. The ability to grow and adapt at the neuronal level is called neuroplasticity. It is thought that neuroplasticity may be altered by environmental stress-induced inflammation and HPA axis dysfunctions (Malhi and Mann, 2018). The neurogenesis is regulated by proteins such as brain-derived neurotrophic factor (BDNF). Neurotrophic factors are small peptides or proteins that promote the growth, survival, and differentiation of developing neurons. BDNF is one of these factors that has been studied in detail. It has been shown that BDNF expression is downregulated in the brain and blood tissues of depressive disorders patients (Wang et al., 2019). It has been observed that the BDNF level can be increased again with antidepressant treatment and psychological interventions (Molendijk et al., 2014). Postmortem analyzes shown that depressed patients having treatment have more dividing neuronal precursor cells in the dentate gyrus region than those who are not treated (Gururajan et al., 2016).

4. Cytokine Theory

Cytokines are small proteins playing critical roles in cell signaling. These proteins including interferons, chemokines, interleukins (ILs) and tumor necrosis factors (TNFs) have an important place in inflammation theory (Himmerich et al., 2019). While peripheral cytokines can directly cross the blood-brain barrier and affect neurons and glial cells, they can also exert their effects through afferent pathways such as the vagus nerve (Miller and Raison, 2016).

Brain cytokine levels increase during chronic stress and depressive disorders. Activation of cytokines such as IL-6 and TNF-α can cause a decreased serotonin level. Activation of indoleamine-2,3-dioxygenase contributes to this process by triggering the stimulation of monoamine oxidase. Cytokines can also alter the excitability and synaptic scaling of neurons through their direct effects. For example, the effect of IL-1β on the HPA axis can increase the release of stress hormones such as cortisol, which may affect the activity of microglia to drive synaptic remodeling (Chan et al., 2019). It is thought that the effects of early life stressors on microglia may lead to long-term consequences (Catale et al., 2020). The long-term effects of early life stressors and the increased risk of depression in individuals with autoimmune diseases or a history of severe infections are important findings that support this theory. Higher childhood IL-6 levels increase the risk of depression in adulthood. The microglial activation and neuroinflammation observed in the brains of depressed patients support this theory (Setiawan et al., 2015).

Concept of Epigenetics and Epigenetic Mechanisms

The term *"epigenetics"* is formed by the Greek prefix *"epi"* meaning on the top of or in addition to genetics. In general terms, it refers to changes on the DNA backbone (Keverne and Binder, 2020). This term was first used by Conrad Waddington in 1939 and stated that the interactions of the material in the fertilized egg trigger development and this process creates new phenomena (Kuehner et al., 2019). Today, epigenetics is used as a concept that describes the mechanisms that affect the function of the genome without changing the DNA sequence. These mechanisms include processes such as regulation of gene expression and how DNA is packaged in the nucleus. In particular, these mechanisms have critical effects on the function of post-mitotic neurons and brain development (Keverne and Binder, 2020). The best-known epigenetic mechanisms are DNA methylation (DNAm), histone modifications, chromatin remodeling and noncoding RNAs.

1. Histone Modifications

There is need to access to certain regions of the packaged DNA in the nucleus for essential biological processes such as transcription and translation (Patterson et al., 2021). DNA forms chromatin structure by wrapping around a histone octamer consisting of H2A, H2B, H3 and H4 histone types (Kornberg, 1974). Histone proteins are small positively charged molecules that interact with the negatively charged DNA to form a coiled structure. Histone chaperones play a vital role in this process, building the octameric structure of histone proteins, and this structure is called nucleosome (Türkel, 2014). The functionality of the nucleosome structure is regulated by various modifications occurring in histone proteins (Patterson et al., 2021). The most common among these modifications are histone methylation and acetylation.

Histone acetylation is a critical chromatin modification regulated by the addition of acetyl groups to lysine residues by histone acetyltransferases (HATs). Histone deacetylases (HDACs) remove these groups (Kuehner et al., 2019). This process affects the structure of chromatin and thereby gene activity. The acetyl groups added by HATs change the nucleosome structure, facilitating access of transcription factors to DNA. In contrast, removal of acetyl groups by HDACs returns histones to their original positively charged state, resulting in tighter chromatin and reduced gene expression (Kuehner et al., 2019; Yang et al., 2021). Increased acetylation (hyperacetylation) leads to loosening of chromatin and increased gene activity, while decrease (hypoacetylation) causes chromatin to tighten and gene activity to decrease.(Erol et al., 2010). HDAC inhibitors can improve learning and memory in neurodegenerative models and induce neural differentiation in embryonic cortical cells (Erol et al., 2010; Fischer et al., 2007). These findings suggest that HDAC inhibitors have potential applications in the treatment of neurodegenerative

and neuropsychiatric diseases. Regulation of acetylation is important for understanding changes in gene expression and its role in therapeutic interventions (Kuehner et al., 2019).

Histone methylation occurs by inclusion of methyl groups to histone lysine residues, and this process is catalyzed by histone methyltransferases (HMTs). Methylation is often associated with activation of gene expression. Although histone methylation was formerly considered an irreversible modification, the discovery of histone demethylase enzymes has changed this understanding (Shi et al., 2004).

2. Chromatin Remodeling

It is necessary to fit the genomic DNA, which is approximately 2 meters long, into a cell nucleus that is only 6 μm in size. The nucleosome is a DNA molecule of approximately 146 bp wrapped around a histone octamer to form the chromatin. One of the factors that have a central influence in this process is ATP-dependent chromatin rearrangement complexes. These complexes alter the density of chromatin by modifying the interactions between DNA and histones. These modifications increase or decrease the ability of transcription factors to bind to DNA, which has an important role in gene regulation. The BAF (mammalian SWI/SNF) complex is an example of these ATP-dependent chromatin rearrangement complexes (Son and Crabtree, 2014). BAF complexes are associated with neuropsychiatric disorders including autism spectrum disorder and schizophrenia (Koga et al., 2009).

3. Non-coding RNAs (ncRNAs)

An important way of epigenetic regulation is through non-protein-coding RNA (ncRNA) molecules. Only less than 2% of the transcripts in the human genome code for proteins. The remaining is designated as non-protein coding RNAs. ncRNAs are divided into two main categories according to their sizes: short and long ncRNAs. siRNA, piRNA, miRNA and lncRNAs have variable lengths from 21 to 100,000 nucleotides. ncRNAs are effective in gene regulation through transcriptional and post-transcriptional mechanisms.

microRNAs (miRNAs) are around 22 bp long and affect gene expression at the post-transcriptional level (Bartel, 2004). A miRNA can target multiple mRNAs or mRNA can be targeted by several miRNAs (Lim et al., 2005). Studies have identified the relationship of miRNAs with brain development and various diseases. For example, miR-124 is the most abundant miRNA in the mammalian brain and plays a critical role in neuronal differentiation and maturation (Lagos-Quintana et al., 2002). Also, miR-137 is essential in neurogenesis and neuronal maturation processes and has been associated with neuropsychiatric disorders such as schizophrenia (Kwon et al., 2013). lncRNAs consist of at least 200 nucleotides and have no protein-coding properties

(Kapranov et al., 2007). These RNAs generally do not contain protein coding regions and intronic regions are few and short (Pang et al., 2006; Ravasi et al., 2006). lncRNAs can regulate the transcription of neighboring genes and act on distant transcription activators and repressors. As a IncRNAs, X-inactive specific transcript (XIST) play important roles in X chromosome inactivation (Brockdorff et al., 1991) and HOTAIR suppresses the transcription of the HOXD locus (Mercer et al., 2010). More than 800 IncRNAs have been discovered in mouse brain, and these are thought to have critical roles in neuronal processes and synaptic functions (Lipovich et al., 2012; Piwecka et al., 2017).

4. DNA Methylation (DNAm)

DNAm is a modification that arises when a methyl group is covalently attached to the 5th carbon of the cytosine base in the DNA molecule and 5-methyl cytosine (5m-C) occurs (Bird, 2002). DNA methylation usually takes place in the cytosine-guanine (CpG) sites of genome. CpG residues in the promoter regions of genes are frequent sites of DNA methylation. However, DNA methylation can also be observed outside CpG islands. It has been observed that non-CpG methylation occurs especially in neurons, glial and pluripotent stem cells. Hydroxymethylation and other chemical DNA modifications were also identified. Hydroxymethylation is a derivative of DNA methylation and occurs by adding a hydroxymethyl group to the 5th carbon of the cytosine base. Hydroxymethylation, especially observed in neurons, is associated with increased gene expression (Keverne and Binder, 2020). These findings emphasize that DNA methylation is an important mechanism in regulating gene expression.

The effect of the methylation mechanism from the transcription initiation regions, exons, introns, regulatory regions and repetitive sequences are investigated. In studies conducted on the X-chromosome, it has been determined that increased methylation is associated with gene expression (Güler and Balcı Peynircioğlu, 2016). The region of the gene where DNA methylation occurs is a factor affecting gene expression. For example, it has been suggested that DNA methylation may affect splicing (Maunakea et al., 2013). Hypermethylation downregulates gene expression, while hypomethylation upregulates gene expression. DNA hypermethylation utilize 2 different mechanisms to downregulate gene expression including “direct mechanisms” and “indirect mechanisms” (Figure 2).

In direct mechanisms, methylated CpG islands located at the transcription factor binding sites structurally prevent transcription factors from these sites and consequently repress gene expression. This process plays a critical role in the regulation of gene expression. In indirect mechanisms, methylation of CpG sites in the promoter region promotes the binding of methyl CpG binding proteins (MeCPs) to this site. Binding of these proteins brings other

proteins such as HDACs and HMTs to the region. HDACs facilitate the transition of chromatin to an inactive structure by deacetylation of histones. HMTs, especially SUV39H, add methyl groups to the 9th lysine residue of histone H3, which supports the inactive structure of chromatin. Methylation at H3K9 recruits heterochromatin protein (HP1) and chromatin remodeling proteins (BRM, SIN3A) to the region, leading to repression of gene expression (Güler and Balcı Peynircioğlu, 2016).

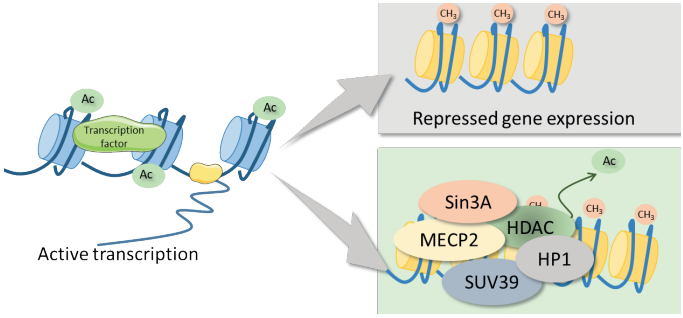

Figure 2. *Mechanisms of DNA methylation*

DNA Methyltransferases

Enzymes responsible for adding methyl groups to CpG sites in DNA are called DNA methyltransferases (DNMTs). Each of these enzymes has evolved to suit its own characteristics (Kuehner et al., 2019). These enzymes catalyze the reaction during DNAm and S-Adenosyl methionine is used as a methyl provider (Jurkowska et al., 2011).

DNMT1, DNMT3A, DNMT3B, DNMT3L and DNMT2 are defined in eukaryotes. Among these, DNMT1 was first discovered in 1983 and responsible for maintaining methyl groups during DNA replication. DNMT1 has a large N-terminal regulatory domain and a C-terminal catalytic domain. The regulatory domain comprises several protein-protein interaction domains that are involved in targeting DNMT1 to specific regions of the genome. The catalytic domain is responsible for the methylation of DNA. By methylating the other chain of DNA whose single chain is methylated during replication, DNA can be inherited and thus transmitted between generations as an epigenetic marker through cell division (Jurkowska et al., 2011).

DNMT2 is thought to play a role in DNA damage detection, DNA recombination and mutation repair (Turek-Plawa and Jagodzinski, 2005). Although DNMT2 belongs to the methyltransferase family, it is mainly involved in tRNA methylation. The methylation activity of this enzyme protects tRNA molecules from the ribonuclease degradation. The precise function of DNMT2 has not yet been fully determined, but it is suggested that it contributes to tRNA stabilization and enhanced translation efficiency (Jeltsch et al.,

2017). The structure of DNMT2 is similar to other DNMTs, but consists of a catalytic domain that lacks the N-terminal regulatory domain that is found in DNMT1 and DNMT3 family (Jurkowska et al., 2011).

DNMT3A and DNMT3B carry out *de novo* methylation, the attachment of new methyl groups to the DNA molecule. The structure of DNMT3A and DNMT3B consists of a catalytic domain, a regulatory domain, and a C-terminal domain. While the catalytic domain is responsible for the transfer of the methyl group to the cytosine residue, the regulatory domain plays a role in the recognition of the DNA substrate. The C-terminal domain is involved in protein-protein interactions and is important for the stability and function of the enzyme (Jurkowska et al., 2011). Its role in the formation of the first methylation patterns established in the early stages of development is important (Güler and Balcı Peynircioğlu, 2016). How *de novo* DNA methylation is directed to specific gene regions is still poorly understood. RNA interferase, transcription factors and other mechanisms are thought to play a role in this process. The most fundamental feature that distinguishes DNMT3A and DNMT3B is the gene expression pattern. DNMT3A is often widely expressed. DNMT3B is however expressed at lower levels in mostly differentiated tissues such as thyroid, testis and bone marrow. DNMT3B is required in the early developmental stage. DNMT3A is essential for normal cellular differentiation (Moore et al., 2013).

Unlike DNMT3A and DNMT3B, DNMT3L lacks the catalytic domain. DNMT3L does not have DNA methyltransferase active site motifs and its function depend on its association with other methyltransferases. For this reason, it plays an auxiliary role in the activity of DNMT3A and DNMT3B enzymes. It interacts with these enzymes to increase their activity, which results in elevated DNAm (Jurkowska et al., 2011; Turek-Plawa and Jagodzinski, 2005). Consistent with its role in early development, it is required for maternal and paternal genomic imprinting, methylation of retrotransposons, and X-chromosome inactivation. DNMT3L is expressed in the developing brain, while its expression level decreases during nerve cell differentiation. In the postnatal period, its expression in the brain is not observed (Moore et al., 2013).

Functions of DNA Methylation

DNAm plays a critical role in gene regulation. This process is assessed by presence and distribution of methyl groups in various regions of the genetic material (Güler and Balcı Peynircioğlu, 2016). Moreover, the methylation status of CpG sites may differ depending on tissues and cell types. For example, the HT2RA gene, which is associated with many neuropsychiatric diseases, is expressed differently in the cerebellum and cortex. HT2RA expression is regulated by DNAm (Ladd-Acosta et al., 2007). Moreover, the methylated CpG

islands affecting HT2RA expression are not located in the promoter region, but in a region more than 1 Kbp away from the promoter region. Therefore, DNAm plays an important role in different transcriptome profiles in various region of brain. DNAm is involved in regulating the activation of protein-coding genes as well as non-coding RNAs such as lncRNA.

DNAm also plays a role in genomic stability by controlling the expression of repetitive genomic regions, retrotransposons and satellite DNA (Walsh et al., 1998). Genome instability has been associated with many neuropsychiatric diseases such as Rett syndrome, autism spectrum disorder and schizophrenia (Smith et al., 2010). DNAm also plays a role in silencing retroviral elements. The mammalian genome contains transposable and viral elements silenced by mass methylation (Moore et al., 2013). If these elements are expressed, replication and splicing processes may cause DNA mutation (Ukai et al., 2003).

DNAm plays a fundamental role in early developmental processes such as gene imprinting. This process involves special markings to determine on which chromosome of parental origin and thereby the genes will be active. Some genes are active only in maternal genomic regions, and some are active only in paternal genomic regions. Dynamic regulation of DNAm is vital for cellular differentiation (Kuehner et al., 2019). In a study examining DNAm patterns during cellular differentiation, it was observed that the most dynamic changes occur when stem cells lose their pluripotency properties and differentiate into neuronal progenitor cells. In contrast, very little methylation or demethylation profiles were observed during the transformation of neuronal progenitor cells into mature neurons. (Mohn et al., 2008).

Information on the regulatory effects of DNAm on axon and dendrite elongation and neural migration, which are critical processes of brain development, is quite limited (Kuehner et al., 2019). Previous studies suggest that DNMT1 may have a regulatory role in migration of immature GABAergic interneuron (Pensold et al., 2017; Symmank et al., 2018). DNAm contributes to the survival and migration of these interneurons by downregulating PAK6 expression (Pensold et al., 2017). It has been determined that *de novo* methylation by DNMT3b during early embryonic development has a critical role in the regulation of clustered protocadherin (PCD4) genes (Toyoda et al., 2014). Protocadherins are adhesion proteins that play critical roles in nervous system development and have been associated with neuropsychiatric disorders such as depression, schizophrenia and autism spectrum disorder (Kuehner et al., 2019).

DNAm plays also a central role in memory formation and storage. Compounds suppressing DNAm (i.e. zebularine and 5-aza-2-deoxytidine) can cause methylation changes in BDNF and Reelin genes, which promote neuronal plasticity (Levenson et al., 2006). Contextual fear conditioning (CFC)

induces changes in DNAm pattern during memory formation in mice. After conditioning, DNMT3a and DNMT3b mRNA levels were increased in brain tissue. Hippocampal injection of DNMT suppressors (such as zebularine or 5-aza-2-deoxytidine) immediately after conditioning abolishes the fear response. These results suggest that DNAm is required for memory formation (Miller and Sweatt, 2007). In addition, DNAm is thought to have a potential role in long-term memory storage. CFC affects the DNA status of memory-related genes such as FGR1, reelin and calcineurin. Unlike other genes, calcineurin maintains its hypermethylated state for 30 days after fear conditioning. These findings suggests that DNAm may be also required for the formation of long-term memory (Miller et al., 2010).

DNA Methylation and Depression

Epigenetic mechanisms have been studied in different tissues of depressed patients, postmortem brain tissues of depressed patients who committed suicide and animal models (Šalamon Arčan et al., 2022).

Depression and Global DNA Methylation

Global DNAm is the ratio of the total amount of 5m-C to the total cytosine (C) content in a DNA sample. There are several studies describe relationship between global DNAm and depression (Table 1). For instance, Duman and Canli (2015) reported a positive relationship between chronic stress and global DNAm. In another study, it was found that people with a history of depression had more methylated genes compared to the control group (Uddin et al., 2011). Genome-wide DNAm analysis using peripheral blood mononuclear cells (PBMCs) from MDD patients who were not receiving any medical treatment showed hypomethylation in 84 of 393 CpG island (Numata et al., 2015). In another global DNAm study conducted using monozygotic twin pairs, lower DNAm levels were detected in female MDD patients than in the control group (Byrne et al., 2013).

Table 1. *Global DNA methylation and depression studies*

Literature	Sample size	Study Design	Sample	Results
Uddin et al., 2011	100	Case-Control	Whole blood	Individuals having a history of depression, the number of methylated genes is significantly higher than in the control group.
Duman et al., 2015	105	Cross-sectional study	Whole blood	A positive relationship was found between chronic stress and global DNAm.

Numata et al., 2015	63	Case-Control	PBMC	Hypomethylation was observed in 84 of 393 CpG sites in depression patients.
Byrne et al., 2013	44	Case-Control	PBMC	Global DNAm level is lower in twin women having MDD than in the control group.

In conclusion, these studies suggest that MDD is associated with epigenetic modifications. It has been observed that chronic stress contributes to the pathophysiology of MDD by increasing global DNA methylation. The increased number of methylated genes in individuals with a history of depression strengthens the relationship between MDD and epigenetic modifications. Furthermore, global DNA methylation levels in monozygotic twins suggest that gender plays an important role in the interaction with epigenetic factors.

Candidate genes

1. BDNF

BDNF is a neurotrophin that is widely expressed and thoroughly studied in the mammalian brain. BDNF plays important roles in the growth and survival of neurons. It has a critical function for the proper development, adaptation, and plasticity of nerve cells, especially at glutamatergic and GABAergic synapses. Furthermore, BDNF regulates the differentiation of neurons by modulating serotonergic and dopaminergic neurotransmission. BDNF acts both pre- and post-synaptically at synapses via paracrine and autocrine mechanisms. BDNF also has a central role in the formation of long-term synaptic memory. As a key regulator of neuroplasticity, it also influences dendritic structures and adult neurogenesis in the hippocampus. In this capacity, BDNF is considered to effect learning and memory processes and potentially contribute to depressive behaviors (Colucci-D'amato et al., 2020). BDNF activates cellular signal transduction cascades by binding to the high-affinity TrkB receptor. In particular, signaling pathways encoding the production of CREB (cAMP response element binding protein) and CBP (CREB binding protein), two important proteins vital for β cell survival, are activated by this interaction. Furthermore, BDNF and IGF-1 (insulin-like growth factor-1) promote cellular survival using similar signaling mechanisms. These factors trigger the production and activation of various proteins to ensure cellular survival and normal functions. These mechanisms help cells cope with stressful situations and maintain the survival and functionality of neurons (Bathina and Das, 2015). Fuchikima et al. (2011) conducted a study in the Japanese population with 20 MDD patients and 18 healthy controls. It was observed that 29 out of 35 CpGs in BDNF CpG1 had increased methylation levels in MDD patients. However, no significant difference was detected in BDNF CpG

4 between both groups. DNA methylation profile of BDNF CpG 1 region may be a potential biomarker for the diagnosis of major depression (Fuchikami et al., 2011). In another study conducted with PBMC genomic DNA, it was found that BDNF exon 1 promoter methylation was higher in the MDD group than in the bipolar patients and control groups. However, no association was found between the symptoms of the disease and BDNF exon 1 methylation in the MDD group. In addition, 12 SNPs were analyzed and none of them showed significant association between the groups (Carlberg et al., 2014). The total methylation level in the BDNF promoter region was found to be higher than in the control group (D'Addario et al., 2013). In a study, interaction of Val66Met polymorphisms and DNAm of different BDNF exons with anhedonia, reward learning and cognitive performance in MDD was evaluated. BDNF promoter 1 methylation was found to be lower in MDD patients and negatively associated with anhedonia (Bakusic et al., 2021). BDNF was hypermethylated in MDD patients with and without serious suicidal ideation. BDNF expression was also lower in the MDD group (Roy et al., 2017).

The relationship between BDNF DNAm and depression treatment has been investigated in different studies. Kim et al. (2015) followed 711 patients with acute coronary syndrome who were prone to depression for 1 year. Within the scope of DSM-4 criteria, patients were categorized into three different time points including prevalent depression at baseline, incident depression at follow-up and persistent depression. In addition, 255 of the 378 participants having prevalent depression were randomized to 24-week double-blind treatment with escitalopram (n=127) or placebo (n=128). Whereas, the other 123 patients received conventional medical treatment. Findings suggested that acute coronary syndrome patients with increased BDNF methylation had higher susceptibility to depression and higher depression persistence. Antidepressant treatment was more effective in patients with high BDNF methylation and preventing the persistence of depression (Kim et al., 2015). In a study conducted on 561 MDD patients, Lieb et al. (2018) investigated how the methylation rate of a specific BDNF region and expression levels can predict the treatment responses of MDD patients. Severe depression patients (HAMD-17 score ≥ 25) having high methylation level at BDNF exon 4 CpG-87 achieved high remission rates, and 75% of these patients showed a positive response to treatment in the first two weeks. In a comparison of patients who showed early recovery and 140 patients who did not, it was determined that early recovery increased the chance of remission by 4.24 times. However, the capacity of early recovery to predict treatment response was evaluated as having low specificity and a high false positive rate. Nevertheless, the increased plasma BDNF level detected on day 14 and the combination of BDNF exon 4 CpG-87 methylation with early recovery improved the specificity of treatment response and reduced the false positive rate (Lieb et al., 2018). Tadić et al. (2014)

reported that hypomethylation in BDNF exon 4 CpG-87 was found to be associated with decreased response to antidepressant treatment.

2. NR3C1

The NR3C1 (nuclear receptor subfamily 3 group C member 1) gene encodes the glucocorticoid receptor (GR). This receptor binds to the promoters of genes containing glucocorticoid response elements (GRE) to regulate gene function. Typically located in the cytoplasm, this receptor is transported to the cell nucleus when the relevant ligand binds. As a result, it has an important role in the inflammatory response, cell proliferation and differentiation (Lu and Cidlowski, 2005). When exposed to stress, the paraventricular nucleus of the hypothalamus is first activated, resulting in the release of CRH. CRH stimulates the release of ACTH from the anterior pituitary to the adrenal glands. As a result, glucocorticoids (cortisol) are secreted. One of the functions of cortisone is the regulation of the HPA axis. As a lipophilic molecule, cortisol passes through the cell membrane by passive diffusion and binds to the cytoplasmic mineralocorticoid receptor (MR) or GR. GR plays a critical role in coping with stress appropriately (Palma-Gudiel et al., 2015).

In a chronic psychosocial stress animal model, chronic stress was found to cause elevated GR methylations in the adrenal and pituitary glands. Chronic stress changed the corticosteroid response to acute stress, which was accompanied by higher methylation levels in organized clusters in the adrenal and pituitary glands (Witzmann et al., 2012). Decreased GR protein levels were observed in juvenile and adult mice having chronic stress. However, this was not associated with methylation of GR promoter region in hippocampus (Desarnaud et al., 2008). In a study conducted with 1149 adolescents in Sweden, internalizing psychopathological symptoms and NR3C1 exon 1F methylation were investigated. The self-report-questionnaire Center for Epidemiologic Studies-Depression Child (CES-DC) was used to measure internalizing psychopathologic symptoms. NR3C1 hypermethylation was found to be cross-sectionally associated with higher scores for internalizing symptoms across the entire group, particularly among female participants. An association between bullying or lack of friends and NR3C1 hypermethylation was also demonstrated (Efstathopoulos et al., 2018). NR3C1 exon 1F hypermethylation was retrospectively associated in adults having childhood traumatic history (Palma-Gudiel et al., 2015).

Using the Beck Depression Inventory (BDI) on 349 volunteers, the prevalence of depression was reported as 19%. NR3C1 DNAm levels of participants with high depression scores were found to be elevated significantly than the other participants (Borçoi et al., 2020). Also, NR3CA1 exon 1F DNAm level was found to be higher in the depression group and was reported to be associated with morning cortisol level (Farrell et al., 2018). The relationship between

epigenetic modifications of stress-related genes and MDD and serious suicidal ideation was investigated. Results suggested that NR3C1 gene was hypermethylated in the MDD group and NR3C1 expression was downregulated in the depression group (Roy et al., 2017). The relationship between NR3C1 methylation and hippocampal subfield volumes in MDD were investigated. MDD patients had lower methylation in CpG3 and CpG4 regions. Also, in MDD patients, methylation levels were positively correlated with bilateral cornu ammonis (CA) 2-3 and CA4-dentate gyrus subfields. In the control group, methylations showed a positive correlation with subiculum and presubiculum. It has been interpreted that there may be a different epigenetic feature in non-psychotic MDD patients, and NR3C1 hypomethylation may have compensatory effects associated with relevant hippocampus regions (Na et al., 2014).

In a Korean-based study, 732 people over the age of 65 were included. The methylation effects of three NR3C1 CpG sites were investigated on late-life depression. Depression was initially reported in 101 people and 521 volunteers without depression were followed for 2 years. During the follow-up phase, 86 depression cases were reported. Mean and increased NR3C1 methylation levels at CpG 2 and 3 and methylation were independently associated with the prevalence of depression in the initial group. In addition to this, an increased NR3C1 methylation level at CpG 2 was associated with the incidence of depression after 2 years. NR3C1 exon 1F, particularly CpG 2 methylations may indicate an association with depression in later life (Kang et al., 2018). In a Brazilian study with mostly female volunteers, food and nutrient insecurity (FNiS) was found to be associated with depression The NR3C1 DNAm of individuals with depressive symptoms and exposed to FNiS were found to be higher than that of healthy individuals who were not exposed to FNiS (Borçoi et al., 2021).

These findings suggest that there may be a relationship between the methylation status of the NR3C1 gene and depression. In different studies, it appears that methylation levels of NR3C1, especially in exon 1F, may be associated with the prevalence and incidence of depression. It was emphasized that the NR3C1 methylation status may be a mediating factor in the relationship between social-environmental factors and depression. The symptom of severe suicidal ideation in MDD patients may also be associated with NR3C1 methylation status.

3. SLC6A4

The SLC6A4 gene (solute carrier family 6 member 4) encodes an integral membrane protein called serotonin transporter (SERT or 5-HTT) which is responsible for the reuptake of serotonin into the presynaptic neurons. The concentration of serotonin in the extracellular environment regulates the

strength of serotonin signals and the duration of the postsynaptic neuron's response to serotonin (Lesch et al., 1996). Genetic and epigenetic variations of the SLC6A4 have been associated with various psychiatric disorders, particularly depression and anxiety disorders. There are two variants of the 5-HTTLPR polymorphism in the SLC6A4 promoter region: short (S) and long (L). The S allele is known to lead to lower gene expression and was associated with depression. DNAm levels in the promoter region of the gene are also associated with depression. Using a total of 84 monozygotic twin pairs, the association between methylation variation in the SLC6A4 promoter region and depressive symptoms was evaluated. Of 20 CpG regions, DNAm variation in 10 regions was significantly associated with the intrapair difference in BDI scores measuring depressive symptoms. These findings suggest that hypermethylation in the SLC6A4 promoter region is associated with higher depressive symptoms. This relationship was found to be unaffected by genetic and other environmental factors between the twins (Zhao et al., 2013). SLC6A4 promoter methylation level was independently found to be higher in women and participants with MDD (Philibert et al., 2008). Iga et al. (2016) investigated the possible effect of 5-HTT methylation, expression, and genotype on clinical symptoms in MDD. 5-HTT promoter region methylation levels of MDD patients were significantly higher than the control group. 5-HTT expression levels were higher in medication-free patients compared to the controls and decreased after 8 weeks of antidepressant medication (Iga et al., 2016). Another study was conducted with 236 MDD patients to examine the role of SLC6A4 promoter methylation in predicting response to serotonergic antidepressants. Patients were evaluated with the Hamilton Depression Scale after 6 weeks of treatment. SLC6A4 hypomethylation has been shown to reduce response to antidepressant treatment. SLC6A4 hypomethylation may lead to overexpression and thus serotonin transport, which may impair drug response by interfering with the efforts of serotonergic antidepressants to increase serotonin levels in the brain (Schiele et al., 2021). In MDD patients, SLC6A4 methylation was investigated for possible association with stressful life events (SLEs), the core emotions SADNESS (emotional response to negative life events), SEEKING (an individual's sense of intrinsic motivation to seek new experiences, learn and enjoy life), depressive symptom severity and age of depression onset. Female patients had higher SADNESS and higher SLC6A4 methylation than male patients. Depression severity and SLC6A4 methylation were also positively associated only in female patients (Sanwald et al., 2021).

The findings suggest a potential relationship between the methylation status of the SLC6A4 gene and depressive symptoms. It is thought that this relationship may provide an important clue that there may be gender-related differences. However, it is also possible that the response to antidepressant treatment may vary depending on the SLC6A4 methylation status.

4. FKBP5

The FKBP5 gene (FK506 binding protein 5), which belongs to the immunophilin protein family, is involved in basic cellular processes such as folding and trafficking of proteins and immunoregulation. FKBP5 is a cis-trans peptidyl-prolyl isomerases that binds to immunosuppressants such as tacrolimus and sirolimus. The FKBP5 gene encodes a molecular accessory protein of the GR complex that regulates glucocorticoid signaling and plays a critical role in the compensatory control of the stress response (Hähle et al., 2019).

The effects of FKBP5 genotypes, childhood abuse and depression on the methylation levels of five CpG islands in FKBP5 7th intron were investigated. Low methylation levels were observed in MDD individuals with the FKBP5 rs1360780 TT genotype (Klinger-König et al., 2019). These findings suggest that childhood adverse life events may induce demethylation of FKBP5 in individuals with high-risk genotype. Furthermore, in all participants, low methylation levels in the FKBP5 7th intron were associated with decreased gray matter concentration in the bilateral inferior frontal orbital gyrus (Tozzi et al., 2018). FKBP5 rs1360780 CC genotype was positively correlated with the thickness of the right transverse frontopolar gyri and DNAm in MDD patients. This results demonstrates that epigenetic changes in the FKBP5 gene may influence morphological changes in brain regions involved in emotion regulation (Han et al., 2017).

5. MAOA

Monoamine oxidase A (MAOA) is a member of the gene family encoding mitochondrial enzymes that accelerate the oxidative deamination reaction of amines such as dopamine, norepinephrine and serotonin. It has been associated with various psychiatric disorders such as antisocial personality disorder, obsessive-compulsive disorder, depression and anxiety disorders (Liu et al., 2013).

It was found that the mean MAOA exon 1 methylation levels of women with depression were lower than the control group. This would theoretically lead to an abundance of MAOA, which metabolizes target neurotransmitters, and seems to be consistent with the mechanism of monoamine oxidase inhibitor (MAOI) (Melas et al., 2013). In a replication study using a smaller sample size, it was found that women with depression showed hypomethylation in MAOA exon 1 compared to the control group. The results seem to be consistent with the monoamine hypothesis of depression and the mechanisms of action of MAOI. Therefore, MAOI seems to be a potential biomarker for future research (Melas and Forsell, 2015). In a study of Swedish women, the MAOA first exon hypermethylation was found to mediate a possible association between sexual abuse and depression. It was also found that methylation levels and sexual abuse independently predicted lifetime depression (Checknita et

al., 2018). MAOA is an important candidate gene in depression studies. However, number of studies describing MAOA methylation levels and its relationship with depression are relatively few and the results seem to be inconsistent.

Conclusion

Depression is a major mental health problem causing individual and economic problems on a global scale. Having a biopsychosocial basis, depression is characterized by different symptoms including depressed mood, decreased desire and loss of pleasure in previously enjoyable activities. There is no single model or mechanism that definitively defines the pathophysiology of depression. Monoamine, stress-induced, neurotrophic and inflammation theories come to the fore (Malhi and Mann, 2018; Šalamon Arčan et al., 2022).

Genetic and other factors have been implicated in approaches to elucidate the physiopathology of depression (Bakusic et al., 2017; Ugartemendia et al., 2021; Zhu et al., 2023). The concept of epigenetics describes the mechanisms involved in the gene regulation (Keverne and Binder, 2020). Previous research describes different candidate genes that explain the relationship between DNA methylation and depression. There are also studies suggesting epigenetic changes in global DNAm associated with depression. Chronic stress may shed light on mechanisms underlying the pathophysiology of MDD by increasing global DNA methylation (Byrne et al., 2013; Numata et al., 2015; Uddin et al., 2011). Methylation level of the BDNF gene is closely related to the pathophysiology of depression and antidepressant treatment response (Lieb et al., 2018; Tadić et al., 2014). The methylation status of the NR3C1 gene may also be associated with the prevalence and incidence of depression, and social factors may play a mediating role in this relationship (Borçoi et al., 2020; Kang et al., 2020). There may be also a potential relationship between the methylation status of the SLC6A4 gene and depressive symptoms. These studies suggest that the relationship and its biological basis may differ between genders. It has also been shown that the response to antidepressant treatment may vary depending on the methylation status of the SLC6A4 gene (Iga et al., 2016; Philibert et al., 2008; Zhao et al., 2013). Although studies have found that MAOA methylation level is associated with depression, the results are not yet consistent (Checknita et al., 2018; Melas and Forsell, 2015). The FKBP5 gene has also been associated with depression and it has been pointed out that this gene may lead to changes in the emotion regulation regions of the brain through epigenetic mechanisms (Han et al., 2017; Klinger-König et al., 2019; Tozzi et al., 2018).

In conclusion, the role of genetic and epigenetic mechanisms in understanding the biological basis of depression is significant. However, there are need for more detailed and comprehensive studies in this field. The results of these studies will contribute to a better understanding of the physiopathology of depression and open up novel and alternative treatment strategies.

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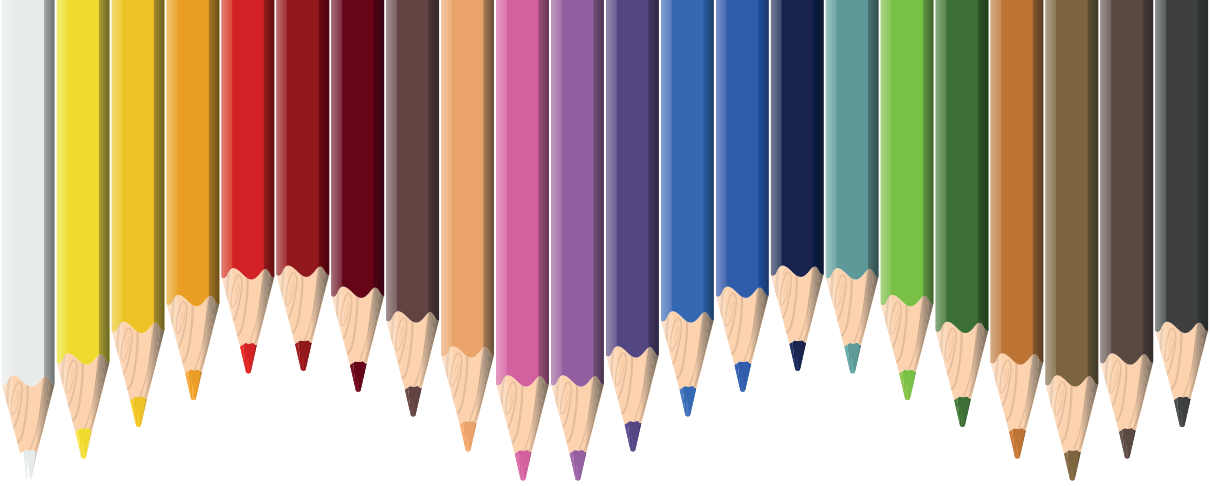

Chapter 10

SIALOLITHIASIS: A COMPARISON OF THE USE OF ULTRASONOGRAPHY AND CONE- BEAM COMPUTED TOMOGRAPHY IN THE DIAGNOSIS OF SALIVARY GLAND STONES

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SIALOLITHIASIS:

Sialolithiasis (also known as salivary calculi, salivary gland stones or salivary stones) is calcified structures that occur within the salivary glands and its ductal system.(Neville BW, 2002) The cause of sialoliths is unclear but the formation of sialoliths primarily arise as microscopic concretions, termed as sialomicroliths start to accumulate during the salivary gland normal secretion. Inflammatory foci helps primary or caused migration of oral bacteria by retrograde.(Harrison, 2009; Marchal, Kurt, Dulguerov, & Lehmann, 2001)

Sialoliths most frequently (80–90%) develop within the submandibular gland ductal system; occuring of calculi within the parotid gland and its duct is less often. The long, winding, upward path of the submandibular (Wharton's) duct and the more mucoid secretions of this salivary gland may be responsible for its tendency to formation for salivary stones. Sialoliths can also develop within the minor salivary glands, occur at almost any age, but they are most often in young and middle-aged adults. (Neville BW, 2002) The prevalence of sialolithiasis is 1.2% and incidence of 2.9–5.5 cases/100,000 of the population.(Schroder et al., 2017)

The etiology of salivary gland stones is still not fully elucidated today. Since the incidence of salivary gland stones is relatively low, studies on the detection of etiological factors remain limited. When we examine the formation mechanism of sialolithiasis, could collect the reasons under two headings. The first is anatomical; caused by such as canal stenosis or inflammation, the other is compositional; caused by different enzyme activity or increased calcium content. (Neville BW, 2002) When the geographical distribution of water hardness and sialolith formation is investigated; no correlation was observed between salivary stone incidence and water hardness. (Sherman & McGurk, 2000) Although sialomicroliths includes calcium and phosphorus minerals as well as necrotic cell residues and organic materials, salivary calculi development is not related to any calcium and phosphorus metabolism disorders. (Neville BW, 2002; Sanchez Barrueco et al., 2022) Also there is no evidence of increased salivary stones with hypercalcemia in some studies.(Huoh & Eisele, 2011) The studies of researching the additional factors of dehydration and pharmacologic side effects that caused to less production saliva (i.e., diuretic use) still continues. Recently, tobacco smoking has been discussed as a potential risk factor for the formation of salivary calculi.(Hammett & Walker, 2023;

Huoh & Eisele, 2011; Marchal & Dulguerov, 2003) Low incidence of symptomatic sialolithiasis makes difficult large epidemiological studies on sialolithiasis. (Patel, Hashemi, & Joshi, 2014)

Sialolithiasis is the most frequent cause of salivary glands swelling and the most obvious symptom is cyclical gland swelling and pain during mealtime.(Hammett & Walker, 2023; Rzymska-Grala et al., 2010) When pressure is applied to the salivary gland, clean saliva is expected to flow from the duct; if this does not happen, a stone may be blocking the flow of saliva. Tenderness to palpation and purulent discharge from the duct increases the concern for acute sialadenitis. (Badash, Raskin, Pei, Soldatova, & Rassekh, 2022)

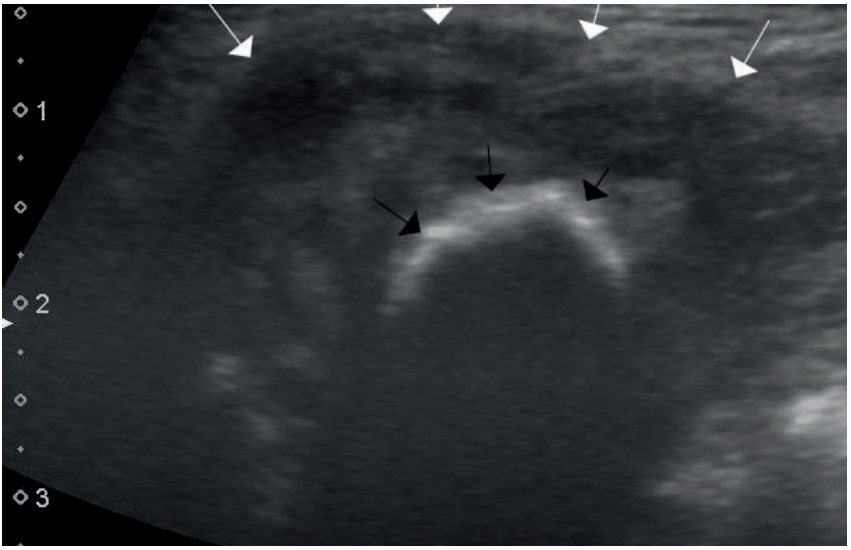
Many diagnostic methods are used to detect sialoliths such as computed tomography, magnetic resonance sialography, ultrasonography, sial-endoscopy, radiographic sialography and cone-beam computed tomography; either all of these imaging modalities are invasive or require ionizing radiation except magnetic resonance sialography and ultrasonography.(El-Rasheedy, Abdalla, Hassanein, Hafez, & Aboel-naga, 2021; Razek & Mukherji, 2018)

Ultrasonography and CBCT are imaging methods frequently used in dentistry faculties today in the diagnosis and treatment planning of sialolith patients who present with pain and swelling during eating.

ULTRASONOGRAPHY

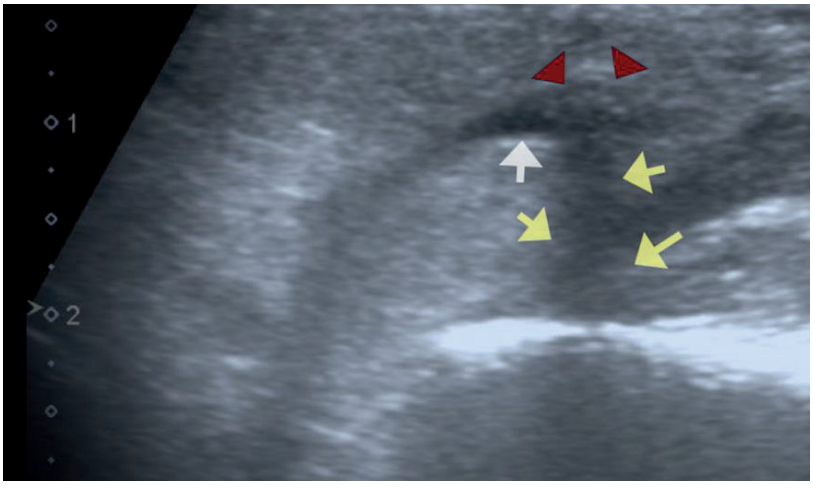
Ultrasonography (USG) is a diagnostic imaging method widely used in many fields of medicine today and provides extremely valuable information in the evaluation of head and neck diseases. It has a particularly high specificity and sensitivity in the detection of salivary gland stones and also offers significant advantages to removal of sialoliths by non-endoscopy methods. Its valuable contribution to the removal and diagnosis of foreign bodies in the head and neck area, has been demonstrated by many recent studies. (Ng & Pinto, 2000; Ng, Songra, & Bradley, 2003; Patel et al., 2014)

USG is accepted as the first imaging method in salivary gland diseases. (Fig. 1,2) It is also used in acute inflammation to investigate the presence of sialolithiasis or abscess. It is a very sensitive imaging method for detecting sialolithiasis and has replaced sialography in many institutions.



Usg is preferred to sialography in patients complaining an acute salivary gland infection due to contraindicated as well.(El-Rasheedy et al., 2021; Razek & Mukherji, 2018) In comparison to other imaging modalities, such as computed tomography, cone-beam computed tomography, Usg still considered a first-line tool for diagnosis. Usg is a non-invasive method that offers dynamic imaging without the use of ionizing radiation.(Patel et al., 2014)

(**Figure 1**. Parenchymal borders of the right submandibular salivary gland (white arrows), large calcified sialolith forming acoustic shadow located within the gland (black arrows))



(**Figure 2**. *Hyperechoic sialolith (red arrowheads) in the stenon duct of the right parotid gland, image of the proximally enlarged duct (white arrow), acoustic shadow of the salivary gland stone (yellow arrows)).*

Usg is recommended by some researchers as a choice in salivary gland calculi detection because of its high sensitivity in sialolithiasis detection amounts to 94%, specificity – 100%, and accuracy – 96%.(Madani & Beale, 2006; Wong, Ahuja, Yuen, & King, 2003; Yousem, Kraut, & Chalian, 2000) Jäger et al. showed the ultrasonography sensitivity in sialolithiasis detection is equal to 59.1–93.7%, and its specificity: 86.7–100% in their study.(Jäger et al., 2000)

Also, Usg is a diagnostic imaging method allowing for non-opaque salivary gland stones detection with sensitivity of 80–96%. A typical Usg image of a sialolith involves: a oval or round structured, revealing strongly hyper echogenic lines or points with distal acoustic shadow. In addition duct occlusion and dilated ducts are monitored in symptomatic cases.(Bialek, Jakubowski, Zajkowski, Szopinski, & Osmolski, 2006; Rzymska-Grala et al., 2010; Sansanwal, Chouhan, Bhateja, & Arora)

Sialolithiasis in salivary ducts may lead to the dilatation of the duct by blocking which may be shown on Usg. Salivary gland calculi smaller than 2 mm may not produce any acoustic shadow.(Alyas et al., 2005) Small stones in intraparenchymal ducts without dilatation may cause diagnostic mistakes. (Madani & Beale, 2006) In addition, hyperechoic minimal air bubbles in the saliva in that area may be confused with small stones on Usg and cause misdiagnosis as well.(Bialek et al., 2006) Sialoliths which located near to the Wharton duct opening in the sublingual caruncles region may sometimes be made more visible during ultrasonography by applying finger pressure from inside the oral cavity. (Bialek et al., 2006)

The submandibular space and its superficial components are accessible to Usg. Salivary gland stones located in the gland or duct system can be easily differentiated from abscess and cellulitis based on ultrasonographic assessment. Additional information can obtain, such as size, number and location of calculi, helps determine prognosis and can guide for initial management. Because of its high sensitivity and specificity, Usg has become the first step in the evaluation of suspected salivary calculi.(Oliveira, Hurst, & Magajna, 2014)

Terraz et al. detected 53 sialolithiasis in 44 salivary glands (11 parotid glands and 33 submandibular glands) in their study .The majority (57%) of salivary gland stones diameter was greater than 3 mm, 20% was 3 mm and 23% was less than 3 mm. They observed that the sensitivity of ultrasonography in diagnosing sialolithiasis depends on the salivary stone diameter which smaller than 3 mm (sensitivity, 10%) and in 33 of 34 glands with calculi 3 mm or larger (sensitivity, 97%). Also as a result of their study, they observed the specificity, sensitivity, negative predictive value, positive predictive value and accuracy of ultrasonography in the detection of sialolithiasis were; 95%, 77%, 78% 94% and 85%.(Terraz et al., 2013)

Rasheedy et al. in their study on the evaluation of salivary gland stones with USG in 2021, obtained results including 34 true-positive and 36 true-negative readings. Also, they observed false-negative results in ten salivary glands and false-positive results in two glands. They were able to observe just ductal dilatation or acoustic shadow in 10 salivary gland stones with a diameter of less than 3 mm. (El-Rasheedy et al., 2021)

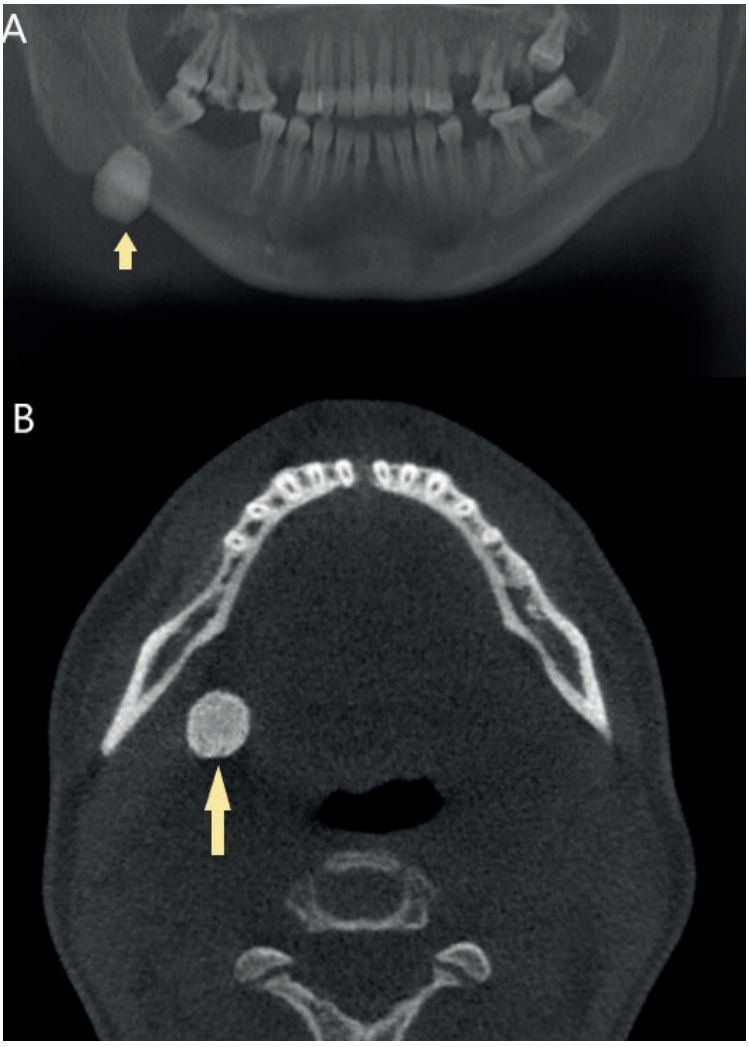
Thomas et al. revealed the sensitivity and specificity were 65% and 80% for USg on 48 sialolith cases. Also, they claimed that Usg had a lower sensitivity (65%) than what has been reported in the literature (70%-94%).(Thomas, Douglas, & Rassekh, 2017)

Kim et al. revealed the sensitivity, specificity, negative predictive value and positive predictive value of Usg as 79.9%, 65.6%, 80.4%, 77.4% and concluded that the ultrasonography usefully detects of submandibular and parotid gland stones, also has the highest diagnostic accuracy,of the parotid gland calculi.(Kim et al., 2022) Grale et al. in their study observed that about 50% of the sialolithiasis cases which showed features of inflammation in Usg images. (Rzymska-Grala et al., 2010)

CONE- BEAM COMPUTED TOMOGRAPHY

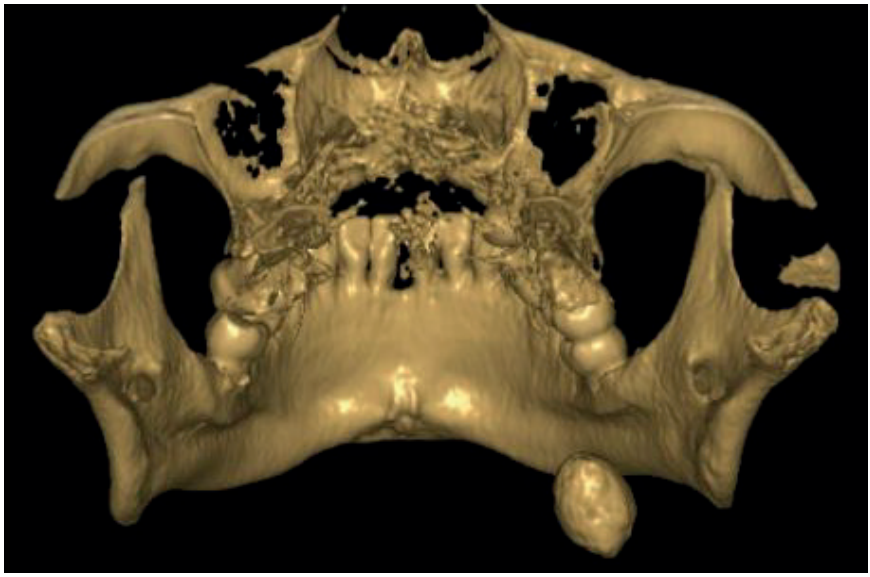
Cone-beam computed tomography (CBCT) is an imaging modality which was originally developed for angiography in the 1980s with the first dental and maxillofacial units produced in the late 1990s and early 2000s. Unlike other extraoral dental imaging techniques, such as cephalometric and panoramic radiography, CBCT acquires volumetric data providing three-dimensional (3D) radiographic imaging for the evaluation of the dental and maxillofacial regional anatomical and pathological changes. CBCT imaging is a volumetric image-capture technology ensuring data set from which digital images are presented and reformatted on a monitor. Image display should be formatted and dynamic according to task-specific display protocols. Several software applications contributes the capability to expand the use of this volumetric data set to facilitate treatment planning, image guidance of surgical and operative procedures and additive manufacturing. (Mallya, 2018)

Recently, using of CBCT in head and neck region and diagnosis and visualization of the dento-maxillofacial disorders has been significantly increased. (Fig. 3,4)



*(**Figure 3**. (**A**) Radiopaque oval-shaped calcified formation superimposed on the right mandibular angulus region in panoramic radiography (short arrow). (**B**) Axial CBCT image shows a round shaped large hyperdense calcified mass in the right submandibular region (long arrow).)*

It provides relatively high spatial resolution of bony structures in a single rotation about 9-40 seconds.(Yajima et al., 2006) CBCT imaging is an important imaging modality for detecting sialolithiasis due to high sensitivity and specificity for salivary calculi, superior diagnostic performance through without superimposition imaging, less radiation-dose ratio comparing to computed tomography. (Dreiseidler et al., 2010)



(**Figure 4.** 3D CBCT remodelling image shows large oval shape sialolit extending below the level of the basis mandibula.)

Also, specificity and sensitivity for CBCT are superior to conventional radiographs, sialography, Usg, sialendoscopy and CBCT is comparable with imaging modalities such as medical CT and MRI sialography. (Stanley, Bardales, Beneke, Korourian, & Stern, 1996; Szalma, Olasz, Toth, Acs, & Szabo, 2007; Varghese et al., 1999)

Dreiseidler et al. in their study on 29 sialolith patients; obtained 1 false positive and 1 false negative result and achieved both sensitivity and specificity rates of 98.85% in CBCT.(Dreiseidler et al., 2010)

Meij et al. in their study revealed that the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the detection of salivary stones on CBCT as 94%, 90% 84%, 97% and 92%. Also, they observed the inadequacy of CBCT only three sialoliths which missed because of prominent scattering due to the presence of dental restorations.(van der Meij, Karagozoglu, & de Visscher, 2018)

Costan et al. observed that using of CBCT imaging presented for optimal determination of the number, location, and shape of the sialoliths with an optimal understanding of the 3D location of the sialoliths and its surrounding structures. In addition, they mentioned the informations obtained from CBCT images increased the accuracy of calculi retrieval.(Costan, Ciocan-Pendefunda, Sulea, Popescu, & Boisteanu, 2019)

Schwarz et al. obtained heterogeneous results; sensitivity, specificity, positive and negative predictive values of the Usg and CBCT imaging to detect

sialolithiasis in their study. They didn't observe any statistical difference for comparing the sialolith diameter between Usg and CBCT. They revealed the sialolith detection rate as 73% for Usg (24/33), 82% for CBCT (27/33).(Schwarz et al., 2015)

Whereas the specificity (90% and 90%) and the positive predictive value (96% and 96%) showed similar values for these diagnostic tools, the results of the negative predictive value and sensitivity showed differentiations. The sensitivity of Usg was (70%) and CBCT (79%). The negative predictive value of Usg was (47%) and CBCT (56%). (Schwarz et al., 2015)

In their study, Kraaij et al. state that when CBCT scans are used to detect submandibular salivary stones, it could be noted that the sialoliths are much smaller than the measurement results obtained. This finding was important when cut-off values of sizes of stones are used in stone removal planning. Also they suggested that to overcome this limitation and to ensure closest measures the actual volume of the calculi, should use the smallest voxel size possible. (Kraaij, Brand, van der Meij, & de Visscher, 2021)

CONCLUSION

Ultrasonography may be the first choice in detecting salivary gland stones due to its easily accessible, cheaper, dynamic image acquisition and without ionizing radiation features. It also provides important informations in terms of evaluating salivary stones and imaging the duct structure during acute salivary gland infections caused by sialolith. If we suspect salivary gland stones during the clinical examination of the patient and we could not observe this situation in the ultrasonography, in this case, we can visualize the opaque calculi in the CBCT images for diagnosig correctly.

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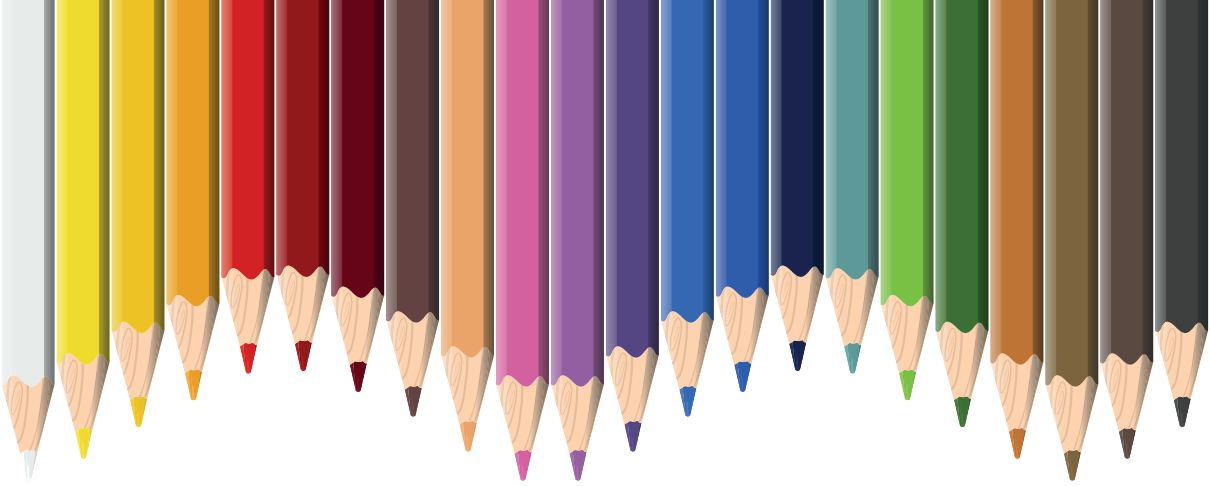

Chapter 11

THE EFFECT OF DIODE LASER WITH MECHANICAL DEBRIDEMENT ON PERI-IMPLANT SULCULAR FLUID LEVELS OF PERIOSTIN AND VITAMIN 'K'*

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* Mekanik Tedavi ve Diyot Lazer Uygulanmış Peri-İmplantitisli Hastalarda Peri-İmplant Sulkus Sıvısı Periostin Ve K Vitamini Seviyesi, Gardenya Çevik Ünalan, Tez Danışmanı: Prof. Dr. Ebru OLGUN Tarih: 08.11.2021 Uzmanlık Tezi, Kırıkkale

1. INTRODUCTION

Peri-implantitis treatment is based on regimens of infection control, detoxification of implant surfaces, regeneration of lost tissues, and plaque control by mechanical debridement with or without flap surgery (Bautista & Huynh-Ba, 2013; Schou et al., 2004). One of the main goals of treatment is to detoxify contaminated implant surfaces. Reducing the bacterial load on the implant surfaces may sometimes not be sufficient with the non-surgical mechanical treatment option. In this case, additional treatment options such as antibiotics, antiseptic and laser treatments are recommended. In recent years, the use of lasers has become very popular in dental implantology. Since laser therapy is a treatment option that can control subgingival microorganisms (Karlsson et al., 2008), diode, carbon dioxide (CO2), neodymium-doped yttrium aluminum garnet (Nd:YAG), erbium-doped yttrium aluminum garnet (Er:YAG)) and erbium, chromium doped: yttrium, scandium, gallium, garnet (Er,Cr: YSGG) lasers are frequently used in periodontal and dental implantology fields. Lasers have properties such as ablation, vaporization, hemostasis, microbial inhibition, biostimulation. Studies have shown that diode laser facilitates bacterial elimination from periodontal pockets and provides better healing than the control group. Diode laser basically does not interact with titanium or titanium coated material and it has been shown that the diode laser is effective in the decontamination of implant surfaces without causing problems in the surrounding tissues (Romanos et al., 2000).

Periostin is a member of the fasciclin family and is a vitamin K-bound, glutamate-containing matrix cellular protein synthesized at 93.3 kDa. Periostin was first detected in the mouse osteoblastic cell layer and was originally named osteoblast-specific factor-2 (OSF-2) (Takeshita et al., 1993). Later, its name was changed to periostin due to its presence in the periosteum and periodontal ligament (Horiuchi et al., 1999). Periostin is commonly found in collagen-rich tissues, which is thought to affect the production of collagen fibers. Additionally, periostin is synthesized in tissues exposed to constant mechanical forces such as the periosteum, periodontal ligament, tendon, heart valve and skin (Du & Li, 2017). Periostin mediates inflammation and fibrosis during diseases of various organs, including the heart, lung, kidney, skin, liver, skeletal muscle and retina. It was shown that inhibition of periostin in these diseases has been effective in the development of pathologies in animal models (Nakama et al., 2015; Oka et al., 2007). The importance of periostin in dentistry was revealed by examining periostin null mice. The lack of periostin in mice resulted in severe alveolar bone loss, external root resorption, and enlargement of periodontal ligament (Rios et al., 2008). In mice in which the periostin gene was destroyed, eruption disorders, defective remodeling in periodontal ligament, and periodontal disease-like phenotype were shown (Rios et al., 2008; Kii et al., 2006). In an experimental study, it was report-

ed that the level of periostin in rats decreased after periodontitis induction and was inversely proportional with the amount of bone loss (Padial-Molina et al., 2012). Cell turn-over and differentiation are firmly regulated during periodontal hemostasis and healing. Matrix cellular proteins in periodontal ligament support extracellular matrix mechanically and biologically. Periostin, a matrix cell protein, acts as a highly released adhesion molecule from periodontal ligament to maintain periodontal tissue integrity. This plays an important role in tooth development and eruption (Kruzynska-Frejtag et al., 2004). In addition, periostin expression is increased in occlusal loading and orthodontic tooth movements (Wilde et al., 2003).

Vitamin K is a vitamin necessary for blood clotting and an important vitamin that activates seven protein components that form the basis of the coagulation cascade and acts as an essential cofactor for carboxylase (Hauschka et al., 1975). In addition to its effect on blood coagulation in mammals, vitamin K is also effective in bone metabolism (Ohsaki et al., 2006). γ-carboxylation is a vitamin K-dependent post-translational modification that has profound effects on the structure and function of proteins. γ-carboxylation has similarly important effects on the structure and function of periostin in a vitamin K-dependent manner.

In the literature, there is a limited number of human clinical trials investigating the effects of using dental lasers in the treatment of peri-implantitis at molecular levels. Therefore, in this study, it was aimed to evaluate the effect of diode laser in addition to mechanical debridement on peri-implant sulcular fluid (PISF) levels of periostin and vitamin K.

2. MATERIALS and METHODS

This study was designed as a randomized, prospective clinical trial. Individuals who applied to Kirikkale University, Faculty of Dentistry, Department of Periodontology were included into the study. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by Kirikkale University Clinical Research Ethics Committee (14.11.2019-26/01). Prior to the study, all individuals to be included in the study were given detailed information about the purpose and the method of the research and informed consent forms were obtained.

2.1.Study groups

A total of 42 implants with peri-implantitis were included and divided into three groups. Patients in group 1 (n = 14 implants) received mechanical debridement using titanium curettes while patients allocated in group 2 (n = 14 implants) received diode laser irradiation and patients in group 3 (n=14 implants) received mechanical therapy in combination with diode laser irradiation (settings 940±10 nm, 2.5WCP2 mode). Randomization was provided by

draw up. The inclusion criteria were as follows: (1) Inflammation with probing depth of ≥4 mm in at least one peri-implant site (2) >2 radiographic bone loss (3) bleeding on probing and suppuration (present or absent) (Schwarz et al., 2004). The exclusion criteria were as follows: (1) tobacco use (2) having a systemic disease that may affect the outcome of treatment such as diabetes mellitus (3) pregnancy and lactation (4) using antibiotics and/or anti-inflammatory drugs within the last 3 months.

2.2.Clinical measurements

Before treatment, peri-implant probing depth (PPD), clinical attachment level (CAL), suppuration, modified plaque index (mPI), keratinized mucosa width (KMW), suppuration (S), gingival index (GI), and modified sulcus bleeding index (mSBI) were recorded and PISF samples were collected. PPD, CAL, mPI, GI, and mSKI were recorded from 4 regions of each implant. After applying light pressure to the mucosa, the presence of pus was determined as S (+) or its absence as S (-). The KMW was evaluated in as milimeter and recorded. A standard interval, color-coded, pressure-calibrated (20-25 g) plastic periodontal probe (Click-Probe® Blue, Kerr GmbH, Biberach, Germany) was used for clinical parameters. Panoramic and periapical radiographs were used to determine the level of peri-implant bone loss. All measurements were made by the same examiner (GCU). Before PISF sampling, the implant area was air-dried and plaque was removed to avoid saliva contamination, cotton rolls used for isolation. Two paper strips were used to collect for PICF samples. Paper strips were inserted into the deepest probing until a moderate pressure was felt and kept for 30 s. All clinical measurements and PISF were collected at baseline and the 6^{th} and the 12^{th} weeks after treatment in all groups.

2.3. Peri-implantitis treatment procedure

In group 1 (mechanical debridement only): After the clinical measurements were recorded and the PISF samples were collected, mechanical debridement was carried out with manual instruments (titanium-coated Gracey curettes) to remove all soft and calcified deposits.

In group 2 (diode laser only): The implants in this group were treated with 940-nm ± 10nm diode laser application at 2.5 W in CP2 pulsed mode. The 400-µm optical fiber (E4 7&9mm) was inserted parallel to the longitudinal axis of the implant, up to 1 mm from the most apical portion of the sulcus, and moved, during laser light emission, in apico-coronal and mesio-distal direction for 30 s (Mettraux et al., 2016). This procedure was performed on day 0 (= baseline) and repeated on the 7^{th} and the 14^{th} days.

In group 3 (both mechanical debriment and diode laser): Following the recording of clinical measurements and collection of PISF samples, after mechanical debridement, diode laser application was performed as described above.

2.4. Statistical analysis

In this study, the changes in the parameters that did not show normal distribution in the evaluated time periods and the differences between the groups were determined with the Kruskal Wallis test, and the Mann-Whitney U test was used as a post-hoc analysis to determine the groups with differences. Intra-group differences in the changes of these parameters in the evaluated time periods were determined by the Friedman test, and the Mann-Whitney U test was used as a post-hoc analysis to determine the time periods with differences. Tukey and Duncan tests were used as post-hoc analysis for the determination of the groups with differences, by determining the changes in the normally distributed variables in the evaluated time periods with the help of the One-Way ANOVA test between the groups. Intra-group differences in the changes of these variables in the evaluated time periods were analyzed with the T-test. The relations of the existing parameters were evaluated with the Spearmen correlation test. Statistical analyzes were performed in IBM SPSS Statistics 23 software and $p<0.05$ was considered statistically significant.

3.RESULTS

All the clinical parameters and treatment oucomes are presented in Table 1. At the 6th week, there were no significant differences among groups for PPD, CAL and KDW. mSBI and mPI were signifcantly higher in Group 1 than the other groups. GI and S were significantly different in Group 2 than the other groups. At the 12th week, there were no significant differences among groups for mSBI and KDW. PPD was signifantly lower in Group 2 than the other groups. mPI and GI were signifantly different in Group 1 than the other groups. S was signifantly different in Group 2 than the other groups. In all groups, PPD and CAL were significantly decreased from baseline to the 6th and the 12th weeks. mSBI was significantly decreased from baseline to the 12th week in all groups. In Group 2 and 3, GI values were significantly decreased from baseline to the 12th week after therapy.

In all groups, PISF significantly decreased from baseline to the the 12th week. At the 6th and the 12th weeks, the periostin level was significantly lower in Group 2 than the other groups. At the 12th week, it was significantly lower in Group 1 than Group 3. Vitamin K level was significantly higher in Group 2 than the other groups at the 6th week whereas lower at the 12th week. In Group 1 and 2, periostin level was signifiacntly decreased from baseline to the 12th week and from the 6th week to the 12th week. Vitamine K level was significantly decreased from baseline to the 6th and 12th weeks in Group 2. (Table 2)

4.DISCUSSION

Periostin is a mechanical stress-sensitive molecule that is secreted mainly by fibroblasts during wound healing. Periostin is thought to be critical for

maintaining the integrity of periodontal ligament and is very important for postpartum development and that periostin can play a crucial role in the cross-linking and distribution of collagen or non-collagen extracellular matrix proteins. In this study, the effect of diode laser used in addition to mechanical debridement in the treatment of peri-implantitis on periostin and vitamin K levels in the PISF was examined. According to our results, all three treatment methods provided a successful recovery while no additional benefit was observed in the diode laser-assisted mechanical debridement in terms of molecular levels.

Many treatment protocols have been suggested in the treatment of peri-implantitis, among which non-surgical mechanical instrumentation methods and antibacterial agents have been frequently used. These treatment approaches mainly consist of mechanical debridement with an additional therapy aimed at removing the biofilm attached to the implant surface (Zitzmann et al., 2001). Recently, lasers have been used with increasing frequency in the treatment of peri-implant diseases. It has been reported that diode laser is an effective therapeutic alternative in the control of bacterial infection (Maiorana et al., 2002). It has been reported that the diode laser does not cause any damage to the titanium surface after irradiation and has the capacity to clean the rough implant surfaces (Romanos et al., 2000). The diode laser, which provides detoxification of the implant surface with irregular areas, is not an ablative instrument and can directly contact the implant surfaces without causing melting, cracking or crater formation (Kreisler et al., 2002). Diode laser may be a more practical option for intraoral applications due to its small size and convenience. There are studies in the literature that differ in wavelength, energy density, frequency of application and evaluation techniques (Schar et al., 2013; Roncati et al., 2013). Considering the studies using diode laser in periodontitis and peri-implantitis, 940 nm diode laser with settings 940±10 nm, 2.5W CP2 mode parameters was used in our study. Analysis of PISF provides a non-invasive method to examine host response in peri-implant diseases and may facilitate early diagnosis of patients at risk for active disease (Petkovic et al., 2010). The levels of biochemical mediators released into the PICF have been evaluated in various studies with the aim of revealing a diagnostic marker to evaluate peri-implant health and diseases (Paknejad et al., 2006).

Unlike structural matrix proteins, matrix cellular proteins describe a family of extracellular macromolecules that do not play a primary role in cell structure but are induced following injury and do not function structurally, which regulate cell-cell and cell-matrix interactions (Ozbek et al., 2010). Periostin is a matricellular protein that acts a part in the cell–matrix interactions and cell functions. Based on these properties, periostin provides cell migration, recruitment, adhesion, proliferation, and binding of various tissues to healing sites. It promotes the migration of fibroblasts and osteoblasts and can

play an important role in remodeling of the periodontal ligament and bone (Du & Li, 2017). Periostin has two important roles, physiological protective actions and pathological events. Periostin is required for the collection of suitable collagen in the skin. Increased mechanical stres and damage of the periosteal expression contribute to physiological extracellular matrix remodeling and wound repair. Periostin stimulates keratinocyte proliferation, and in the absence of periostin, wound repair on the skin is delayed (Nishiyama et al., 2011). Similar to these findings, in case of damage to periodontal tissues, it may be expected that the level of periostin increases and contribute to tissue repair and remodeling. In addition, periostin is thought to be an effective regulator in future periods in increasing regeneration of periodontal tissues (Du & Li, 2017).

In a study evaluating levels of periostin, pyridinoline crosslinked carboxyterminal telopeptide of Type I collagen, and C-terminal crosslinked telopeptide of Type I collagen levels for dental implants and natural teeth, it was suggested collagen breakdown products may be used as markers to evaluate peri-implant metabolism. (Akman et al., 2018) Findings in this study may only reflect the gingivitis/peri-implant mucositis in which early inflammatory mechanisms.

Periostin, a vitamin K-bound, glutamate-containing matrix cellular protein, is synthesized at 93.3 kDa, especially in tissues exposed to constant mechanical forces and in connective tissue rich in collagen (Takeshita et al., 1993; Horiuchi et al., 1999). However, it mediates inflammation and fibrosis during diseases of various organs (Du & Li, 2017). Periostin encoded by the human POSTN gene; It has been reported that its expression is induced by TGF β, BMP 2 and BMP 4, VEGF, valsartan and IL-3, IL-4, IL-6, IL-13 and vitamin K (Norris et al., 2009).

Production of blood coagulation factors or ECM proteins, matrix-Gla protein and osteocalcin is vitamin K dependent via post-translational modification (Berkner, 2008). Vitamin K-dependent proteins are commonly coagulation proteins, while other vitamin K-dependent proteins have been discovered in a few tissues other than the liver and have important physiological functions such as soft tissue calcification and bone metabolism (Schultz & Arnold, 1990). Based on these and similar findings, we aimed to examine the changes in periostin and vitamin K together in our study. Padial Molina et al. (2013) investigated the synthesis of messenger RNA and periostin levels in the PDL structure exposed to biomechanical loading and bacterial virulence factors (TNF-α and P. *gingivalis*). According to their results, under biomechanical loading and bacterial virulence factors, both the synthesis and protein levels of periostin increased in the early period of exposure to the agent, followed by a significant decrease during and after disease progression.

There are controversial results in the literature regarding the additional benefits of lasers compared to conventional treatment. Some authors have suggested that lasers may not provide more clinical benefit than scaling and root planing (De Micheli et al., 2011), while others have suggested that they provide superior results compared to scaling and root planing alone (Saglam et al., 2012; Qadri et al., 2010; Giannelli et al., 2012). The reason for the incompatibility can be attributed to many factors such as differences in laser settings, wavelengths and study designs.

The form of the healing in all tissues of the body and the biochemical events after the injury is very similar. In many tissues, periostin was indicated for repair and remodeling after the damage on the surface, and it was seen in the minimal level in healthy tissue. When these findings were addressed, it was not surprising that similar results were observed in periodontal tissues. The dual functions of periostin as an extracellular matrix and a matricellular protein are important also for the onset of inflammation. Periostin is deposited in inflamed sites showing fibrosis, whereas it activates immune and nonimmune cells as a matricellular protein, further augmenting inflammation. The epithelial/mesenchymal interaction and/ or the immune cell/nonimmune cell interaction is important for periostin to exert its effects in the setting of inflammation. Recent studies have demonstrated that some inflammatory mediators, such as IL-13 and TGF-β, can induce periostin production (Sidhu et al., 2010). Our findings suggest that periostin may play an important role in the inflammatory microenvironment.

In our previous study, we evaluated the effect of non-surgical periodontal treatment on gingival crevicular fluid (GCF) periostin levels in patients with gingivitis and periodontitis. The results of this study suggest that GCF periostin plays a role as a reliable biological marker in the pathogenesis of periodontal disease and non-surgical periodontal treatment is effective in decreasing GCF periostin levels (Arslan et al., 2021). Similarly, in this study, periostin level decreased following treatment protocols in all groups. Periostin can also regulate inflammatory responses (Prakoura et al., 2017; Sidhu et al., 2010). According to the data of our study, it may be thought that periostin may have increased as a protective mechanism and response to periodontal diseases. Periostin may be expected to increase in order to provide repair and remodeling of tissues during peri-implant diseases.

More studies are thought to be needed to determine possible specific mechanisms related to periostin and its full role in chronic inflammation. A better understanding of periostin and its function may support new diagnostic and treatment strategies for peri-implant diseases.

As a result of our study, the vitamin K level in PISF samples taken from peri-implantitis and inflamed areas were decreased after the treatment in all

groups. Our study shows that with the reduction of inflammation during recovery, the total amount of vitamin K also decreases. This finding is in line with the data in the literature (Reddi et al., 1995; Ohsaki et al., 2006). The analysis that emerged as a result of our study confirmed that there is a proportional relationship between the amount of vitamin K and periostin. This finding supports studies reporting that the expression of periostin, which is encoded by the POSTN gene in humans, is induced by vitamin K (Norris et al., 2009; Berkner et al., 2008). Our study is the first clinical study to compare the effects of three different treatment modalities on periostin and vitamin K levels in peri-implantitis and is an original study in its field.

More studies are thought to be needed to determine possible specific mechanisms related to periostin and its full role in chronic inflammation. A better understanding of periostin and its function may support new diagnostic and treatment strategies for periodontal diseases.

5.CONCLUSION

In conclusion, within the limits of this trial, all three treatment procedures provided a successful improvement in the treatment of peri-implantitis. Similar treatment outcomes were obtained in three groups. Based on these findings, it was suggested that addition of diode laser to mechanical debridement does not provide any additional benefits for molecular level in peri-implantitis treatment.

Acknowledgments

The authors express their thanks to assistant professors Mehmet Selim YILDIZ in the Department of Periodontology, Faculty of Dentistry, University of Altınbaş, Turkey, for providing support in statistical analysis. This study was supported by Kirikkale University Scientific Research Projects Coordination Unit (Project no: 2019/023).

Conflict of Interest and source of funding statement

The authors declare that they have no conflict of interest.

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Table 1: Comparison of clinical parameters at baseline and after treatment among groups

		Baseline	6th week	12th week	P#
PPD Median (min-max)	**Group 1**	5.7 (4.5 - 6.5)[a]	4.5 (3.0 - 6.0)[b]	3.6 (0.9 - 5.8)[c]	**,000**
	Group 2	4.7 (3.5 - 7.2)[a]	3.6 (3.0 - 5.5)[b]	3.0 (2.2 - 4.2)[b]	**,000**
	Group 3	5.2 (4.0 - 7.2)[a]	4.2 (2.7 - 5.5)[b]	4.0 (2.7 - 5.2)[c]	**,000**
	P##	**,066**	**,503**	**,030**	
CAL Median (min-max)	**Group 1**	6.0 (4.5 - 7.5)[a]	4.5 (3.0 - 6.0)[b]	3.7 (2.7 - 5.7)[c]	**,000**
	Group 2	5.1 (3.5 - 7.2)[a]	3.8 (3.0 - 5.5)[b]	3.0 (2.2 - 4.2)[c]	**,000**
	Group 3	5.2 (4.0 - 7.2)[c]	4.2 (2.7 - 5.5)[b]	4.0 (2.7 - 5.2)[a]	**,000**
	P##	**,221**	**,806**	**,038**	
mSBİ Median (min-max)	**Group 1**	2.0 (1.0 - 3.0)[a]	2.0 (1.0 - 3.0)[b]	1.0 (0.0 - 1.0)[b]	**,000**
	Group 2	1.0 (0.5 - 3.0)[a]	1.0 (0.0 - 1.0)[a]	0.0 (0.0 - 1.0)[b]	**,000**
	Group 3	1.0 (0.0 - 2.0)[a]	0.0 (0.0 - 1.0)[a]	0.0 (0.0 - 1.0)[b]	**,000**
	P##	**,055**	**,051**	**,000**	
mPİ Median (min-max)	**Group 1**	1.0 (1.0 - 2.0)[a]	1.0 (0.0 - 1.0)[b]	0.0 (0.0 - 2.0)[b]	**,001**
	Group 2	1.0 (0.0 - 2.0)[a]	1.0 (0.0 - 1.0)[a]	0.0 (0.0 - 1.0)[a]	**,005**
	Group 3	1.0 (0.0 - 1.0)[a]	1.0 (0.0 - 1.0)[a]	0.0 (0.0 - 1.0)[a]	**,016**
	P##	**,059**	**,674**	**,171**	
Gİ Median (min-max)	**Group 1**	2.0 (1.0 - 2.0)[a]	1.0 (1.0 - 2.0)[a]	1.0 (1.0 - 2.0)[a]	**,004**
	Group 2	2.0 (1.0 - 2.0)[a]	1.0 (0.0 - 1.0)[b]	1.0 (0.0 - 1.0)[b]	**,000**
	Group 3	2.0 (1.0 - 2.0)[a]	1.0 (1.0 - 2.0)[a]	1.0 (1.0- 2.0)[b]	**,000**
	P##	**,533**	**,108**	**,037**	
S Median (min-max)	**Group 1**	1.0 (1.0 - 2.0)[a]	1.0 (1.0 - 2.0)[b]	1.0 (1.0 - 2.0)[b]	**,000**
	Group 2	2.0 (1.0 - 2.0)[a]	2.0 (1.0 -2.0)[a]	2.0 (1.0 -2.0)[a]	**,022**
	Group 3	2.0 (1.0 - 2.0)[a]	2.0 (1.0 - 2.0)[b]	2.0 (1.0 - 2.0)[b]	**,000**
	P##	**,055**	**,129**	**1,000**	

KMW Median (min-max)	**Group 1**	3.0 (2.0 - 5.0)[a]	3.0 (2.0 - 5.0)[a]	3.0 (2.0 - 5.0)[a]	**1,000**
	Group 2	3.5 (2.0 - 7.0)[a]	3.5 (2.0 - 7.0)[a]	3.5 (1.0 - 7.0)[a]	**,268**
	Group 3	3.5 (1.0 - 6.0)[a]	3.5 (1.0 - 6.0)[a]	3.5 (1.0 - 6.0)[a]	**1,000**
	P[##]	**,803**	**,803**	**,858**	

PPD: Peri-implant probing depth, **CAL:** Clinical attachment level, **mSBI**: Modified sulcus bleeding index,

S: Suppuration, **mPI**: Modified plaque index, **GI**: Gingival index, **KMW**: Keratinized mucosa width.

P[#]: İntragroup comparison results, **P[##]:** İntergroup comparison results

The different letters indicate the difference in those two groups

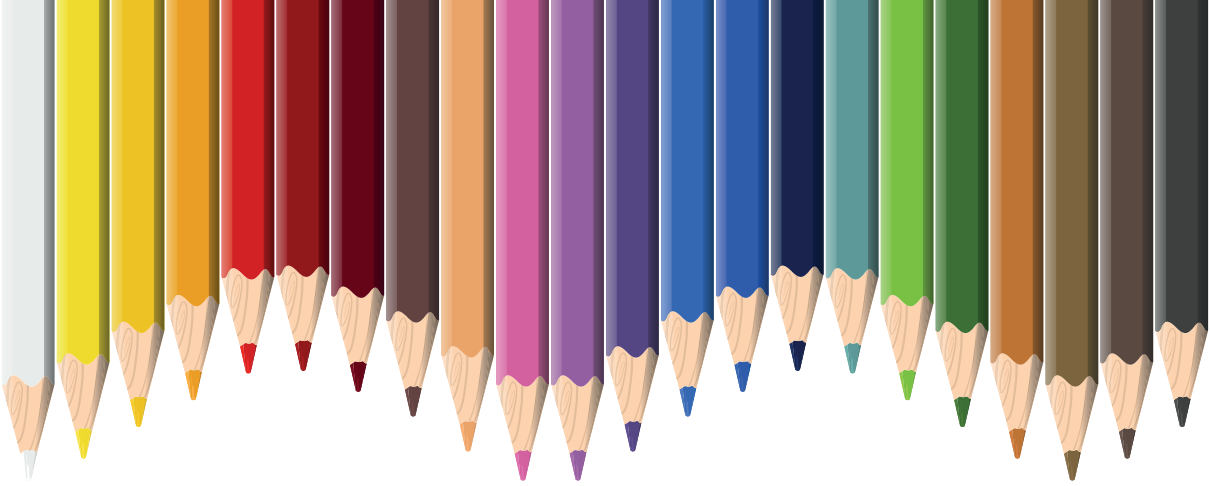
Table 2: Comparison of PISF Periostin and Vitamin K levels at baseline and after treatment among groups

		Baseline	**6th week**	**12th week**	**P[*]**
PISF Periostin level	**Group 1**	2121 (282-2552)[a]	2431 (234-2928)[a]	1393 (214-2236)[b]	**,030**
	Group 2	1864 (1346-2254)[a]	1946 (402-2548)[b]	1243 (38-2172)[c]	**,000**
	Group 3	2086 (240-2564)[a]	2396 (762-2804)[a]	1782 (306-2154)[a]	**,395**
	P[**]	**,439**	**,388**	**,191**	
PISF Vitamin K level	**Group 1**	587 (434- 704)[a]	538 (500- 662)[a]	502 (406-644)[a]	**,319**
	Group 2	611 (402- 710)[a]	574 (474- 700)[b]	483 (402-620)[c]	**,000**
	Group 3	586 (482- 682)[a]	532 (450- 694)[a]	565 (462-684)[a]	**,000**
	P[**]	**,834**	**,731**	**,074**	

PISF: Peri-implant sulcular fluid

P[#]: İntragroup comparison results, **P[##]:** İntergroup comparison results

The different letters indicate the difference in those two groups



Chapter 12

SYSTEMATIC REVIEW OF RESEARCH ON CHEESE MITES IN TURKEY

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Foods of animal origin are of great importance for a healthy and balanced diet (Önganer and Kırbağ, 2009).

Cheese is one of the animal food products that stands out with its nutritional value and diversity. Cheese, which is obtained by coagulating milk and separating the whey, goes through a certain maturation process and acquires its fresh or variety-specific taste, aroma and texture and meets the consumer (Koçak, 1994).

While different types of cheese are produced around the world, almost every country has its own cheese types. While varieties such as white cheese, kashar cheese and tulum cheese are produced intensively in Turkey, there are also traditional cheeses produced according to local characteristics. However, due to reasons such as the generally low education level of the population engaged in agricultural activities in our country, the dispersed structure of agricultural enterprises and the maintenance of animal husbandry as a side business, it is difficult to obtain accurate information about the types of cheese produced (Tekinşen, 2000).

Cheese types, which are widely produced and consumed with pleasure throughout the world, can pose a serious danger to public health if hygienic standards are not complied with at every stage, starting from the supply of milk used in production to the consumption stage (Şimşek and Arıcı, 1991; Çağlar et al., 1996).

Mites, one of the indoor allergens to which people are exposed in home and work environments, can be found in every corner of living spaces (Feng et al., 2009).

Mites represent a subclass within the Arachnida class that is critical for human health (Cevizci et al., 2010). The number of mites in the class Arachnida exceeds 45,000, with approximately 100 of these species tending to infest foodstuffs such as storage foods and cheese (Melnyk et al., 2010).

Adult forms of cheese mites are characterized as being 0.5 mm long, with 8 legs (hairless), and with a white body. These mites have strong, thick, slightly wrinkled leg structures and have layered mouthparts. Female mites do not differ significantly from male mites, except that they are generally larger. Their life phase varies between 15 and 18 days and their ideal living temperature is 22.7°C. At room temperature, it can usually take 10 days for them to transition from egg to adult form. A female mite can lay up to 800 eggs per day during its lifespan, which varies between 20 and 30 days. The larval form initially has 6 legs, but as they transition to the nymph form, they molt and become 8-legged like adults. Adult mites usually live between 60 and 70 days. These mites can cause food spoilage and irritation to the skin and digestive system. People prefer ripe cheeses to fresh cheeses. When people consume

these cheeses, which contain thousands of mites, they usually first experience complaints related to the gastrointestinal system. These mites prefer warm and humid environments, so they cannot survive in refrigerators or cooling systems (Cevizci et al., 2010).

Mites are used to ripen cheese as a very old and widespread tradition in France and Germany. In France and Germany, mites have a long tradition in cheese ripening. In Germany, cheeses such as Würchwitzer Milbenkäse or locally known as Spinnenkäse, Altenburger Ziegenkäse cheese, known as "spider cheese", and in France, various cheeses such as Mimolette and Artisou are produced by adding cheese mites called Tyrolichus casei and Acarus siro. While Milbenkäse cheese is ripened by Tyrolichus casei, cheeses such as Mimolette, Cantal vieux and Artisou are ripened by mites called Acarus siro. Cheeses ripened with mites often acquire a nutty, fruity taste and aroma (Shimano et al., 2022; Shimizu et al., 2022).

Thickening of the crust, color changes and appearance defects are observed in cheeses infected with mites. These parasites can cause economic damage and serious losses by carrying pathogenic microorganisms on the outer surface of the cheese to its interior (Teğin and Özer, 1971; Yaman et al., 2000).

Foodborne mites pose a major threat to human health. Cheese, peanuts, flour, cereals and other grain products are among the risky foods. These foodstuffs can become infested with many species of mites during the preservation process. These mite species are often called storage mites. Various mite species can be found in places containing nuts, tobacco, seeds, flour, grains, dried fruits and vegetables, animal feed, stored foods such as cheese, milk powder, sugar, pepper, and other organic residues. Some mites found in warehouse products are shown in Table 1. (Olsen, 1998; Mehlhorn, 2001; Thind and Clarke, 2001; Aygün, 2007a).

Table 1. *Some mites found in warehouse products*

Family name	Species name
Acaridae	*Tyrophagus putrescentiae*
	Tyrophagus longior
	Thyreophagus entomophagus
	Acarus siro
	Acarus farris
Suidasiidae	*Suidasia pontifica*
Pyroglyphidae	*Dermatophagoides pteronyssinus*
	Dermatophagoides farine
	Euroglyphus maynei
Glycypagidae	*Glycyphagus domesticus,*
	Lepidoglyphus destructor
	Gohieria fusca

Cheese is an extremely delicate food item. While the mites feed and multiply on the surface of the cheese, the dead mites, shed skins, eggs, secretions and food residues accumulate on the surface of the cheese and appear as a layer of light brown dust with a sharp mint smell. In severe infestations, this deposit may be 2 cm or more thick. If control measures are not taken, weight loss of cheeses can increase by 25% due to mite growth on cheeses (McClymont Peace, 1983). This may cause significant damage to mite-infested cheeses, leading to serious economic losses (Yaman et al., 2000).

Inflammatory symptoms and the development of the immune response in individuals exposed to mite allergens are due to the protease, phosphatase, esterase, aminopeptidase and glycosidase enzymes found in high levels, especially in the feces. Food allergies can pose a high risk of morbidity by affecting individuals through inhalation. Although this situation usually occurs in the work environment, it actually often arises from non-work environments such as home, school, grocery store or market. These sometimes seemingly insignificant allergic reactions can cause severe, life-threatening clinical symptoms, especially if a strong predisposing factor such as asthma is added (Morgan and Arlian, 2006; Ramirez and Bahna, 2009).

The most common symptoms in humans include severe gastrointestinal disorders such as dermatitis, conjunctivitis, acute enteritis and diarrhea, urinary tract disorders, and various allergic reactions, including systemic anaphylaxis (Aygün et al., 2007b).

There is no established safety limit or legal limit to protect against allergic reactions or sensitization caused by mites in food. It is stated that 75 mites that can be found in one hundred grams of canned mushrooms or 15 grams of dried mushrooms are harmless for individuals who are not sensitive to mite allergens (Olsen, 1998). However, considering that mites cause IgE-related reactions in humans, it is stated that individuals who are sensitive to mite allergens may be at risk when they consume foods contaminated with mites (Olsen, 1998; Umur, 1995).

Diseases and clinical findings caused by some mite species are shown in Table 2 (Cevizci et al., 2010).

Table 2. *Diseases and clinical findings caused by mite species in some occupational groups*

Mite species	Occupational group	Disease/Clinical finding
Lepidoglyphus destructor	Grain workers	Allergy
Lepidoglyphus destructor	Silo workers	Allergy
Lepidoglyphus destructor	Seed warehouse workers	Cough, cold
Lepidoglyphus destructor	Grain workers	Allergy
Lepidoglyphus destructor	Grain workers	Asthma
Acurus siro	Grain workers	Allergy
Acurus siro	Seed warehouse workers	Cough, cold
Acurus siro	Silo workers	Allergy
Glycyphagus domesticus	Seed warehouse workers	Cough, cold
Tyrophagus putrescentiae	Silo workers	Allergy
Tyrophagus putrescentiae	Seed warehouse workers	Cough, cold
Tyrophagus putrescentiae	Grain workers	Allergy
Chortoglyphus arcuatus	Grain workers	Allergy

Turkey is a country that varies according to its regions in terms of cheese production and consumption (Tiğin and Özer, 1971). Studies on mites seen in cheese in Turkey are systematically given in Table 3.

Table 3. Studies on mites seen in cheese in Turkey

Cheese type	Mite type	Researchers
Kashar cheese	*Tyraglyphus farinea*	Mimioğlu (1959)
Kashar cheese	*Tyraglyphus farinea*	Oytun (1969)
Kashar cheese	*Acarus siro ve Caloglyphus rhizoglyphoides*	Tiğin and Özer (1971)
Kashar cheese	*Acarus immobilis, Tyrophagus longior and Glycophagus domesticus*	Çobanoğlu and Toros (1988)
Aged kashar cheese	*Acarus siro* (%85)	Umur (1995)
Moldy cheese and tulum cheese	*Acarus siro* (%10.34) and *Acarus siro* (%3.27)	Yaman et al. (2000)
Civil cheese	*Acarus siro* (%0.05)	Aygün et al. (2007a)
Cottage cheese	*Tyrophagus putrescentia*	Aygün et al. (2007b)
Kashar and tulum cheese	*Acarus siro* (%0.88)	Karatepe et al. (2017)
Kashar and tulum cheese	*Acarus siro* (%0.84)	Karadere and Karatepe (2019)
Kashar and tulum cheese	The mite could not be detected.	Solmaz and Karatepe (2020)

Considering that foods may be contaminated with mites under improper storage conditions, it is recommended that the environmental humidity be kept below 60% and the humidity rate of the stored food should be below 13.4% to prevent this contamination. Additionally, it is stated that various methods such as radiation application, vacuum packaging or modified atmosphere storage can be used to prevent food contamination with mites (Hill, 2002). In addition, it is stated that storing food in the refrigerator is a precaution that can prevent the proliferation of storage mites (Matsumoto and Satoh, 2004).

Hazard Analysis of Critical Control Points, one of the food safety measures defined as "Hazard Analysis at Critical Control Points" in Turkish, consists of the initials of the phrase HACCP, is a protective and preventive food

safety system that aims at food safety from field to table and prevents potential dangers in these processes before they ocur (Baş et al., 2007).

HACCP is a reliable method to ensure food safety and offers many advantages to businesses and consumers. These advantages can be listed as follows:

a) It allows safe food production.

b) Raises awareness of product safety by training business personnel on hygiene and HACCP.

c) It ensures that critical tests are carried out on-site and quickly.

d) It contributes to keeping records and documentation organized in the business.

e) It can correct sudden changes in production parameters without causing product loss, which reduces the risk of defective products, ensures effective use of resources and reduces costs.

f) Prevents product security problems.

g) It increases the marketing power of the product through economical production and provides competitive advantage in the market by gaining customer trust.

h) It reduces the economic losses of consumers and businesses against diseases caused by food.

i) It supports healthy food production.

j) HACCP practices ensure compliance of food production with European Union Directives, Codex Alimentarius standards and international legislation (Özçiçek, 2002; Altun, 2011).

In conclusion;

1. The importance of mites in terms of public health should be known and public awareness should be raised.

2. It is of great importance to train and raise awareness of the personnel working in the cheese production process, as they will be working in an environment where mites can easily reproduce.

3. Full compliance with HACCP rules must be ensured during the production, packaging, marketing and especially storage stages of cheese.

4. Defects in the appearance of mite-infested cheeses create negative economic effects.

5. The effects of cheese mites on public health should be known and the society should be informed about this issue.

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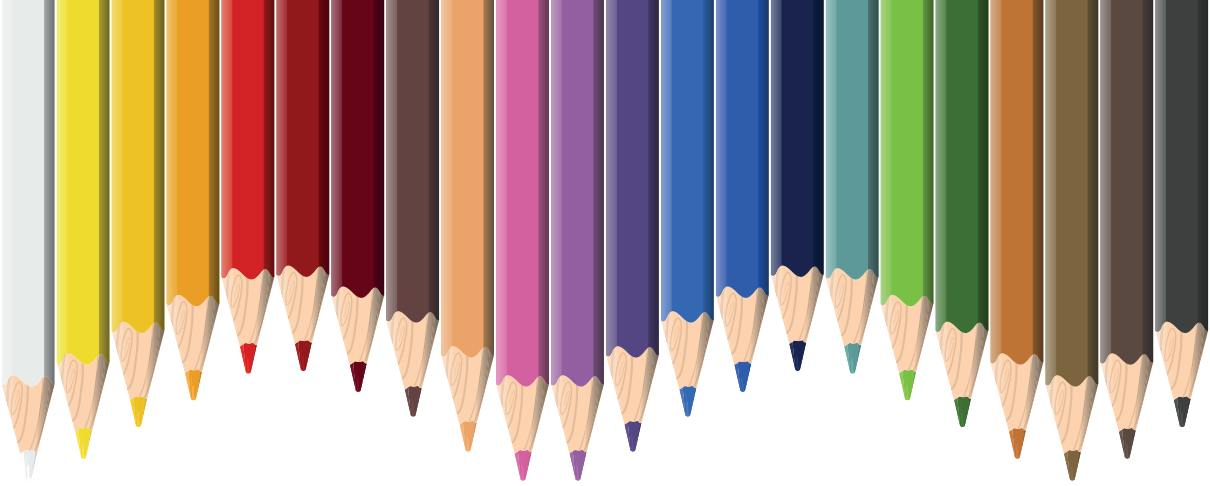

Chapter 13

EFFECTS OF TOTAL PARENTERAL NUTRITION SOLUTIONS BASED ON OLIVE OIL ON OXIDANT-ANTIOXIDANT SYSTEM AND ARTERIAL STIFFNESS

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Effects of Total Parenteral Nutrition Solutions Based on Olive Oil on Oxidant-Antioxidant System and Arterial Stiffness

INTRODUCTION

Malnutrition is one of the important causes of morbidity and mortality. Patients followed up in hospitals or palliative care centers are at high risk of malnutrition development. If the patient is not given timely and sufficient nutrition support, energy and protein deficiency is inevitable (1). For patients with functional gastrointestinal system, preferred feding route is the enteral nutrition. But in patients whom enteral nutrition is not applicable or insufficient, parenteral nutrition (PN) should be used. PN solutions contain water, carbohydrates, amino acids, lipids, electrolytes, trace elements, vitamins and other additives. These nutrition solutions can be soybean oil-based or olive oil-based (%80 olive oil, %20 soybean oil content).

The studies conducted on this topic generally discuss the protective effects of lipids containing olive oil, consumed through diet, on the cardiovascular system (2,3,4). In addition to the metabolic beneficial effects and anticarcinogenic effects of the Mediterranean diet and olive oil consumption in particular, it has been emphasized that it reduces the risk of coronary heart disease. After regular infusions of emulsions from soybean, a decrease in the plasma alpha-tocopherol level and a decrease in the antioxidant capacity have been observed, whereas the fatty acid pattern in the cell membranes has not changed with the emulsions formed by mixing olive and soy oil in an 80/20 ratio. It has been stated that, especially in cases where they are enriched with alpha-tocopherol, oxidative damage can be more easily prevented (5). In this study, we aimed to investigate the effects of olive oil-based nutrition solutions on arterial stiffness and total antioxidant-oxidative system in patients who received olive oil-based total parenteral nutrition (TPN).

MATERIAL AND METHODS

This prospective study included patients who receive TPN in intensive care unit of Ahmet Nejdet Sezer Research and Application Hospital of Afyon Kocatepe University between 2013 and 2014. Patients who were between 20 and 75 years old and need TPN due to malnutrition were evaluated. This study was approved by the ethical committee of our instution. The parenteral nutrition solutions contained the same sources in terms of composition of carbohydrates, fats, proteins, minerals and trace elements. All patients were given Oliclinomel® TPN solution containing 80% olive oil and 20% soybean oil. Parenteral nutrition was administered in the form of 24-hour infusion according to the total calorie needs of the patients calculated by Harris-Benedict formula. Our patients constituted a single group and we applied the TPN pro-

tocol based on olive oil to this group. We planned to determine the changes from day 0 to day 1 and day 3 of TPN.

Measurement of Total Oxidant Status and Total Antioxidant Status

To determine the effects of nutrition solutions based on olive oil, which are thought to have antioxidant effects, serum total antioxidant capacity (TAS) and serum total oxidant capacity (TOS) values were recorded on the 0th, 1st and 3rd days after parenteral nutrition was initiated. The 0th day measurements were taken immediately before the treatment started, and the 1st and 3rd day measurements were taken at the same time as the 0th day measurements.

TOS: Venous blood was taken from the individuals and centrifuged in a cooled centrifuge (Nüve NF 800 R, TR). The obtained serums were stored at -20°C. The collected serums were evaluated with a single measurement.

TOS: The Total Oxidant Status (TOS) Assay Kit (Rel Assay Diagnostics, TR) was used for the measurement of the total oxidant status.

TOS = μmol H2O2 Equiv/L

TAS: The Total Antioxidant Status (TAS) Assay Kit (Rel Assay Diagnostics, TR) was used for measuring the total antioxidant status.

TAS = mmol Trolox Equiv/L

Oxidative stress index (OSI): Obtained by dividing TOS value by TAS value and multiplying the result by a constant of 100.

OSİ: TAS/TOS X 100

Arterial Stifness Measurement

Arterial stifness measurement was done by Bio Clip Plus (International Antiaging Systems, USA), a finger device that works with pulse wave analysis. The test was performed in an environment free of noise and with appropriate temperature. The device was prepared for use by entering the person's name, surname, actual date of birth, height, weight, blood pressure and daily smoking amount into the program. Day 0 measurements were taken before starting TPN, while the day 1 and 3 measurements were taken at the same hour as day 0. Three seperate measurements were taken from the patients and the best results were evaluated. The measurement was done by attaching the finger device to the second finger of the right hand of the person in a lying position at the same level as the heart. After short pulse wave recording (10-30 seconds) with the Bio Clip Plus finger device, the system calculated the person's average pulse rate, stiffness index (SI), reflection index (RI), vascular age (VA) and oxygen saturation (SPO2). The device was able to automatically generate typical pulse

waves and show the SI aortic reflective wave speed in m/s. A decrease in SI value is accepted as an indication of decreased arterial stiffness. The faster the wave, the stiffer the artery. If the speed is less than 6 m/s, it is very good, 6-9 is good, 9-12 is normal, 12-15 is bad, and more than 15 is very bad.

Statistical Analysis of Data

Descriptive statistical methods (Mean, Standard deviation) were used to evaluate the study data, and Student t test was used for the comparison of parameters that showed normal distribution between groups, while Mann Whitney U test was used for the comparison of parameters that did not show normal distribution between groups. Paired-t test was used to analyze the changes between days for the parameters. Pearson correlation test was used to analyze the relationships between parameters. The results were evaluated at 95% confidence interval, with significance level $p<0.05$.

DISCUSSION

In our study, we did not find a significant difference in SI, RI, VA parameters for assessing arterial stiffness in 35 patients. This situation may be due to the progression of the existing infection tables on day 3, or due to the frequent immobilization of the patients and the presence of comorbid conditions that could affect arterial stiffness. The study conducted by Pilor et al. showed that feeding with 500 mg olive oil extract for 11 days caused a decrease in arterial stiffness compared to placebo. They have linked this to an increase in high-density lipoprotein levels in blood and a decrease in triglyceride levels (6).

We investigated the effects of the olive oil-based lipid emulsion used on antioxidant and oxidant system through TAS, TOS, and OSI parameters. As a result of our study, no significant changes were observed in the TAS, TOS, and OSI values both in 35 patients evaluated for day 1 compared to the baseline and in 25 patients evaluated for the day 3 compared to the baseline. In previous studies, statistically significant increase in TAS levels were detected. In one of these studies, olive oil, soybean oil, saline, and fat-free nutrition solutions were applied to 12 healthy individuals for 24 hours by infusion. They reported a significant decrease in the oxidative parameters, glutathione redox potential, in the 12th and 24th hours in the patients receiving olive oil-based parenteral nutrition infusion (7). In an another study, Demirer and colleagues conducted a study to investigate the effects of different fat emulsions with parenteral nutrition in patients undergoing major abdominal surgical interventions, using medium chain triglycerides/ long chain triglycerides, soybean oil / olive oil, soybean oil / olive oil / fish oil as fat sources, and evaluated the patients in terms of inflammatory response and antioxidant capacity (8). After a minimum of four days of nutrition, they found that OXLDL3 levels, which was

used to assess antioxidant changes, were significantly lower and the TBARS levels, which was used to evaluate plasma antioxidant capacity and is a marker of lipid peroxidation, were significantly higher in the group using lipid emulsions containing a mixture of 20% soybean and 80% olive oil. They noted that soybean oil / olive oil-based lipid emulsions had beneficial effects in patients undergoing major abdominal surgery considering the inflammatory response and antioxidant capacity (8).

Katerina and colleagues recently conducted the Olivous study in Australia, which included 50 healthy individuals. As a result of using 60 ml/day high density olive oil for 3 weeks, they detected a significant decrease in ox-LDL and hs-CRP levels and slight increase in total antioxidant capacity level. (9)

We did not detect a significant change in serum TAS, TOS and OSI values after nutrition with olive oil-based TPN solution. The results of our study were different from the results of Demirer's , Siqueira's and Katerina's studies. The lack of findings in our study that olive oil-based nutrition increases antioxidant capacity as shown in previous studies may be due to the presence of multiple comorbidities such as diabetes, hypertension, congestive heart failure, the addition of infection signs that were not present at the beginning of the treatment. If our study had a longer duration, in other words, if the patients had been able to receive olive oil-based TPN therapy for a longer period of time and if the infection that developed during the follow-up period could be excluded, we could have obtained different results. Nevertheless, our findings were different from the examples in the literature in terms of the effects of olive oil-based nutrition on the oxidative-antioxidant system. We hypothesized that this could be related to comorbid conditions such as infection, limited mobilization of patients, associated diabetes and hypertension, which compromise vascular structure and elasticity. We also think that one of the reasons why the results of the data we obtained did not match those of the studies in the literature could be due to the short duration of our nutrition support.

Olive-based TPN may reduce arterial stiffness in patients who need PN. But, in order to show the beneficial effects of olive-based solutions, studies with larger and homogenized sample size, and with longer follow period were needed.

RESULTS

Thirty five patients were included in this study. Average age was 61.71 ± 15.34. There were 16 (45.7%) females and 19 (54.3%) males. The patients had comorbidities such as hypertension (37.1%), malignancy (28.6%), diabetes (25.7%), coronary artery disease (20%), chronic obstructive pulmonary disease (17.1%), and congestive heart failure (2.9%).

There were 2 or more comorbidities in 15 (42.9%) patients while there was only one comoborbidity in 17 (48.6%) patients. Three patients had no comorbidity. There was not any significant change in terms of TAS, TOS, OSI, SI, RI, VA between day 0 and day 1 (Table-1).

Out of the 35 patients, 10 were excluded since their treatment could not reach to day 3. Data from the remaining 25 patients on day 3 day was evaluated. There was not any significant change in terms of TAS, TOS, OSI, SI, RI, VA between day 0 and day 3 (Table-2).

Table-1: Comparisons between day 0 and day 1 in terms of TAS, TOS, OSI, SI, RI, VA, systolic BP, diastolic BP and arterial oxygen saturation.

Parameters	0th day	1th day	P value
TAS	2.36±1.70	2.45±1.53	0.782
TOS	8.96±4.58	8.67±3.74	0.776
OSİ	590.43±500.23	494.76±356.69	0.229
SI	9.62±1.45	9.55±1.57	0.694
RI	58.97±13.34	58.31±15.01	0.734
VA	57.37±12.14	57.05±12.94	0.617
Systolic blood pressure	114.57±19.19	119.14±24.89	0.199
Diastolic blood pressure	68.14±11.50	70.00±13.71	0.402
SpO2	93.02±3.32	93.65±2.66	0.240

Table-2: Comparisons between day 0 and day 3 in terms of TAS, TOS, OSI, SI, RI, VA, systolic BP, diastolic BP and arterial oxygen saturation.

Parameters	0th day	3rd day	P value
TAS	2.18±1.50	2.41±1.60	0.552
TOS	8.52±3.53	8.09±3.26	0.656
OSİ	607.08±534.63	434.08±234.23	0.093
SI	9.77±1.51	10.15±2.72	0.437
RI	61.64±12.34	62.64±13.69	0.678
VA	58.72±11.93	59.32±14.65	0.604
Systolic blood pressure	116.80±19.08	114.88±19.33	0.711
Diastolic blood pressure	69.00±12.24	67.60±10.51	0.596
SpO2	93.16±3.55	93.60±3.68	0.573

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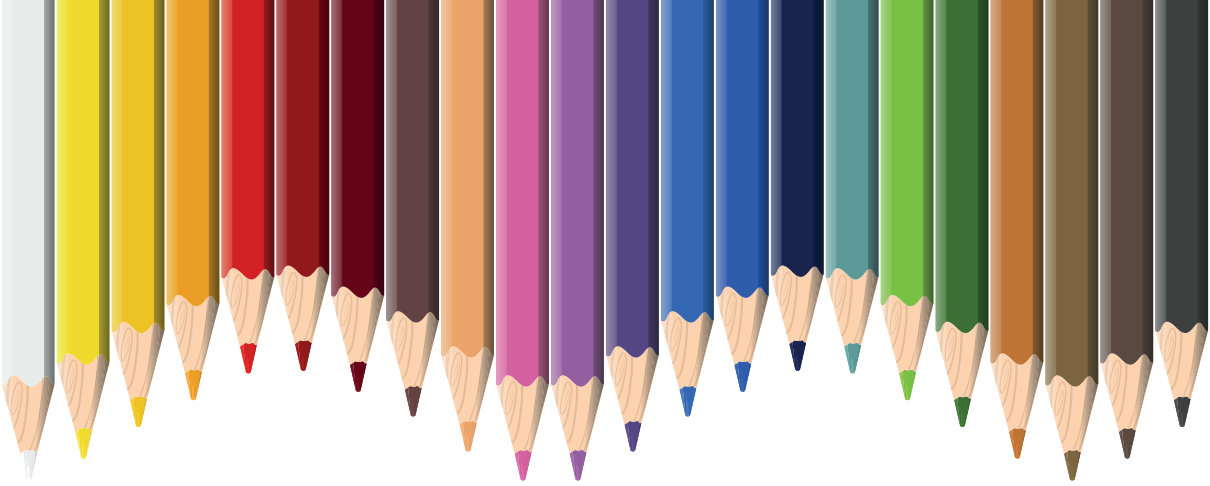

Chapter 14

ROLE OF KERATINOCYTES AND IMMUNE CELLS AS KEY ACTORS IN PSORIASIS

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Role of Keratinocytes and Immune Cells as Key Actors in Psoriasis

Psoriasis as a chronic and autoimmune disorder

The development and maintenance of psoriatic inflammation are due to concern with the skin's innate and adaptive immune responses. The innate immune system is activated by endogenous danger signals and cytokines. In some patients, this situaition can show up as autoinflammatory and T-cell-driven autoimmune reactions. So, psoriasis has all the signs of an autoimmune disease on top of a (auto)inflammatory background, with causing the disease worse. In psoriasis, immune system cells, especially T cells, become overactive, and attack the body's own cells in a way that isn't normal, which leads to a constant inflammatory response (Guo et al., 2023). In a healthy body, T cells fight off infections. But in psoriasis, these cells attack healthy skin cells because they are damaged or sending the wrong signals, and causes skin cells to divide quickly and out of control, which is called keratinocyte proliferation, and causes thick, scaly plaques to form on the skin. When immune cells like T cells are activated, they release chemicals (cytokines) that cause inflammation. These cytokines help skin cells divide quickly and keep the inflammatory response going (Mateu-Arrom & Puig, 2023). Because of all of these things, psoriasis is considered an autoimmune disease.

Pathogenesis process of psoriasis

Understanding how psoriasis starts is important for treating and managing this complicated and multifactorial condition. Psoriasis is a long-lasting skin disease that is caused by inflammation and can be affected by both genetic and environmental factors (Zhong, Luo, Zhong, Xu, & Hao, 2022). By understanding the pathogenesis, we can learn more about the underlying causes and mechanisms of the disease, and helps us come up with better ways to treat patients and make their lives better overall. When someone gets psoriasis, their innate and acquired immune systems, as well as genetic and environmental factors, work together in a complicated way (Sonkodi, 2022). The innate immune response is the body's first line of defense against pathogens and damage to tissues. In psoriatic patients, this response can be set off by cuts, infections, some medicines, or even stress (Lee, Park, Van Kaer, & Hong, 2022). Keratinocytes, which are the main type of cell in the epidermis, are very important indicators and drivers in pathogenesis of psoriasis. These cells can be set off by different things, and the production of cytokines and chemokines causes inflammation (Pasquali et al., 2019). Keratinocytes that are activated release TNF-α, IL-1β, IL-6, and antimicrobial peptides such as LL-37. More immune cells, like dendritic cells and neutrophils, are drawn to activated by these mediators (Ayala-Fontánez, Soler, & McCormick, 2016). Dendritic cells in the skin become active and move to lymph nodes in people with psoriasis. In that place, they show antigens to T cells, which connects the natural and

learned immune systems. Dendritic cells turn on naïve T cells in the lymph nodes. Certain types of cells, especially Th1 and Th17, are overactive in psoriatic inflammation process. Th17 cells make IL-17, IL-22, and IL-23, while Th1 cells make interferon-gamma (IFN-γ) (Kim & Krueger, 2015). These cytokines are crucial in psoriasis, due to cause inflammation and keratinocytes hyperproliferation. The acquired immune response in psoriasis is mostly driven by T cells, but B cells and autoantibodies also play a part in some people. Both T cells and keratinocytes continue to produce proinflammatory cytokines, which drive the inflammation cycle. This leads the immune system to activate more and results in the formation of psoriatic plaques (Bos, De Rie, Teunissen, & Piskin, 2005; Coimbra, Figueiredo, Castro, Rocha-Pereira, & Santos-Silva, 2012; Lorscheid et al., 2019).

Role of keratinocytes in the psoriasis

In the pathogenesis of psoriasis, alterations in the keratinocytes and their abnormal behaviors are fundamental features of the disease, contributing to the characteristic appearance of psoriatic lesions. Patients with psoriasis exhibit rapid proliferation of keratinocytes (Bos et al., 2005). Normally, the maturation of keratinocytes to form the skin's outermost layer (epidermis) takes weeks. In psoriasis, this process can shorten to a few days, leading to the formation of thick, scaly lesions on the skin's surface (Baadsgaard, Fisher, Voorhees, & Cooper, 1990). Activated keratinocytes produce proinflammatory cytokines (TNF-α, IL-1, IL-6) and chemokines, attracting and activating more immune cells (T cells, dendritic cells) to the skin region. These cytokines and chemokines trigger the immune response in the skin and play a significant role in sustaining psoriatic inflammation (Ni & Lai, 2020). Signaling pathways such as NF-kB and STAT in keratinocytes are known to be excessively activated. These pathways are associated with inflammation and cell proliferation. TGF-β plays a role in the normal differentiation of skin cells and regulation of immune responses. Irregularities in this signaling pathway have been reported in psoriasis patients (Guo et al., 2023; Kodali, Blanchard, Kunamneni, & Lebwohl, 2023; Takahashi & Yamasaki, 2020). Accelerated cell proliferation and disrupted differentiation lead to a weakened epidermal barrier, making the skin more susceptible to environmental irritants and pathogenic microorganisms. Due to the central role of keratinocytes in psoriasis pathogenesis, changes in these cells play a crucial part in the development of clinical features and psoriatic plaque lesions. The disease can be treated in various ways, including inducing these changes. Research in this field contributes to developing innovative approaches for managing psoriasis (Takahashi & Yamasaki, 2020; Vijayapoopathi et al., 2023). Furthermore, imbalances in the proliferation of keratinocyte stem cells, reductions in lipids and keratohyalin granules in keratinocytes, and rapid proliferation

of mature keratinocytes critically escalate psoriasis pathogenesis. Keratinocytes play an active role in macroscopic clinical findings, including the visible formation of psoriatic plaques in the skin's outermost layer. However, the development of the psoriatic plaque is not limited to epidermal inflammation but is shaped by the interaction of keratinocytes with various cell types in the dermal layer (innate and adaptive immune cells, vascular system). The pathogenesis of psoriasis can be characterized by an initial trigger phase, induced by trauma (Koebner phenomenon), infection, or drugs, followed by a chronic progression phase. Microscopic findings in psoriatic plaques show inflammatory infiltrates composed of dermal dendritic cells, macrophages, T cells, and neutrophils overlying acanthosis (epidermal hyperplasia) (Liu et al., 2022; Sonkodi, 2022; Takahashi & Yamasaki, 2020; Wu, Dai, & Zeng, 2023; Zhang et al., 2023). Neovascularization is another prominent and significant feature. The degree of histological changes in psoriatic inflammation is influenced by the severity, duration, and specific signaling pathways involved in disrupting natural and adaptive cutaneous immune responses (Ghaffarinia et al., 2023).

Role of Immune Cells in the Psoriasis

Psoriasis is classified as an inflammatory disorder that is sustained by regulatory T (Treg) cells and subsets of T cells (Th1, Th2, Th17, and Th22 cells). The contribution of these T cells to the pathogenesis of psoriasis is significant in its development and progression (Albanesi, Madonna, Gisondi, & Girolomoni, 2018). A multitude of recent discoveries concerning the role of T cells in disease progression have prompted a departure from the conventional understanding of psoriasis as a skin disorder mediated by T-helper (Th)1. Consequently, the functions of T cells in psoriasis have to be reassessed (Kim & Krueger, 2015).

Activation of T Cells

A critical question that has intrigued researchers in the context of psoriasis, an autoimmune skin disease mediated by T cells, is understanding how pathogenic T cells are activated during disease development. The close relationship between streptococcal infection and psoriasis has highlighted streptococcal antigens as primary candidates for activating T cells (Ji et al., 2023; Kim & Krueger, 2015; Kuczyńska, Gabig-Cimińska, & Moskot, 2023). According to several studies, the concept of superantigen has been proposed as the initial target molecule due to its limited T-cell receptor (TCR) Vβ usage in both the peripheral blood and lesions of patients with psoriasis. Additionally, streptococcal exotoxins have the ability to stimulate T cell expression of cutaneous lymphocyte-associated antigen, a skin homing receptor (Sato, Ogawa, & Okuyama, 2020). Nevertheless, the presence of oligoclonal T-cell proliferation in psoriasis lesions and the examination of TCR utilization by

distinct T cell groups infiltrating the lesions suggest that the local lesion elicits an antigen-specific T-cell response. Consistent presence of the same T-cell clone despite recurrent disease and the detection of preserved clonal TCR rearrangements in skin lesions from various patients indicate that chronic psoriasis is regulated by T cells that are sustained by a common antigen(s) (Lorscheid et al., 2019; Qiao et al., 2019).

In conjunction with the high structural similarity between streptococcal M protein and type I keratins, these results suggest molecular mimicry or disease imitation as a new theory regarding psoriasis. The concept of molecular mimicry explains that anti-streptococcal T cells can become activated against specific tissue antigens in the skin, consequently causing disease (Ayala-Fontánez et al., 2016; Kim & Krueger, 2015). Moreover, in contrast to healthy controls, peripheral blood T cells derived from individuals with psoriasis have demonstrated an IFN-γ-producing response to multiple synthetic peptides that correspond to these homologous sequences (Baliwag, Barnes, & Johnston, 2015). Further research has demonstrated that CD8+ T cells in the peripheral blood of psoriasis patients carrying the HLA-Cw6 allele exhibit a substantial IFN-γ response when exposed to peptides derived from the shared sequence of keratin 17 and M6 protein, which also predicted HLA-Cw6 binding (Morelli et al., 2021). The vast majority of the responding cells display the cutaneous lymphocyte-associated antigen determinant, which constitutes over 90% of the total. Autoantigen candidates that have been investigated include Heat Shock Protein 27 (HSP27), Ezrin, Maspin, and Peroxiredoxin 2 (Wang & Jin, 2019). These candidates have been identified as streptococcal protein homogeneous and have been reported to react with the sera of psoriasis patients (Besgen, Trommler, Vollmer, & Prinz, 2010).

Nevertheless, research conducted by Fry et al. (2015) indicates that recombinant M protein did not induce proliferation in T-cell lines derived from lesional dermis, suggesting that streptococcal M protein might not be the target of these cells. On the contrary, a minimum of 50% of the Th1 cells that select for the streptococcal cell wall in psoriasis lesions do so in response to streptococcal peptidoglycan (PG), which serves as the primary constituent of the cell wall of Gram (+) bacteria (Fry, Baker, Powles, & Engstrand, 2015). An elevated count of macrophages harboring streptococcal PG has been detected within dermal lesions, specifically in dermal papillae or clusters of dermal T cells. PG is commonly recognized for its potent pro-inflammatory effects in chronic inflammation, which it achieves by interacting with pattern recognition receptors on dendritic cells (DCs) and monocytes (Toll-like receptor 2, nucleotide-binding oligomerization domain 1 and 2, and PG recognition proteins 1-4). It is noteworthy that the genes responsible for encoding these PG recognition receptors are all situated in linkage regions that are linked to psoriasis (Lewis, Chan, Hinojosa, Hsu, & Feldman, 2019; Valdimarsson,

Thorleifsdottir, Sigurdardottir, Gudjonsson, & Johnston, 2009). A group has postulated, on the basis of these results, that PG serves as the principal etiological agent in psoriasis and that a modified innate response targeting PG could potentially facilitate the activation and proliferation of pathogenic T-cells within the psoriasis lesion. Nevertheless, the inability of the results to adequately elucidate the function of CD8+ T-cells in psoriasis has led to the conclusion that this factor is crucial in the pathogenesis of psoriatic lesions (Baker et al., 2006). In their recent study, Valdimarsson et al. (2009) shed light on the possible involvement of CD4+ and CD8+ T cells in the pathogenesis of psoriasis lesions, as well as the mechanism by which streptococcal infection initiates and maintains the condition (Valdimarsson et al., 2009). Furthermore, research has shown that streptococcal CpG DNA, not streptococcal PG, can stimulate the proliferation and activation of peripheral T cells derived from individuals with psoriasis when exposed to streptococcal antigen. This finding underscores the comprehensive role that streptococcal antigen, and specifically streptococcal DNA, play in the development of psoriasis (Ying Luo et al., 2021). Controversial are the (auto)antigens that are accountable for the activation of psoriatic T cells. Future research focusing on the discovery of the autoantigen that triggers psoriatic T cells could have a significant influence on the treatment of the disease, potentially leading to the creation of a vaccine therapy (Ni & Lai, 2020).

Formation of Th1 and/or Th17 Cell-Mediated Chronic Inflammation

For decades, psoriasis has been characterized as a skin inflammation induced by Th1 and Th17 cells. The classification of T cells as Th1 or Th2 cells, based on the release of signature cytokines IFN-γ and interleukin (IL)-4, has been in place for over 30 years (Sato et al., 2020). Psoriatic plaques and the peripheral blood of psoriatic patients have been found to contain elevated quantities of CD4+ Th1 and CD8+ cytotoxic T cells type 1 (Tc1), which are known to produce cytokines including IL-12, TNF- and IFN-γ. As a result, it is generally accepted that the interaction between T cells and DCs results in the secretion of substantial quantities of Th1-type cytokines, which establish a «type 1» inflammatory milieu and contribute to the progression of psoriasis (Kodali et al., 2023; Purzycka-Bohdan et al., 2022; Sato et al., 2020). Psoriasis has been unequivocally classified as a Th1 cell-mediated disease in light of these results.

Th17, a novel subpopulation of CD4+ Th cells that generate IL-17, has been identified and demonstrated to function in models of autoimmune and inflammatory diseases (Liu et al., 2022). It is well-established that IL-23 is a crucial cytokine for the development and maintenance of Th17 cells in both mice and humans. IL-23p19, a heterodimeric cytokine, is composed of a distinct subunit that is combined with IL-12p40, which is also present in IL-12 (Levin & Gottlieb, 2014). Th17 cells and associated cytokines, IL-17A, IL-17F, IL-22, IL-21, and IL-26 in critical, are essential in the pathogenesis of numer-

ous chronic inflammatory diseases, including psoriasis. Elevated quantities of Th17 cells and their effector molecules have been identified in the circulation and skin of psoriatic patients. Furthermore, in comparison to lesion-free and normal skin, psoriatic skin lesions contain elevated levels of IL-23 mRNA and IL-23 protein. IL-23 is predominantly produced by activated macrophages and DCs (Benhadou, Mintoff, & Del Marmol, 2019; Teng et al., 2021). An investigation was conducted wherein the treatment of IL-23 or IL-21 via intradermal injection to mice induced keratinocyte proliferation and resulted in epidermal hyperplasia (acanthosis), a characteristic that is particularly conspicuous in human psoriasis (Mylle, Grine, Speeckaert, Lambert, & van Geel, 2018; Rizzo et al., 2011). In addition, CC chemokine receptor (CCR) 6, IL-22, and IL-17A are all necessary for IL-23-induced psoriasiform skin inflammation. Moreover, in the mouse psoriasiform model induced by topical administration of the Toll-like receptor 7/8 agonist imiquimod (IMQ), IL-22 is indispensable (Zhong et al., 2022). IL-17A has been implicated in the upregulation of keratin 17 expression in keratinocytes, a characteristic feature associated with psoriasis. Contributing to the psoriatic phenotype, IL-17A and IL-22 increase the expression of antimicrobial peptides in keratinocytes in a synergistic manner. These peptides regulate distinct pathways of inflammation and keratinocyte response (Zhang et al., 2023). As previously discussed, the provided data tentatively support the classification of psoriasis as a Th1 cell-mediated disease, by demonstrating the involvement of the IL-23/Th17 pathway in its characterization (Wu et al., 2023).

A novel paradigm has surfaced suggesting that Th17-type T cells, in addition to the Th2-type immune system, may play a role in autoimmunity and chronic inflammation. It has been demonstrated in animal models that this novel subset of T cells, which is capable of producing IL-17 and IL-22, is crucial in the pathogenesis of autoimmune inflammatory diseases. These cells are thought to have a unique population of T helper cells, which are crucial for adaptive immunity mediated by CD4+ T cells. IL-1, IL-6, and transforming growth factor-β (TGF-β) are mediators of the Th17 immune system. These factors promote the transformation of naive CD4+ T cells into activated memory Th17 cells (Furue, Furue, Tsuji, & Nakahara, 2020; Ghaffarinia et al., 2023). It has been reported that the production of IL-6 and IL-23 are associated with the induction of Th17 cells, whereas IL-12 production appears to be necessary for the induction of Th1 cells (Furue et al., 2020; Ghaffarinia et al., 2023; Park, Gupta, Kim, & Dziarski, 2011; Rizzo et al., 2011). Together with TGF-β, IL-6 regulates a series of cytokine-dependent signaling pathways that promote the differentiation of Th17 cells that are RORt-dependent. It has been determined that orphan nuclear receptor RORγt is the initial transcription factor to be expressed exclusively in Th17 cells. IL-6 stimulates the production of IL-21, which in turn activates IL-21 and IL-23 receptors on naive CD4+

T cells (Elbana, Elgamal, Hashim, Emran, & Alkhrsawy, 2022). In vivo differentiation of Th17 cells requires the transcription factor signal transducer and activator of transcription 3 (STAT3), which is required for the effects of IL-6 and IL-21. In addition to other requirements, STAT3 is necessary for the production of RORγt. Through their interaction with STAT3, IL-21 and IL-23 stimulate RORγt, a factor that promotes the expression of IL-17 (Miyoshi et al., 2011; Sano et al., 2005). Characterized by the synthesis of IL-17, IL-17F, IL-6, and TNF-. T cells dependent on IL-23 exhibit a high degree of pathogenicity. At present, there is speculation that the development of inflammation in psoriasis is facilitated by the infiltration of Th1/Th17 cells into the skin, which induces dermal dendritic cells and macrophages to secrete mediators that induce aberrant keratinocyte proliferation (Rizzo et al., 2011; Zhou, Bian, & Wu, 2023). Potential therapeutic targets, Th17 cells are likely to be the proximal regulators of psoriatic skin inflammation.

IL-22-producing Th cells, a recent subpopulation of Th (Th22) cells that express CCR10, CCR6, and CCR4 and produce only IL-22 but not IL-17 or IFN-, have been identified (Di Cesare, Di Meglio, & Nestle, 2009; Nickoloff et al., 2000). A distinct subset of human skin-homing memory T cells, these cells play a role in pathology, epidermal immunity and remodeling, and skin homeostasis. Additionally, it has been documented that psoriatic patients have a higher concentration of Th22 cells in their bloodstream, in addition to Th1 and Th17 cells (Liu et al., 2022; Purzycka-Bohdan et al., 2022). Piskin et al. (2010) have demonstrated that psoriatic lesions contain an increased number of Tc17 (IL-17-producing CD8+ T cells) and Tc22 (IL-22-producing CD8+ T cells). The possibility exists that these Th22 and Tc22 cells originate from IL-17-producing cells (Th17 and Tc17 cells), which would indicate that these cells develop plasticity. This would preclude the classification of psoriasis as a condition mediated exclusively by a single subgroup of Th cells (Piskin et al., 2010). Conversely, every pathogenic Th cell that is capable of interacting with DCs, neutrophils, and other T cell subtypes establishes a persistent inflammatory environment that sustains psoriatic plaques (Luan, Ding, Han, Zhang, & Liu, 2014).

Subsets of T cells are not distributed uniformly within psoriasis lesions. A fraction of epidermal T cells that are CD8+ T cells migrate to the epithelium via the expression of integrin Eβ7, which is linked to E-cadherin and desmosomes. In contrast, dermal T lymphocytes consist of both CD4+ and CD8+ cells, with a CD4+ predominance comparable to that observed in peripheral blood (Caruso et al., 2009; Fry, Baker, & Powles, 2006; Luan et al., 2014). In psoriatic lesions, natural killer (NK)-T cells have been identified through immunohistochemistry (IHC) using NK surface markers; however, further comprehensive investigations are required to validate this finding. In most cases, T cell infiltration into skin lesions is quite extensive. For example, if an indi-

vidual with psoriatic lesions covering 20% of their body surface is assumed to have eight billion T cells in their bloodstream, the estimated number of T cells in the epidermis and dermis of psoriatic plaques would be twenty billion. Skin lesions contain the majority of memory T cells that express cutaneous lymphocyte antigen (CLA). CLA+ T lymphocytes comprise less than ten percent of the total T lymphocytes in circulation. As a result, inflammatory psoriatic lesions are impressively and selectively targeted by CLA+ T cells (Caruso et al., 2009; Chandra, Senapati, Roy, Chatterjee, & Chatterjee, 2018; Duan et al., 2020; Fry et al., 2006; Y Luo et al., 2020; Wu et al., 2023; Zhang et al., 2023).

Conclusion

Psoriasis is one of the most extensively studied chronic inflammatory skin diseases. There is not a significant hypothesis describing the unique mechanisms that elucidate the particular typical features of psoriasis. Conversely, the fluctuating behavior of psoriasis, with its remission and relapse phases, might be suggestive of its association with existing regulatory and effector immune mechanisms. Additionally, while the fact that activated T cells are crucial for the development and persistence of psoriatic lesions is undisputed, the pathophysiology of the disease can only be partly explained by the roles of keratinocytes and T lymphocytes. Other resident skin cells, including keratinocytes and dendritic cells, contribute to the development of psoriatic plaques. This cellular contribution is associated with strong and dynamic epigenetic effects. Various studies have indicated that structural defects in keratinocytes are fundamental to the development of psoriasis. A growing number of specific target molecules found in genes controlling proliferation and differentiation processes in the epidermis as well as T cell recruitment and keratinocyte inflammatory activation have been associated with the disease.

In the current review, we covered research on the pathophysiology of psoriasis within the framework of signaling between keratinocytes and immune cells. Our aim is to delineate how disordered immune responses that contribute to the development of psoriatic plaques and inflammation emerge from the axis of keratinocyte-immune cell interactions. Despite the precise sequence of events that leads to the formation of psoriatic plaques and the cytokine cascade remaining unknown, identifying early triggers and the roles of keratinocytes and T cells might provide novel, promising targets for the prevention and effective treatment of psoriasis.

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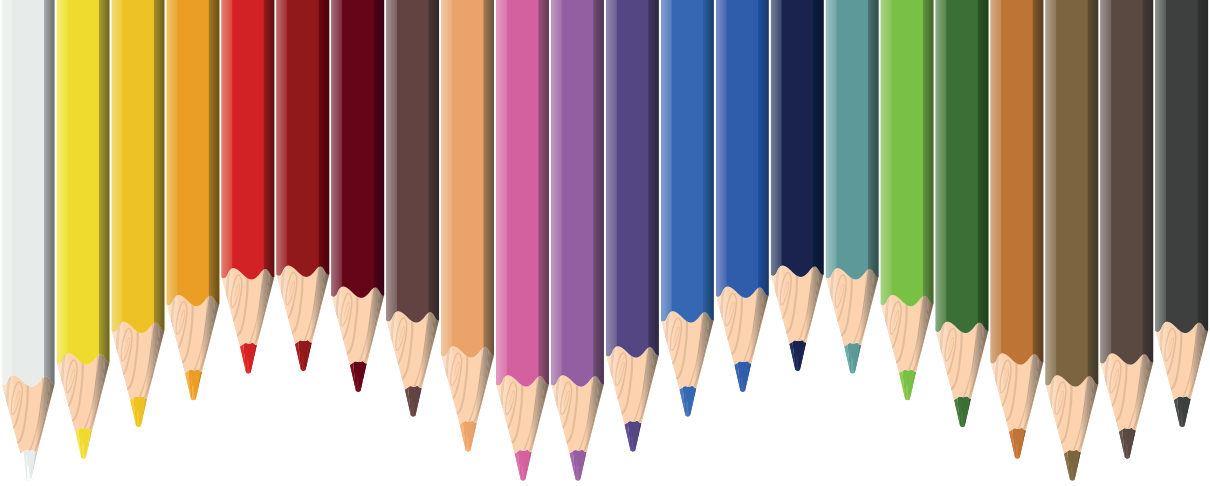

Chapter 15

CLINICAL FEATURES AND PSYCHIATRIC REFLECTIONS IN CHILDREN DIAGNOSED WITH CEREBRAL PALSY

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1. Introduction

Cerebral Palsy (CP) describes a neurological disorder that occurs in developing fetus, newborn or early childhood and has a permanent effect but is not progressive (Rosenbaum, vd.,2007). It first took its place in the literature in 1862 with the definition of spastic rigidity caused by prematurity and birth complications by Dr William Little. The name "Cerebral Palsy" was coined by Winthrop Phelps in 1947 (Morris, 2007). According to the 2004 international CP Definition and Classification Workshop, CP is defined as a permanent loss of motor function, posture and movement disorder that is non-progressive, inhibits movement, and occurs as a result of damage to the brain structure that is developing during the fetal or infantile period (Bax, vd.,2005). Posture disorders in which motor dysregulation is seen are common in infancy and early childhood, and although the clinical course varies with the development of the individual, the fact that the damage to the cerebrum, cerebellum and brainstem is not progressive are the four main elements that bring together the diagnoses. Along with movement disorders in CP; it may also be accompanied by cognitive deficiencies, language speech disorders, vision and hearing problems, convulsions, gastrointestinal disorders, oral and dental problems and psychiatric disorders (Rosenbaum, vd.,2007).

2. Epidemiology

It is reported that the biggest cause of physical disabilities in childhood is CP, and its prevalence varies in all countries (Graham, vd.,2016). The prevalence of CP was found to be 1.04-2.52/1000 live births in Europe and 2.11/1000 live births worldwide. In a study conducted in Turkey, the prevalence of CP was found to be 4.4/1000 live births (Oskoui, Coutinho, Dykeman, Jetté, Pringsheim, 2013; Serdaroğlu, Cansu, Özkan, Tezcan, 2006; Tecer, 2017). Studies have reported that the incidence of CP decreases every year in countries with high income levels (Novak, vd.,2017 & Galea, vd.,2019). It has been reported that improvements in intensive care conditions and advances in neonatal care and the survival of premature and very low birth weight babies have reduced neonatal mortality, while causing an increase in babies at risk of CP (Tecer, 2017). Although CP is a disease that affects both genders, it can be seen more frequently in boys than girls. In a study conducted in Turkey, the male/female ratio was found to be 1.54 and 1.33 in Europe (Fidan, Baysal, 2014 & SCPE, 2002).

3. Aetiology and Risk Factors

Many different mechanisms play a role in the development of CP, which is multifactorial and affects the developing brain or sometimes just the disease may cause cerebral palsy. While the aetiology cannot be revealed in some cases, it is known that 70–80% prenatal, 10–20% natal and 4 postnatal risk factors play role in the aetiology (Yakut, 2006). In a study investigating the

prevalence of CP in Turkey, prenatal causes were found to be 26.6%, perinatal/neonatal causes 18.5% and postnatal causes 5.9% in children aged 2-16 with CP, while 48.9% had unclassifiable causes (Serdaroğlu, vd.,2006). CP risk factors are listed in Table 1 (Sadowska, Sarecka-Hujar, Kopyta, 2020). In a study, it was reported that prenatal and perinatal causes were among the first etiological factors of CP with a rate of 88%, and perinatal asphyxia and/or the combination of prematurity with a rate of 74%. This has been reported that prenatal and perinatal risk factors are of critical importance in the development of CP, and perinatal risk factors can be reduced by improving prenatal and baby care conditions through close pregnancy follow-up (Kabakuş, vd.,2005).

Table 1. *Cerebral Palsy aetiology & risk factors (Sadowska, vd.,2020)*

Before pregnancy	**Prenatal**	**Natal**	**Perinatal – infant**
Presence of systemic disease in mother	Pregnancy bleeding	Prematurity	Respiratory distress syndrome
Medications, drug use	Placental anomalies	Use of vacuum-forceps	Intracranial haemorrhage
Immune system disorders before pregnancy (type 1 diabetes mellitus, celiac disease, Chrohn's disease, lupus disease, ulcerative colitis, multiple sclerosis, etc.)	Multiple pregnancy	Post maturity	Hypoxic ischemic encephalopathy
Infections	Systemic infection in mother	Placental infarction	Infections
Infertility treatment	Intrauterine	Asphyxia	Hyperbilirubinemia
Spontaneous abortion	Disturbance in fetal heartbeat	Meconium aspiration syndrome	Hypoglycaemia
Socio-economic factors	Tocolytic drugs	Chorioamnionitis	Convulsions
	Oligopolyhydramniosis	Abnormal presentation	Polistemi
	Intrauterine developmental delay	Low Apgar score	Coagulopathy
	Intrauterine hypoxia	Premature membrane rupture	Fluid-electrolyte imbalances
	Pregnancy toxaemia		Traumas
	Thrombosis		Poisonings
	Consanguineous marriage		Vascular events

4. Pathogenesis

CP develops as a result of damage to the first motor neurons in the cerebral cortex. While the first motor neurons initiate voluntary movement, they also act to suppress the functions of the second motor neuron in the spinal anterior horn. As a result of injury, the inhibitory functions of the first motor neurons are reduced. The stimuli coming from the cortex through the corticospinal and reticulospinal pathways decrease, muscle control is impaired and muscle tone increases.

As a result of this damage, the balance between upper and lower motor neurons is disrupted and the following clinical conditions occur (Yakut, 2008):

a) Since the upper motor neuron cannot act as a suppressor, movement control is impaired.

b) When there is hyperactivity, muscle tone and deep tendon reflexes (DTR) increase.

c) In case of hypoactivity, muscle weakness occurs and can often be confused with flaccid paralysis due to spinal cord damage or second motor neuron injury. Hypoactivity usually turns into hyperactivity in the future.

d) As a result of autoregulation disorder, temperature balance, respiration, swallowing, chewing, bowel and bladder functions and homeostasis are disrupted.

5. Clinic and Diagnosis

There is no specific diagnostic system for CP, clinical diagnosis results by taking a personal history by questioning risk factors before, during and after birth, without relying on etiological reasons.

Findings such as spasticity, involuntary, uncontrollable, repetitive, stereotypical movements, hyperkinesia and hypotonia, hypokinesia and hypertonia, and coordination disorder during movement are important in clinical classification (Vitrikas, Dalton, Breish, 2020). The signs and symptoms of CP are given in Table 2 (Brandenburg, Fogarty, Sieck, 2019).

Table 2. *The signs and symptoms of CP (Brandenburg, vd.,2019)*

Neurological	**Orthopaedic**	**Cognitive**	**Visual / Audio**	**Respiratory and Gastrointestinal System**
Spasticity	Femoral anteversion	Autism	Conductive hearing impairment	Aspiration pneumonia
Ataxia	Hip subluxation/ dislocation	Mental retardation	Cortical visual impairment	Dysarthria
Athetosis	Hip dysplasia	Epilepsy	Dyskinetic strabismus	Constipation
Dystonia	Joint contractures	Learning disabilities	High myopia	Gastroesophageal reflux
Gait disorder	Scoliosis		Retinopathy of prematurity	Obstructive sleep apnea
Hyper-reflexia	Tibial torsion		Sensorineural hearing impairment	Respiratory disorders (decreased forced vital capacity, forced expiratory volume, peak expiratory flow)
Hypotonia				Drooling
Impaired gross and fine motor coordination				

Although diagnosis can be challenging due to the variable and uncertain muscle structure before the age of 2, diagnosis can be made in the first years of life as a result of anamnesis and detailed examination.

Predictive tools to detect CP risk in infants ≤5 months (corrected age); neonatal brain MRI (86-89% sensitivity), Prechtl Qualitative Assessment of General Movements (98% sensitivity) and Hammersmith Infant Neurological Examination (90% sensitivity) (Patel, Neelakantan, Pandher, Merrick, 2020).

Predictive tools to detect CP risk in infants >5 months (corrected age); brain MRI (86-89% sensitivity), Hammersmith Infant Neurological Examination (90% sensitivity) and Developmental Assessment of Young Children (83% sensitivity) (Patel, vd.,2020).

As in many diseases, early diagnosis is very important in CP, and a good neurological examination and detailed history are essential for a correct diagnosis. In case of an injury in the months when the brain develops rapidly, such as infancy, the greater the brain's ability to adapt to the new situation (plasticity), the better the injury can be controlled (Yakut, 2010). For this reason, knowing the developmental stages of infancy well is the basis for an accurate neurological diagnosis. Expected motor development stages and CP clues according to age are given in Table 3 (Yakut, 2010).

Table 3. *Motor development stages and CP clues (Yakut, 2010).*

3 Months old	Normal	While lying face down, can stand on his arms, hold his head and lift it.
	Abnormal	Cannot lift the head from the ground, throw the head back, stiffness in the legs, not smiling, not looking at the mother's face
6 Months old	Normal	While standing, puts his weight on his legs, turns on his stomach, and sits with support from his hands.
	Abnormal	Sitting slumped over, inability to lift head from lying down, inability to stretch arms forward, stiffness in legs
9 Months old	Normal	Can sit, reach for objects, crawl, roll over from prone position.
	Abnormal	Hunched posture when seated, difficulty crawling, inability to stand on legs, inability to bear body weight, hand preference
12 Months old	Normal	Takes steps by holding on and passes the object from hand to hand.
	Abnormal	Climbing is difficult due to spasticity in the legs, stiffness in the arms, gets support from his arms while sitting, sits with his weight on one side, and stands on his tiptoes.
15 Months old	Normal	Can walk and squat without assistance.
	Abnormal	Cannot walk without help, stands on tiptoe and stands unsteadily.

6. Psychiatric Disorders

Cerebral palsy (CP), resulting from damage or inadequate development of the immature brain; is a permanent but non-progressive disorder that often brings comorbid emotional and behavioural problems (Rosenbaum, vd.,2007). Numerous research findings show that the prevalence of mental health disorders in individuals with a chronic brain disorder is higher than in their peer group without any brain disorder (Goodman, 2002). Psychological disorders in CP were first investigated in 1990, and it was concluded that more than half of the sample group of 428 people had a mental disorder (Goodman & Graham, 1996). It is necessary to evaluate psychological health as a whole, together with the general structure of the brain, the presence and level of mental disability, executive function and social reasoning areas (Bottcher, 2010). According to studies, the most common accompanying psychiatric disorder is attention deficit hyperactivity disorder, followed by behavioural disorders, mood disorders and anxiety disorders (Bjorgaas, vd.,2012 & Parker, vd.,2008). Although it is not a sufficient assumption to say that the cause of the mental or behavioural disorder is directly related to the damage in the brain, it reveals that the exclusion of individuals diagnosed with CP from social environments and the peer bullying they are exposed to constitute the basis for a number of psychological disorders (Yude, Goodman, 1999; Yude, Goodman & McConanchie, 1998). According to a study conducted with school-age individuals diagnosed with mild CP, individuals with CP are unable to participate in sports activities due to the disease or are often the last ones, cannot perform self-care skills without support, and think that the attention of the people around them is on them due to the inadequacy in all these. It results in high levels of stress (Goodman & Yude, 2000). Since CP is a neurological disorder often accompanied by pain that requires hospitalization and surgery, this condition can cause increased depression and anxiety in individuals (Kjersti, Reidun, Ola, Skjeldal & Trond, 2012). In a study conducted with 1174 CP patients between the ages of 8 and 12, it was concluded that the presence of a comorbid psychological disorder significantly limits the participation of individuals (Dang, vd.,2015). In this context, the existence of physical disability and other comorbid conditions, limitations in social participation, and vital difficulties arising from disability require a multidisciplinary approach (Rosenbaum & Gorter, 2012). Along with the accompanying mental disorders, isolation of children with CP from social environments is seen as a possible outcome. In research conducted; it was found that children with CP had fewer friends, experienced rejection more frequently, and were victimized more than the healthy age group (Yude, vd.,1998). According to literature data, behavioural problems in children diagnosed with CP are reported to be 5 times more common than in the matched control group without any mental illness (McDermott, vd.,1996). There are also studies supporting that conduct disorder and anxiety in indi-

viduals arise from the difficult relationships of children diagnosed with CP with their peers (Bjorgaas, Elgen & Hysing, 2021). A recent longitudinal study found that there is a significant relationship between behavioural disorders in individuals with CP at the age of seven and emotional problems at the age of eleven, and that behavioural disorders in early childhood pose a potential risk for emotional problems that may occur in the future (Bjorgaas, vd.,2021). Considering that social interaction is directly proportional to psycho-social well-being, it would be effective to increase the studies to be carried out in line with the needs of children diagnosed with CP regarding social relations that will support their individual development (Bottcher & Dammeyer, 2013). Respiratory system disorders, which are considered the biggest cause of early deaths in individuals diagnosed with CP, are a serious cause of morbidity and are seen in approximately 30% of individuals with CP (Allen, vd.,2021; Pinto, Alves, Mendes, Ciamponi, 2016). Dyspnoea and anxiety due to fear of death are common basic emotions in individuals with respiratory system disorders. In individuals with anxiety, impairments occur in the stimulus perception system, higher levels of reactive sensitivity are shown against warnings, a high level of anxiety occurs in the way of perceiving the current dyspnoea, resulting in a worsening of the clinical course of the disease and an increase in the need for medication (Chetta, vd.,1998; Güzelhan, vd.,1999).

7. Result

It appears that many individuals diagnosed with CP do not have adequate defence against additional mental health problems that bring functional difficulties in their lives. Although motor deficits are the most commonly reported complaints, children with cerebral palsy also experience higher rates of conduct disorders, concentration difficulties, problems in social functioning, and mood disorders compared to the healthy population. Even though mental health disorders and symptoms are present in individuals diagnosed with CP, services focus mainly on physical needs. Cerebral palsy is a neurological disease that can affect individuals emotionally and socially as well as physically. It is important to develop intervention programs that can increase individuals' long-term social interactions with their peers. As a result of practices aimed at correcting behavioural problems in individuals with CP in the early stages, emotional disorders that are likely to be seen in individuals in the future will be prevented. It is important to consider the high prevalence of mental health disorders and symptoms in CP when planning diagnosis and treatment strategies, and to use accordingly multifactorial treatment methods.

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