RESEARCH & REVIEWS IN HEALTH SCIENCES

October 2022

EDITORS
Prof. Dr. Zeliha SELAMOĞLU
Prof. Dr. Hasan AKGÜL
Doç. Dr. İlhan BAHŞİ

gece kitaplığı

İmtiyaz Sahibi / Publisher • Yaşar Hız

Genel Yayın Yönetmeni / Editor in Chief • Eda Altunel

Kapak & İç Tasarım / Cover & Interior Design • Gece Kitaplığı

Editörler / Edıtors • Assoc. Prof. Dr. Zeliha SELAMOĞLU
Prof. Dr. Hasan AKGÜL
Doç. Dr. İlhan BAHŞİ

Birinci Basım / First Edition • © Ekim 2022

ISBN • 978-625-430-443-9

Gece Kitaplığı / Gece Publishing
Türkiye Adres / Turkey Address: Kızılay Mah. Fevzi Çakmak 1. Sokak
Ümit Apt. No: 22/A Çankaya / Ankara / TR
Telefon / Phone: +90 312 384 80 40
web: www.gecekitapligi.com
e-mail: gecekitapligi@gmail.com

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

Research & Reviews in Health Sciences

October 2022

EDITORS

Prof. Dr. Zeliha SELAMOĞLU

Prof. Dr. Hasan AKGÜL

Doç. Dr. İlhan BAHŞİ

gece kitaplığı

CONTENTS

CHAPTER 1

OVERVIEW OF RENAL TRANSPLANT IN THE COVID19 PANDEMIC

***Bekir VOYVODA*[1]**

1 Dr.Öğr.Üyesi Sağlık Blimleri Üniversitesi Kocaeli Derince Eğitim ve Araştırma Hastanesi Üroloji-Organ Nakli Merkezi, Kocaeli ORCID: 0000-0002-0696-7381

Introduction

Caused by severe acute respiratory syndrome that originated in Wuhan and caused the pandemic, China in December 2019, Covid19 spread rapidly worldwide. COVID-19 is transmitted through the respiratory tract during the prodromal period and during illness. Moderate-to-severe pneumonia develops in 20% of infected patients and requires hospitalization. 5-10% of these patients are hospitalized in the intensive care unit for ventilation support (1). Reasons such as male gender, old age, obesity, hypertension, history of cardiovascular disease, chronic lung and kidney diseases may be the cause of high mortality for Covid19 (2-3). Reports from literature indicated that solid organ (SON) recipients are a higher risk group for the development of COVID-19 more severe and mortal. Mortality rates in these patients is 25% compared to 1-14% in the general population. and 35% (4-5-6).

The decrease in the number of covid19 donors has also decreased the number of cadaveric transplants in most countries due to the increasing patient density in hospital intensive care units, and it has risen again in the course of the next pandemic wave (7).

As in the whole world, the number of transplantations performed during the pandemic period decreased in our country (Graph-1-2).

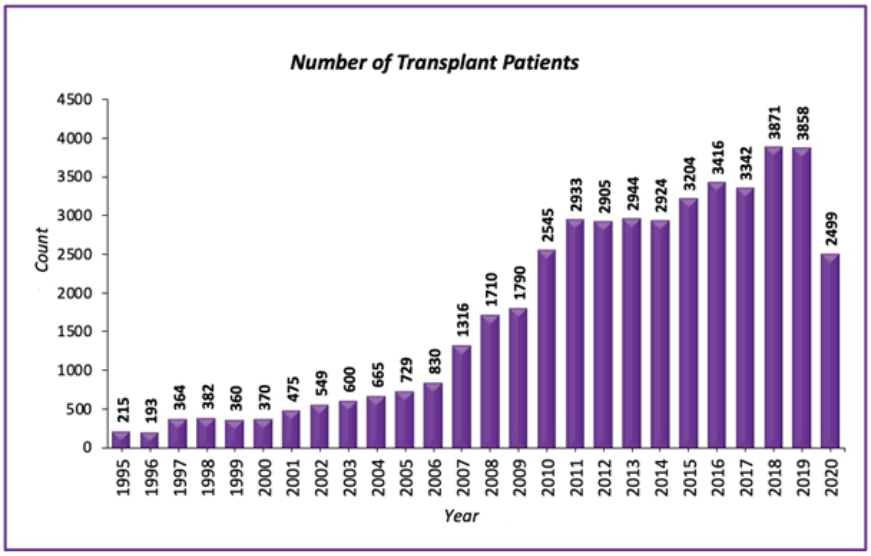


Chart 1 : Number of kidney transplant patients by years: Data from the Ministry of Health, Tissue and Organ Transplantation Center

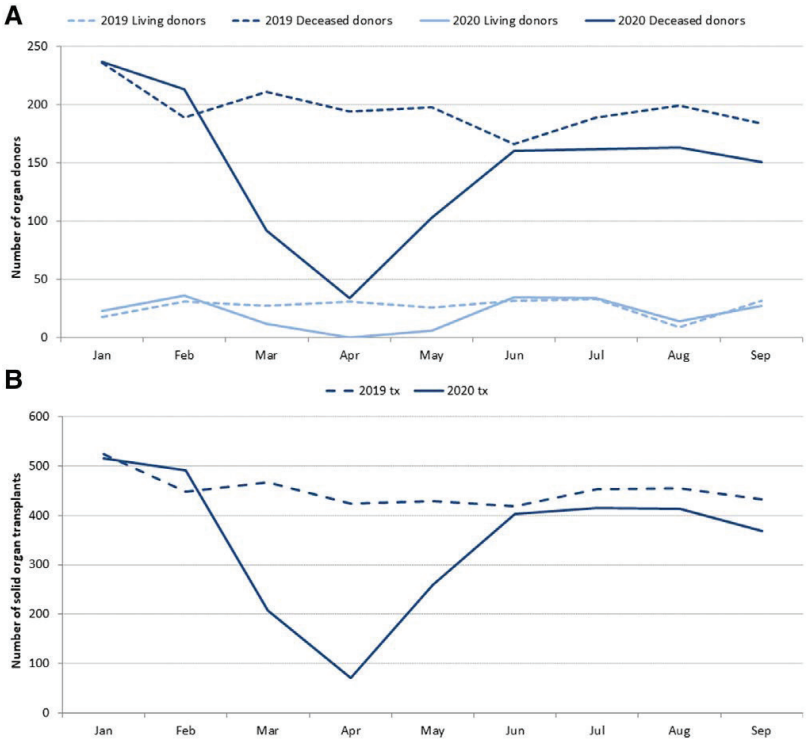


Chart 2: Monthly evolution of organ donation and transplantation in Spain during 2019 and 2020. (A) the number of cadaveric and living donors, (B) the number of solid organ transplants. Between January and September 2020, the number of organ donations decreased by 26% and solid organ transplants by 22% compared to the relevant period of 2019 (7).

In patients undergoing renal transplantation, COVID-19 has been noted in some studies. Multicenter studies provided clearer information on data from transplant patients with COVID-19. The Posttransplant Glomerular Disease Study, a large international data registry from 12 transplant centers from the United States, Italy and Spain, published the course of the disease in 144 hospitalized COVID-19 patients from 9845 adult kidney transplant recipients. (8).

Clinical Findings of Covid-19 in SOT Recipients:

In the general patient group, 50-80% of Covid-19 PCR positive patients are asymptomatic or mildly symptomatic (9).

The clinical manifestations in transplant recipients compared to the general population are as follows.

1- Dyspnea: Dyspnea among symptomatic patients is seen with the

same frequency in transplant patients and non-transplant patients.

2- Cough, weakness, myalgia: It is more common in transplant patients.

3- Fire

4- Gastrointestinal complaints: Diarrhea, especially, is more common in transplant recipients. This situation may also be associated with immunosuppressive therapy side effects.

The presence of an inflammatory syndrome characterized by elevated serum levels of inflammatory markers such as ferritin, C-reactive protein, fibrinogen and d-dimer and elevated Interleukin-6 (IL6) levels have been demonstrated in many patients. IL6 appears to be an important factor in the "cytokine storm" underlying the occurrence of acute respiratory distress syndrome (7). Thrombocytopenia and lymphopenia are the most common hematological abnormalities observed in more than one third of the patients (8,10).

Diagnosis in Covid 19:

The routinely used diagnostic test for COVID-19 is the demonstration of the virus by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of secretions taken by nasopharyngeal swab. (11). However, in Covid 19 RT-PCR can have a high rate of false negative results, serological testing of SARS CoV-2 IgM and IgG antibodies can help to the diagnosis of patients whose RT-PCR is negative despite symptoms similar to COVID, as well as diagnose of previous asymptomatic infection. It can help to identify those (12,13). For example, in a study conducted at Montefiore Medical Center, SARS CoV-2 IgG antibodies of 912 kidney transplant recipients were screened during their routine clinical controls. Results of 152 patients (16.6%) were positive (14).

COVID19 in Solid Organ Transplant Patients

The first report on the course of posttransplant Covid19 came from Wuhan, China, and mortality was reported in 1 out of 10 patients. Studies from various countries have reported a mortality rate of 19-50% in kidney transplant patients (15). Hospitalization rates and intubation rates in the presence of acute respiratory distress are high in patients undergoing renal transplantation due to Covid19, and acute kidney injury may develop in nearly 50% of the patients (8). In a study conducted in our clinic, a patient who had a kidney transplant 8 years before a living donor, who developed acute renal damage and resulted in mortality, was presented to the literature (16-Figure-1). However, the importance of pre-transplant renal failure and concomitant diseases is also emphasized. In studies, 50-100% of patients

had hypertension, 15-90% diabetes mellitus, 8-50% cardiovascular disease, 8-20% chronic lung disease, and 29-69% obesity.

Prevelance of COVID-19 is higher in African-American and Hispanic patients. Hospitalizations and mortality are more frequent in these patients. Mortality rates are also higher in patients with low socioeconomic status. (17). Men also have a higher mortality rate. In a study on blood group, A blood group has a higher risk compared to other blood groups and a protective effect in O blood group (18).

The role of immunosuppression on the progress of COVID-19 is unclear. A report from Turkey including 40 patients with COVID 19 stated that the immunosuppression protocol was associated with high mortality at admission (19).

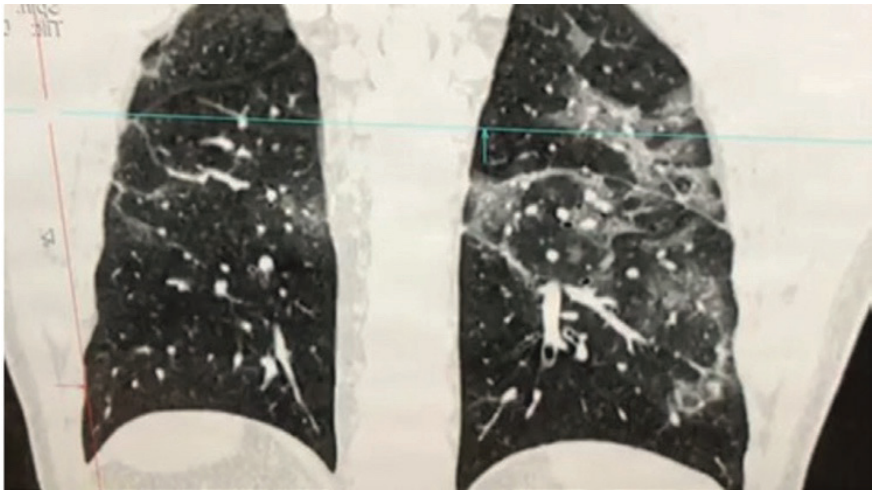


Figure 1: COVID-19 pneumonia on chest CT of a renal transplant patient

Immunity Response in COVID 19

Humans and animals can be infected by Coronaviruses. Typically 4 species of human coronaviruses (NL63, 229E, HKU1 and OC43) especially infect the upper respiratory tract and cause minor symptoms. However, severe acute respiratory syndrome coronavirus-1 (severe acute respiratory syndrome coronavirus-1 -SARS-CoV-1), middle east respiratory syndrome coronavirus (middle east respiratory syndrome coronavirus -MERS-CoV) and SARS-CoV-2 can multiply in the lower respiratory tract and can cause pneumonia (20,21)

S1 and S2 subunit of the spike (S) protein are expressed on the surface of viral particles and gives the coronaviruses a spesific “crown” appearance. Expression of angiotensin converting enzyme 2 and

TMPRSS2 (transmembrane serine protease 2) surface receptors are necessary for SARS-CoV 2 to infects the cells. Proinflammatory cytokines are secreted from innate immune cells that stop viral replication, induct adaptive immunity response and migration of other immune cells to the infection site (21, 22). Neutralizing antibodies can stop infection caused by viruses. Pathogen-derived antigens are presented by activated dendritic cells to naive T helper cells which start an adaptive immunity response which eliminates infected cells before the virus spreads. Immune response of B and T cell to COVID-19 can be detected in the blood approximately one week after the start of COVID-19 symptoms (23,24). T cells (CD8+) are mandatory for killing infected cells directly, whereas CD4+ T cells are responsible in the activation of both CD8+ T cells and B cells and product cytokine to recruit other immune cells. The infected cells are killed by Natural killer (NK) cells by degranulation, receptor-mediated apoptosis and antibody dependent cell mediated cytotoxicity. In addition, the complement system plays a complementary role in stimulating immune cells and eliminating pathogens. These immune-initiated initial responses ensure that the pathogen is eradicated with minimal lung damage.

Uncontrolled inflammation by cytokine storm causes multiple organ damage leading to liver, heart and kidney failure. IL 6 has been proven to be a cytokine that plays a role in the pathogenesis, and increased serum IL-6 levels have been found to be associated with mortality (8).

Lymphocytopenia and decreased CD3, CD4, CD8 counts are common in COVID-19 patients and are associated with disease severity (25). For this, many possible mechanisms such as migration of lymphocytes to the lungs, direct killing of lymphocytes by the virus, and T cell apoptosis are discussed (26).

Immunosuppression in Solid Organ Transplant Recipients

SOT recipients require lifetime immunosuppression, including both rejection prevention and risk of adverse events, to balance efficacy and safety. During the initial outbreak and in the absence of an effective treatment for SARS-CoV-2 infection, most Transplant and Nephrology associations published recommendations to reduce immunosuppression levels. Although immunosuppressive protocols are different in many transplantation centers, calcineurin inhibitors such as cyclosporine and tacrolimus in the maintenance treatment of the world and in our country; mTOR (mammalian target of rapamycin) inhibitors such as everolimus, sirolimus; steroids and antimetabolites such as MMF/MPA (Mycophenolate mofetil / mycophenolic acid) are used. Remarkably, calcineurin inhibitors were reduced in 52% of patients with severe Covid19, in another US study the antimetabolite was continued in 56% and reduced in 10%, with all

immunosuppressive therapy discontinued in only <1% of patients (10). In another international study, mycophenolate or everolimus was reduced or discontinued in 68% of patients, and calcineurin inhibitors were discontinued in 23% of cases (8).

Remarkably, no relationship could be identified between the intensity of baseline immunosuppression or cessation of immunosuppression and the outcome of COVID-19 (27).

As a result; The COVID-19 pandemic has severely impacted transplantation practices worldwide. The outcomes of SON recipients infected with SARS-CoV-2 vary according to general approaches to the management of immunosuppression. A drastic decline in organ transplants worldwide has been accompanied by early decisions to delay non-urgent transplant programs. Such a situation has been worrisome because of the risk of death of patients on the waiting list, and great efforts are being made to identify effective strategies to overcome these ethical issues.

REFERENCES

Mohamad Zaidan, MD, PhD and Christophe Legendre, Solid Organ Transplantation in the Era of COVID-19: Lessons from France. Transplantation January 2021 Volume 105 Number 1. DOI: 10.1097/TP.0000000000003536

Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054–1062.

Cummings MJ, Baldwin MR, Abrams D, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. Lancet. 2020;395:1763–1770.

Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1061–1069.

Guan WJ, Ni ZY, Hu Y, et al; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708–1720.

Goyal P, Choi JJ, Pinheiro LC, et al. Clinical characteristics of Covid- 19 in New York City. N Engl J Med. 2020;382:2372–2374.

Beatriz Domínguez-Gil, Mario Fernández-Ruiz, Domingo Hernández, Marta Crespo, Jordi Colmenero, Elisabeth Coll, and Juan José Rubio. Organ Donation and Transplantation During the COVID-19 Pandemic: A Summary of the Spanish Experience. Transplantation January 2021 Volume 105 Number 1. DOI:10.1097/TP.0000000000003528

Cravedi P, Suraj SM, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium. Am J Transplant. [Epub ahead of print. August 4, 2020]. doi:10.1111/ajt.16185

Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054–1062.

Caillard S, Anglicheau D, Matignon M, et al. An initial report from the French SOT COVID Registry suggests high mortality due to Covid-19 in recipients of kidney transplants. Kidney Int. [Online ahead of print. August 24, 2020]. doi:10.1016/j.kint.2020.08.005

Hanson KE, Caliendo AM, Arias CA, et al. Infectious diseases society of america guidelines on the diagnosis of COVID-19. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2020;ciaa760.

Liu R, Han H, Liu F, et al. Positive rate of RT-PCR detection of SARSCoV- 2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin Chim Acta. 2020;505:172–175.

Xie J, Ding C, Li J, et al. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. J Med Virol. 2020. [Epub ahead of print. April 24, 2020]. doi:10.1002/jmv.25930

Azzi Y, Parides M, Alani O, et al. COVID-19 infection in kidney transplant recipients at the epicenter of pandemics. Kidney international. 2020;S0085-2538(20)31202-3. doi:10.1016/j.kint.2020.10.004

Alberici F, Delbarba E, Manenti C, et al. A report from the Brescia Renal COVID task force on the clinical characteristics and short-term outcome of hemodialysis patients with SARS-CoV-2 infection. Kidney Int. 2020;98:20–26.

Bekir Voyvoda . COVID-19 with a Fatal Outcome in a Kidney Transplant Recipient: Case Report. Journal of Urological Surgery, 2020;7(4):328-330. Doi: 10.4274/jus.galenos.2020.3778

Wadhera RK, Joynt Maddox KE, Yeh RW. CMS quality measure development-reply. JAMA. 2020;324:1214–1215.

Ellinghaus D, Degenhardt F, Bujanda L, et al. Genomewide association study of severe Covid-19 with respiratory failure. N Engl J Med. 2020;383:1522–1534.

Demir E, Uyar M, Parmaksiz E, et al. COVID-19 in kidney transplant recipients: A multicenter experience in Istanbul. Transpl Infect Dis. 2020:e13371 [Epub ahead of print. July 13, 2020]. doi:10.1111/ tid.13371

Morens DM, Fauci AS. Emerging pandemic diseases: How we got to COVID-19. Cell. 2020;182:1077–1092.

Tay MZ, Poh CM, Rénia L, et al. The trinity of COVID-19: Immunity, inflammation and intervention. Nat Rev Immunol. 2020;20:363–374.

McKechnie JL, Blish CA. The Innate Immune System: Fighting on the front lines or fanning the flames of COVID-19? Cell Host Microbe. 2020;27:863–869.

Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. 2020;181:1489–1501.e15.

Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature. 2020;587:270–274

Akalin E, Azzi Y, Bartash R, et al. Covid-19 and kidney transplantation. N Engl J Med. 2020;382:2475–2477.

De Biasi S, Meschiari M, Gibellini L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun. 2020;11:3434.

Kates OS, Haydel BM, Florman SS, et al; UW COVID-19 SOT Study Team. COVID-19 in solid organ transplant: a multi-center cohort study. Clin Infect Dis. [Online ahead of print. August 7, 2020]. doi:10.1093/cid/ciaa1097

CHAPTER 2

THE PREVALENCE OF GIARDIA SPP. IN ANIMALS IN TURKEY

Burçak ASLAN ÇELİK[1]
Özgür YAŞAR ÇELİK[2]

1 Assist.Prof. Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, ORCID: 0000-0002-0130-970X
2 Assoc.Prof., Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, ORCID: 0000-0001-6365-2688

Introduction

Giardia sp. is an enteric protozoan found in the intestines of humans, as well as domestic and wild animals (Cacciò, Thompson, McLauchlin, & Smith, 2005; McGlade, Robertson, Elliot, & Thompson, 2003; Naguib et al., 2018; Tangtrongsup & Scorza, 2010). Even though the giardia parasite was defined in detail as far back as 1859, its clinical significance was not acknowledged until the late 20th century (Geurden, Vercruysse, & Claerebout, 2010). The parasite is currently spread worldwide, which is troubling considering that it's considered a zoonotic agent (McGlade et al., 2003; Thompson, 2000). Giardiasis is now considered the most common parasitological cause of diarrhea in humans with 280 million infections per year (Geurden et al., 2010), and it has been included in the Neglected Disease Initiative by the World Health Organization (Savioli, Smith, & Thompson, 2006). There is a growing interest in giardia since the 1990s in veterinary medicine, which has mainly been due to public health concerns. Giardia is considered a potential cause of diarrhea in companion animals, while for farm animals it's considered a potential reservoir for human giardia infections. As such, studies on Giardiasis are focused on molecular characterization of the isolates obtained from various hosts, and on the parasite prevalence to illuminate the real scope of the zoonotic threat (Geurden et al., 2010).

Etiology

Since 1859, more than 50 species of Giardia have been identified, mostly based on the hosts' specificity. This host-based taxonomy was later replaced with a morphological taxonomy, which is instead based on the morphological properties of trophozoites and median bodies like shape or length (Geurden et al., 2010). Based on their morphological properties, species of the Giardia genus are classified into 8 groups as follows: *Giardia agilis*, *Giardia ardeae, Giardia psittaci, Giardia microti, Giardia muris, Giardia duodenalis* (syn. *Giardia intestinalis* and *Giardia lamblia*), *Giardia peramelis,* and *Giardia cricetidarum* (De Liberato et al., 2021). *G. duodenalis* has 8 different assemblages (from A to H) categorized based on genetic characterization and host specificity (Cai, Ryan, Xiao, & Feng, 2021; Nguyen, Fukuda, Nguyen, Tada, & Nakai, 2016). Assemblages C and D have been determined in dogs, cats, wolves, and hyenas, while E was determined in even-toed ungulates, F was determined in cats, G was determined in rats, and H was determined in seals (Ayan et al., 2019; Cacciò & Ryan, 2008; Cai et al., 2021; Hamnes, Gjerde, & Robertson, 2006; Lalle et al., 2005; Lee et al., 2018; Naguib et al., 2018). Since only Assemblages A and B were encountered in humans, only these two assemblages are considered zoonotic (Hamnes et al., 2006; Lee et al., 2018; Santin, Dargatz, & Fayer, 2012).

Giardia species have a mobile trophozoite (vegetative) form that can colonize the intestinal tract and cause diarrhea, and a cyst form that can survive outside of the host which is also the cause of the spread of infection (Farthing, 1996).

Trophozoites are pear-shaped, bilateral, and symmetric in form (Yukarı & Şahinduran, 2013), with a length of 12-20 µm and a width of 5-10 µm. The parasite has a convex dorsal area and a flat belly that contains a spiral organelle or a sucking disc. Trophozoites also have two symmetric nuclei and four pairs of flagella (Leung, Leung, Wong, Sergi, & Kam, 2019). When there is no clinical diarrhea, trophozoites are seldom encountered in the feces (Küçüködük & Şahinoğlu, 1989). Meanwhile, the cyst form is oval, has approximate dimensions of 8 x 12 µm, and has four nuclei (Adam, 2020).

Transmission

The transmission of this parasite from one host to another can occur directly through the fecal-oral route, by coming into direct contact with infected individuals, or by ingestion of fecal-contaminated food or water (Ayan et al., 2016; Cacciò et al., 2005; Hamnes et al., 2006; Lee et al., 2018; Naguib et al., 2018; Olson, O'Handley, Ralston, McAllister, & Thompson, 2004; Thompson, Palmer, & O'Handley, 2008). The spread of cysts through the feces of infected animals causes environmental contamination (Ayan et al., 2016; Hamnes et al., 2006). Cysts can remain infectious for months in cool and humid area (Thompson et al., 2008).

When a host ingests the cysts, these transform into motile trophozoite form at the proximal small intestine, which is triggered by the acidic environment of the stomach and the stimulant effect of the trypsin and choler in the duodenum (Lagunas-Rangel, Yee, & Bermúdez-Cruz, 2021; Lebwohl, Deckelbaum, & Green, 2003).

In the high-nutrition low-oxygen environment of the small intestines, the trophozoites multiply in number through binary fission (Farthing, 1996; Lagunas-Rangel et al., 2021). Trophozoites are mobile thanks to their four pairs of flagella which they beat around to move, and they can attach to the small intestine walls using their ventral sucking discs (Adam, 2020; Farthing, 1996).

As the parasite concentration inside the host increase and the trophozoites advance to the lower intestinal path, they encounter low cholesterol, increased pH, and increased choler and lactic acid concentration. These conditions cause the trophozoites to transform into infectious cysts, which are excreted with the feces and act as new sources for further infections (Lagunas-Rangel et al., 2021).

Symptoms

Clinical symptoms of the disease include diarrhea, swelling, malnutrition, and weight loss, and most infections are self-limiting (Cai et al., 2021). Since the agent causes damage to the intestinal lumen, malabsorption is frequently observed (Çamkerten et al., 2019).

While the disease has a sub-clinic or asymptomatic course in adult bovines, it causes slower weight gain, lower feed yield, lower carcass weight, diarrhea, and sometimes even death in younger animals (Geurden et al., 2010; Gillhuber et al., 2013; Hamnes et al., 2006; Lee et al., 2018; Nguyen et al., 2016).

In lambs, weight loss, depression, lethargy, tenesmus, abdominal swelling, brown diarrhea, hypothermia, and in advanced cases, death, can be observed (Kırbaş, Balkaya, & Temur, 2012; Ozdal, Tanritanir, GOZ, Deger, & Kozat, 2009; Özmen, Yukari, Haligur, & Sahinduran, 2006; Thompson, 2000).

In cats, the disease may have an asymptomatic course, or maldigestion, malabsorption, and diarrhea due to increased motility might be observed (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010; McGlade et al., 2003; Saleh et al., 2019). In horses, Giardiasis agents are considered non-pathogenic as they cause no clinical symptoms (Altay, 2013).

In humans, the course of the disease can range from asymptomatic to acute and severe form. Diarrhea, nausea, abdominal cramps, anorexia, and weight loss can be observed as well (Ballweber et al., 2010).

It has been noted in the literature that the clinical course of a Giardiasis infection can vary based on the resistance of the agent to environmental conditions, infectious dose, the age of the animal, and the raising conditions (Kırbaş et al., 2012).

Diagnosis

Traditionally, Giardia infections are diagnosed by inspecting the fecal samples under the microscope and identifying the trophozoites and cysts (Soares & Tasca, 2016). Used with the zinc sulfate centrifuging method, the light microscope is still considered the most practical method when working with fecal samples for the diagnosis of Giardiasis. Since the cyst excretion is sporadic, several fecal samples should be inspected for a period of 4 to 5 days (Thompson et al., 2008). Besides the microscopic inspections, various ELISA-based methods also exist that can determine the coproantigens and diagnose the disease. While this work well, they are relatively expensive (Mosallanejad, Avizeh, Jalali, & Alborzi, 2010; Saleh et al., 2019; Thompson et al., 2008). Molecular (PCR) techniques are more

sensitive in the diagnosis of the disease. However, these are mostly limited to epidemiological studies due to their high costs (McGlade et al., 2003; Mosallanejad et al., 2010; Thompson et al., 2008).

Treatment

In cats and dogs, metronidazole (10-25 mg/kg PO, BID, 5-7 days) or fenbendazole (50 mg/kg/day PO, 3-5 days) can be used. The daily dose of metronidazole shouldn't exceed 50 mg/kg in cats. Furthermore, in dogs, tinidazole (44 mg/kg/day PO, 3 days) or furazolidone (2-4 mg/kg PO, BID, 7 days) can be administered. Albendazole, when used in dogs at the dose of 25 mg/kg PO, BID for 2 days, is almost 100% effective (Traş, Yazar, & Elmas, 2007).

For sheep and goats, Fenbendazole is suggested as the initial choice with a dose of 5-10 mg/kg, for 3 days (Yukarı & Şahinduran, 2013). That being said, there are no studies in the literature that evaluate the success rate of this treatment for sheep and goats (Geurden et al., 2010).

In bovine, Quinacrine 1 mg/kg, twice daily for 7 days, Albendazole 20 mg/kg for 3 days, PO once daily, Fenbendazole 10 mg/kg for 3 days, PO twice daily, Ipronidazole 10 mg/kg, twice daily for 5 gün, and Paromomisin 50-75 mg/kg, PO 5 days, are all valid choices for treatment. Chemotherapeutic agents are reported to be quite efficient against *Giardia duodenalis*, however, it appears getting completely rid of the infection in heavily cyst-contaminated sheds is unlikely due to reinfections (B. Sarı & Arslan, 2013).

The Status of the Disease in Turkey

Various studies have been performed on different animal species in Turkey, to determine the Giardiasis prevalence in the country (Table 1).

In studies performed on calves, the prevalences for different cities have been reported as follows: 9.34%-64.79% in Van (Ayan et al., 2019; Göz, Altug, Yuksek, & Ozkan, 2006; Gül, Ciçek, & Kilinç, 2008), 17.68% in Aydın (Gultekin, Ural, Aysul, Ayan, Balikci, Toplu, et al., 2017), 4% in Siirt (Kozat & Tuncay, 2018), 4.13% in Sivas (Değerli, Çeliksöz, Kalkan, & ÖZÇELİK, 2005), 16.82% in Tokat (Kaya & Coşkun, 2018). In another study, Giardia spp. was reported in a single calf in Diyarbakır (Koçhan, Şimşek, Sayın-İpek, & İçen, 2020). The studies on the bovine report the following prevalences: 30% in Kayseri (Onder et al., 2020), 28% Nevşehir (Koçhan et al., 2020) 1.43%-32.67% in Sivas (Değerli et al., 2005; Değerli & Özçelik, 2003; Mamak, Özçelik, Değerli, Oğuztürk, & Akın, 2000; Onder et al., 2020).

Meanwhile, the studies on lamb report the prevalence of Giardiasis

in different locations as follows: 32%-48.48% in Van (Ayan et al., 2019; Ozdal et al., 2009),1.14% in the Marmara region (Arslan et al., 2016), A single lamb in Erzurum (Kırbaş et al., 2012), 36.67% in lamb and kids in Burdur (Özmen et al., 2006), 30.30%-36.36% in kids in Van (Ayan et al., 2019).

There are also some studies performed on cats, the prevalence results of which are as follows: 3% in Ankara (Burgu, Tınar, Doğanay, & Toparlak, 1985), 68.63% in the Central Anatolia Region (Sursal, Simsek, & Yildiz, 2020), 8% in Kayseri and Samsun (Önder et al., 2021), 8.33%-16.67% in Mardin (Aslan-Çelik, 2022), and in a single cat in Burdur (Sevgisunar, Şahinduran, & Adanır, 2013).

On the other hand, the studies performed on dogs report the following findings: 7.89% in Elazıg (Dumanlı, 1984), 18.82% in Aydın (Gultekin, Ural, Aysul, Ayan, Balikci, & Akyildiz, 2017), 35.29% in Nevşehir (M. Sarı & Onmaz, 2011), 3.38% in Ankara (Burgu, 1979).

In one study performed on horses in Kayseri, a prevalence of 16.67% was reported (Demircan et al., 2019).

Table 1. The Distribution of Giardiasis in Turkey by provinces and animal species.

Province	Species	n	Positive		Method	Determined species	References
			n	%			
Kayseri	Horse	150	25	16.67%	Nested PCR	G. intestinalis	(Demircan et al., 2019)
Van	Calf	71	41	57.75%	Zinc sulfate	G. duodenalis	
	Calf	71	38	53.52%	ELISA-based Rap.Diag. Kit	G. duodenalis	(Ayan et al., 2019)
	Calf	71	46	64.79%	Nested PCR	G. duodenalis	
	Calf	182	17	9.34%	Zinc sulfate	Giardia spp.	(Gül et al., 2008)
	Calf	231	34	14.72%	Fulleborn's salty water technique	Giardia spp.	(Göz et al., 2006)
Aydın	Calf	198	35	17.68%	Nested PCR	G. duodenalis	(Gultekin, Ural, Aysul, Ayan, Balikci, Toplu, et al., 2017)
Diyarbakır	Calf	1	1	100.00%	Nested PCR	G. duodenalis (Assemblage D)	(Koçhan et al., 2020)
Siirt	Calf	100	4	4.00%	BoviD-5 Ag Test Kit	Giardia lamblia	(Kozat & Tuncay, 2018)
Sivas	Calf	387	16	4.13%	Zinc sulfate in vitro	Giardia spp.	(Değerli et al., 2005)
Tokat	Calf	107	18	16.82%	Anigen Rapid BoviD-5	Giardia lamblia	(Kaya & Coşkun, 2018)
Van	Goat kid	66	23	34.85%	Zinc sulfate	G. duodenalis	
	Goat kid	66	20	30.30%	ELISA-based Rap.Diag. Kit	G. duodenalis	(Ayan et al., 2019)
	Goat kid	66	24	36.36%	Nested PCR	G. duodenalis	
Burdur	Cat	1	1	100.00%	Zinc sulfate	G. duodenalis	(Sevgisunar et al., 2013)
Ankara	Cat	100	3	3.00%	Zinc sulfate	G. Cati	
Central Anatolia Region	Cat	102	70	68.63%	PCR	G. duodenalis (Assemblage B)	(Sursal et al., 2020)
Kayseri + Samsun	Cat	100	8	8.00%	PCR	G. duodenalis (Assemblage B)	(Önder et al., 2021)
Mardin	Cat	48	4	8.33%	Nativ-Lugol	Giardia duodenalis	(Aslan-Çelik, 2022)
	Cat	48	8	16.67%	PCR	Giardia duodenalis	
Elazığ	Dog	38	3	7.89%	Zinc sulfate	Giardia canis	(Dumanlı, 1984)
Aydın	Dog	473	89	18.82%	Nested PCR	G. duodenalis (Assemblage B)	(Gultekin, Ural, Aysul, Ayan, Balikci, & Akyildiz, 2017)
Nevşehir	Dog	170	60	35.29%	Antigenic test kit	Giardia spp.	(M. Sarı & Onmaz, 2011)
Ankara	Dog	237	8	3.38%	Zinc sulfate	G. canis	(Burgu, 1979)
Van	Lamb	50	18	36.00%	Zinc sulfate	G. duodenalis	
	Lamb	50	16	32.00%	ELISA-based Rap.Diag. Kit	G. duodenalis	(Ayan et al., 2019)
	Lamb	50	21	42.00%	Nested PCR	G. duodenalis	
	Lamb	132	64	48.48%	Fulleborn's salty water technique	Giardia spp.	(Ozdal et al., 2009)
Marmara Region	Lamb	88	1	1.14%	Zinc sulfate	Giardia spp.	(Arslan et al., 2016)
Erzurum	Lamb	1	1	100.00%	Zinc sulfate	Giardia spp.	(Kırbaş et al., 2012)
Burdur	Lamb+kid	30	11	36.67%	Carbolfuchsin and Giemsa stain	G. intestinalis	(Özmen et al., 2006)

Kayseri	Cattle	150	45	30.00%	Nested PCR	G. duodenalis (Assemblage A,E)	(Onder et al., 2020)
Nevşehir	Cattle	150	42	28.00%	Nested PCR	G. duodenalis (Assemblage A,E)	
Sivas	Cattle	150	49	32.67%	Nested PCR	G. duodenalis (Assemblage A,E)	
	Cattle	70	1	1.43%	Zinc sulfate	Giardia spp.	(Değerli et al., 2005)
	Cattle	56	1	1.79%	Zinc sulfate	Giardia spp.	(Değerli & Özçelik, 2003)
	Cattle	200	9	1.45%	Kinyoun acid-fast stain	Giardia spp.	(Mamak et al., 2000)

Figure 1. Map of the provinces where the studies were carried out

Prophylaxis

It has not been possible to develop a strategy to protect the animals against Giardia infections (Altay, 2013). Due to the extended period of cyst survival in the outside environment, protection from this disease is quite challenging (B. Sarı & Arslan, 2013). The most important element for protection is to keep the environment clean and abide by the hygiene rules (Yukarı & Şahinduran, 2013). Limiting the environmental contamination with cysts excreted by the hosts is a very significant practice to protect the animals from the disease (B. Sarı & Arslan, 2013). Providing clean feed and water, daily replacement of offspring floor bedding, and keeping diseased animals from the healthy ones, and old animals from younger ones, are all helpful precautions in the protection against the disease (Yukarı & Şahinduran, 2013).

Conclusion

Many studies performed in different regions, cities, and animal species in Turkey report the prevalence of the disease in Turkey. The fact that zoonotic species of the agent have been determined in these studies is

a source of public health concern. In particular, breeders and professional groups in close contact with animals should be informed about this disease, and giardia factors should be taken into consideration in the etiology of diarrhea, especially in young animals. Finally, appropriate treatment and protection protocols should be determined and implemented for the disease.

References

Adam, R. D. (2020). Giardiasis. In Hunter's Tropical Medicine and Emerging Infectious Diseases (pp. 707-711): Elsevier.

Altay, K. (2013). Giardiosis. In M. Özcel (Ed.), Veteriner Hekimliğinde Parazit Hastalıkları Cilt 2 (pp. 1079-1082). İzmir: Türkiye Parazitoloji Derneği.

Arslan, S., Öncel, T., Malal, M. E., Satir, E., Sait, A., Baca-Ünsal, A., & Aydoğan-Yaman, D. (2016). Bacteriological, Virological and Parasitological Etiology in Diarrhea Cases in Determined in Post-mortem Lambs and Kids in Marmara Region. Van Veterinary Journal, 27(3).

Aslan-Çelik, B. (2022). First Detection of Giardia duodenalis in Cats in Mardin Province. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi, 36(2), 108-111.

Ayan, A., Ural, D. A., Erdogan, H., Kilinc, O. O., Gültekin, M., & Ural, K. (2019). Prevalance and molecular characterization of Giardia duodenalis in livestock in Van, Turkey. Int J Ecosyst Ecol Sci, 9(2), 289-296.

Ayan, A., Ural, K., Aysul, N., Gültekin, M., Erdoğan, H., Balıkçı, C., . . . Toros, G. (2016). Natural Cyst Shedding in Calves Infected with Giardia Duodenalis. JAVST, 1(1), 14-19.

Ballweber, L. R., Xiao, L., Bowman, D. D., Kahn, G., & Cama, V. A. (2010). Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends in parasitology, 26(4), 180-189.

Burgu, A. (1979). The distribution of Giardia canis in Ankara dogs and its public health significance. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 26(03), 184-194.

Burgu, A., Tınar, r., Doğanay, A., & Toparlak, M. (1985). A survey for ecto-and endoparasites of stray cats in Ankara. AÜ Vet Fak Derg, 32(2), 288-300.

Cacciò, S. M., & Ryan, U. (2008). Molecular epidemiology of giardiasis. Molecular and biochemical parasitology, 160(2), 75-80.

Cacciò, S. M., Thompson, R. A., McLauchlin, J., & Smith, H. V. (2005). Unravelling cryptosporidium and giardia epidemiology. Trends Parasitol, 21(9), 430-437.

Cai, W., Ryan, U., Xiao, L., & Feng, Y. (2021). Zoonotic giardiasis: an update. Parasitology Research, 120(12), 4199-4218.

Çamkerten, G., Erdoğan, H., Ural, D. A., Çamkerten, İ., Erdoğan, S., & Ural, K. (2019). Levels of serum 25 (OH) D3 in naturally infected lambs with Giardia duodenalis. Kocatepe Veterinary Journal, 12(1), 71-74.

De Liberato, C., Di Filippo, M. M., Sagrafoli, D., Ferraro, D., Procesi, I. G., & Berrilli, F. (2021). Giardia microti in pet Microtus guentheri: Evidence of a parasite never detected in Italy. Parasitology international, 80, 102207.

Değerli, S., Çeliksöz, A., Kalkan, K., & ÖZÇELİK, S. (2005). Prevalence of Cryptosporidium spp. and Giardia spp. in cows and calves in Sivas. Turkish Journal of Veterinary & Animal Sciences, 29(4), 995-999.

Değerli, S., & Özçelik, S. (2003). The first Giardia infection in cattle gall bladder. Turkish Journal of Veterinary & Animal Sciences, 27(5), 1231-1233.

Demircan, K., Onder, Z., Duzlu, O., Yildirim, A., Okur, M., Ciloglu, A., . . . Inci, A. (2019). First molecular detection and phylogenetic analyses of zoonotic Giardia intestinalis in horses in Turkey. Journal of equine veterinary science, 80, 56-60.

Dumanlı, N. (1984). A survey of the incidence of protozoer agents in dogs in Elazığ vicinity. AÜ Vet Fak Derg, 31(3), 383-387.

Farthing, M. J. (1996). Giardiasis. Gastroenterology Clinics, 25(3), 493-515.

Geurden, T., Vercruysse, J., & Claerebout, E. (2010). Is Giardia a significant pathogen in production animals? Exp. Parasitol, 124(1), 98-106.

Gillhuber, J., Pallant, L., Ash, A., Thompson, R. A., Pfister, K., & Scheuerle, M. C. (2013). Molecular identification of zoonotic and livestock-specific Giardia-species in faecal samples of calves in Southern Germany. Parasites Vectors, 6(1), 1-6.

Göz, Y., Altug, N., Yuksek, N., & Ozkan, C. (2006). Parasites detected in neonatal and young calves with diarrhoea. Bulletin-Veterinary Institute in Pulawy, 50(3), 345.

Gultekin, M., Ural, K., Aysul, N., Ayan, A., Balikci, C., & Akyildiz, G. (2017). Prevalence and molecular characterization of Giardia duodenalis in dogs in Aydin, Turkey. International Journal of Environmental Health Research, 27(3), 161-168.

Gultekin, M., Ural, K., Aysul, N., Ayan, A., Balikci, C., Toplu, S., & Akyildiz, G. (2017). Prevalence and molecular characterization of Giardia duodenalis in calves in Turkey. Acta Scientiae Veterinariae, 45(1), 6.

Gül, A., Ciçek, M., & Kilinç, O. (2008). Prevalence of Eimeria spp., Cryptosporidium spp. and Giardia spp. in calves in the Van province. Turkiye Parazitol Derg, 32(3), 202-204.

Hamnes, I. S., Gjerde, B., & Robertson, L. (2006). Prevalence of Giardia and Cryptosporidium in dairy calves in three areas of Norway. Vet. Parasitol, 140(3-4), 204-216.

Kaya, U., & Coşkun, A. (2018). Determination of etiology of neonatal calves diarrhea in Tokat region. Manas J Agr Vet Life Sci, 8(1), 75-80.

Kırbaş, A., Balkaya, İ., & Temur, A. (2012). A giardiosis case in a lamb. Etlik Veteriner Mikrobiyoloji Dergisi, 23(1), 29-31.

Koçhan, A., Şimşek, A., Sayın-İpek, D. N., & İçen, H. (2020). Severe Bloody Diarrhea in a Calf Infected with Giardia duodenalis. Dicle Üniv Vet Fak Derg, 13(2), 179-182.

Kozat, S., & Tuncay, İ. (2018). Prevalance of Rotavirus, Coronavirus, Cryptosprodium spp., Escherichia coli K 99, and Giardia lamblia pathogens in neonatal calves with diarrheic in Siirt Region. Van Vet J, 29(1), 17-22.

Küçüködük, Ş., & Şahinoğlu, M. (1989). Giardiasis. Ondokuz Mayıs Üni. Tıp Fak. Derg, 6(3), 435-444.

Lagunas-Rangel, F. A., Yee, J., & Bermúdez-Cruz, R. M. (2021). An update on cell division of Giardia duodenalis trophozoites. Microbiological Research, 250, 126807.

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., & Cacciò, S. M. (2005). Genetic heterogeneity at the β-giardin locus among human and animal isolates of Giardia duodenalis and identification of potentially zoonotic subgenotypes. Int. J. Parasitol, 35(2), 207-213.

Lebwohl, B., Deckelbaum, R. J., & Green, P. H. (2003). Giardiasis. Gastrointestinal endoscopy, 57(7), 906-913.

Lee, Y.-J., Han, D.-G., Ryu, J.-H., Chae, J.-B., Chae, J.-S., Yu, D.-H., . . . Choi, K.-S. (2018). Identification of zoonotic Giardia duodenalis in Korean native calves with normal feces. Parasitol. Res., 117(6), 1969-1973.

Leung, A. K., Leung, A. A., Wong, A. H., Sergi, C. M., & Kam, J. K. (2019). Giardiasis: an overview. Recent patents on inflammation & allergy drug discovery, 13(2), 134-143.

Mamak, N., Özçelik, S., Değerli, S., Oğuztürk, H., & Akın, Z. (2000). Prevalence of Cryptosporidium Infection in Cattle in the Vicinity of Zara, Sivas. Turkiye Parazitol Derg, 24(4), 401-404.

McGlade, T., Robertson, I., Elliot, A., & Thompson, R. (2003). High prevalence of Giardia detected in cats by PCR. Veterinary Parasitology, 110(3-4), 197-205.

Mosallanejad, B., Avizeh, R., Jalali, M. R., & Alborzi, A. (2010). Prevalence of Giardia duodenalis infection in household cats of Ahvaz District, south-west of Iran. Iranian Journal of Parasitology, 5(3), 27.

Naguib, D., El-Gohary, A. H., Mohamed, A. A., Roellig, D. M., Arafat, N., & Xiao, L. (2018). Age patterns of Cryptosporidium species and Giardia duodenalis in dairy calves in Egypt. Parasitol. Int, 67(6), 736-741.

Nguyen, S. T., Fukuda, Y., Nguyen, D. T., Tada, C., & Nakai, Y. (2016). Prevalence and first genotyping of Giardia duodenalis in beef calves in Vietnam. Trop. Anim. Health Prod, 48(4), 837-841.

Olson, M. E., O'Handley, R. M., Ralston, B. J., McAllister, T. A., & Thompson, R. A. (2004). Update on Cryptosporidium and Giardia infections in cattle. Trends Parasitol, 20(4), 185-191.

Onder, Z., Simsek, E., Duzlu, O., Yetismis, G., Ciloglu, A., Okur, M., . . . Yildirim, A. (2020). Molecular prevalence and genotyping of Giardia duodena-

lis in cattle in Central Anatolia Region of Turkey. Parasitology Research, 119(9), 2927-2934.

Ozdal, N., Tanritanir, P., GOZ, Y., Deger, S., & Kozat, S. (2009). Parasitic protozoans (Eimeria, Giardia, and Cryptosporidium) in lambs. Bull Vet Inst Pulawy, 53, 47-51.

Önder, Z., Yetişmiş, G., Pekmezci, D., Kökçü, N. D., Zafer, G., Pekmezci, A. Ç., . . . Yıldırım, A. (2021). Investigation of Zoonotic Cryptosporidium and Giardia intestinalis Species and Genotypes in Cats (Felis catus). Turkiye Parazitol Derg, 45(4), 252-256.

Özmen, O., Yukari, B., Haligur, M., & Sahinduran, S. (2006). Observations and immunohistochemical detection of Coronavirus, Cryptosporidium parvum and Giardia intestinalis in neonatal diarrhoea in lambs an kids. Schweiz. Arch.Tierheilk, 148(7), 357-364.

Saleh, M. N., Heptinstall, J. R., Johnson, E. M., Ballweber, L. R., Lindsay, D. S., Werre, S., . . . Zajac, A. M. (2019). Comparison of diagnostic techniques for detection of Giardia duodenalis in dogs and cats. Journal of veterinary internal medicine, 33(3), 1272-1277.

Santin, M., Dargatz, D., & Fayer, R. (2012). Prevalence of Giardia duodenalis assemblages in weaned cattle on cow-calf operations in the United States. Vet. Parasitol, 183(3-4), 231-236.

Sarı, B., & Arslan, M. Ö. (2013). Sığırlarda Giardiosis. In M. Özcel (Ed.), Veteriner Hekimliğinde Parazit Hastalıkları Cilt 1 (pp. 143-151). İzmir: Türkiye Parazitoloji Derneği.

Sarı, M., & Onmaz, A. C. (2011). The Evalution of Hematologic and Biochemical Parameters in Dog with Giardiosis. Sağlık Bilimleri Dergisi, 20(2), 129-136.

Savioli, L., Smith, H., & Thompson, A. (2006). Giardia and Cryptosporidium join the 'neglected diseases initiative'. Trends Parasitol, 22(5), 203-208.

Sevgisunar, N., Şahinduran, Ş., & Adanır, R. (2013). Efficacy of Secnidazole in the Treatment of Giardiasis in a Cat. MAKÜ Sag. Bil. Enst. Derg., 1(1), 26-29.

Soares, R., & Tasca, T. (2016). Giardiasis: an update review on sensitivity and specificity of methods for laboratorial diagnosis. Journal of microbiological methods, 129, 98-102.

Sursal, N., Simsek, E., & Yildiz, K. (2020). Feline Giardiasis in Turkey: Prevalence and genetic and haplotype diversity of Giardia duodenalis based on the β-Giardin gene sequence in symptomatic cats. The Journal of Parasitology, 106(5), 699-706.

Tangtrongsup, S., & Scorza, V. (2010). Update on the diagnosis and management of Giardia spp infections in dogs and cats. Topics in companion animal medicine, 25(3), 155-162.

Thompson, R. A. (2000). Giardiasis as a re-emerging infectious disease and its zoonotic potential. International journal for parasitology, 30(12-13), 1259-1267.

Thompson, R. A., Palmer, C. S., & O'Handley, R. (2008). The public health and clinical significance of Giardia and Cryptosporidium in domestic animals. The veterinary journal, 177(1), 18-25.

Traş, B., Yazar, E., & Elmas, M. (2007). Antiprotozoon tedavi. In B. Traş, E. Yazar, & M. Elmas (Eds.), Veteriner hekimliğinde ilaç kullanımına pratik ve akılcı yaklaşım (Vol. 2, pp. 120-121). Konya: Olgun Çelik Ofset Matbaa.

Yukarı, B., & Şahinduran, Ş. (2013). Giardiosis. In M. Özcel (Ed.), Veteriner Hekimliğinde Parazit Hastalıkları Cilt 2 (pp. 833-835). İzmir: Türkiye Parazitoloji Derneği.

CHAPTER 3

IMPRESSION METHODS IN IMPLANT SUPPORTED FIXED PROSTHESES

Büşra KELEŞ[1]
Elifnur GÜZELCE SULTANOĞLU[2]

1 Dt. Büşra Keleş, Sağlık Bilimleri Üniversitesi, Protetik Diş Tedavisi Ana Bilim Dalı, Orcid: 0000-0001- 8342-8675
2 Dr. Öğr. Üyesi, Sağlık Bilimleri Üniversitesi, Protetik Diş Tedavisi Ana Bilim Dalı, Orcid: 0000- 0003-2163-5219

1.INTRODUCTION

The aim in dental implantology is to treat the problems that arise as a result of tooth deficiencies, to protect the stomatognathic system and to provide aesthetics, function and phonation to the patient. Dental implants have been used successfully for decades to treat partially and totally edentulous patients with fixed dentures. (Gallucci et al.,2014, ss. 287-290) However, since osseointegrated implants do not contain periodontium and cannot make orthodontic movements, errors in size are not easily tolerated. For these reasons, the impression phase of implant supported fixed dentures requires more technical precision. (Burns et al.,2003, ss. 250-255; Del'Acqua et al.,2010, ss. 715-721) In treatment options such as implant supported fixed dentures, where the impression phase plays a direct role in success, the correct, precise and clear transfer of the position and angles of the implants in the mouth to the working model It is very important. The dimensional stability of the technique and impression material used is the most effective factor in keeping the angle and position constant. The long-term clinical success of an implant-supported prosthesis depends on the passive fit of the restoration. If the fit of the restoration does not create a static load on the prosthetic system or the surrounding bone tissue, it is called passive fit. . Today, it is accepted that prosthetic mismatch increases occlusal mismatches and mechanical complications that may occur in abutments, screws, prosthetic restorations or implant parts. The passive fit of the prosthesis and the ability to receive the desired support accurately and precisely from the hard or soft tissues starts with the impression stage. (Skalak, 1983, ss. 843-848; Philips, 1994, ss.533-40; Lorenzoni et al., 2000, ss.629-638)

However, most of the researchers agree on the excess of errors made during the impression stage. Prostheses produced based on unclear impression records cannot be expected to be compatible with implant abutments.Starting from this problem, researchers sought ways to eliminate impression items and to obtain clearer records in the impression process. (Samet et al., 2005, ss. 112-117)

The reluctance of patients and physicians to prefer traditional impression methods for different reasons has led researchers to conduct more studies on digital impression systems. (Wismeijer et al.,2014, ss. 1113-1118; Yuzbasioglu et al.,2014)

With the newly developed computer-aided devices, it is aimed to make the impression process simpler for patients and physicians and to minimize the errors that may occur during impression. Computer-assisted devices; They are digital systems based on the principle of impression (CAI-Computer Aided Impression), design (CAD-Computer Aided Design) and

production (CAM-Computer Aided Manufacturing). With CAD/CAM systems, the design of various implant spacers and superstructures is made with special software and these parts are produced on high precision scraping devices. With the rapid developments in digital dental technology, the use of computer aided impression, design and production systems in dentistry has become widespread. With the use of CAD-CAM systems, data collection techniques have been developed as an alternative to the traditional impression method. The unproven hypothesis of this study is that digital and conventional impression methods will affect the precision of impressions taken from parallel or angled implants.

2.Impression in Dental Implantology

In order to provide a passive compatible superstructure, which is important in the clinical success and prognosis of implant-supported prostheses, the impression phase must be error-free. According to the researches, it has been revealed that there are many factors affecting the impression accuracy in the 3D transfer of the implant positions to the model. (Wee, 2000, ss. 323-331):

- Impression technique
- Impression at implant or abutment level
- Splinting of impression copings in the mouth
- Splint material
- Modification of impression coping
- Number of implants and their angles
- Impression material
- Subgingival depth of the implant (De La Cruz et al., 2002, ss.329-336; Holst et al., 2007, ss. 67-73; Del'Acqua et al., 2008, ss. 226,236; Ma et al., 2012, ss.405-410)

2.1.Parts of Current Implants for Impression and Model Preparation

In making prosthesis on implants; After clinical evaluation and planning, the evaluation of the prosthetic parts of the prostheses to be made on the implants comes. These are called superstructure parts of the prosthesis. These; These are the parts between the implant placed in the jawbone and the prosthesis to be made on it. Superstructure elements can be evaluated under 3 groups, taking into account the shapes and laboratory practices of the prostheses planned to be made according to various implant types.

1. Healing caps and screws

2. Prosthetic posts and spacers

3. Impression transfer and laboratory posts and caps

2.2.Healing Heads and Screws

Healing caps and screws, which are referred to by various names such as “healing cap”, “healing screw” or “gingiva former” in the literature, vary according to implant types and surgical applications. Since the implant is completely under the mucosa in implants with dual-phase surgical procedures, healing screws are used in such implants. Approximately 1.5-3 months after the first surgery, the soft tissue is healed by placing the gingiva former selected according to the current gingival height instead of the covering screw of the implant, which is opened with an incision or “tissue-punch”.

At this stage, it is also possible to prepare a temporary crown with “esthetic caps”. (Friadent, 2007)

In one-stage cylindrical implant applications and some implant systems, there is a “transmucosal part”. This piece stays on the implant during the osseointegration circuit and shapes the mucosa. Thus, these healing caps, which prepare the ground for the insertion and removal of prosthetic posts without the need for a second surgical application, are available in the market as prefabricated under various names such as “temporary healing abutment”, “healing cap”, “tissue abutment”. Such spacers can be disposable parts made of polyethylene or multi-use parts made of titanium. (Sandallı, 2000)

The features and benefits of the healing cap are summarized as follows: - Supports soft tissue formation - Provides the formation of a biocompatible epithelial barrier. (Antgogyr, 2007)

Available in different diameters and lengths to increase flexibility. - Its design guarantees flexibility in connection with soft tissues. During the placement and compression process; The healing head is inserted into the implant with light finger force or a torque wrench, checked and tightened. The recommended torque is 10 Ncm. (Astra Tech Dental, 2007)

2.3.Prosthetic Posts and Spacers

Prosthetic posts and spacers, which form the basis of implant superstructure prostheses, vary according to different companies, as well as providing parallelism between posts, aesthetic reasons and fixed or semi-fixed prosthesis type. According to all these usage purposes, it is possible to classify prosthetic posts under 3 main headings:

Plastic cast posts Standard posts (Flat or angled) Spherical posts

2.3.1 Plastic Castable Posts

Plasticly prepared according to implant diameters, they are straight and curved posts that go into casting directly. These posts are used by being cemented on the implant.

2.3.2 Standard Posts.

Straight Posts

-Posts screwed directly from occlusal (Cemented posts (cemented abutment), Standard screwed posts (fixed abutment), Posts screwed from occlusally (straight antirotation post))

-Posts screwed from occlusally with spacer (PME abutment, straight insert)

Angled Posts

-Standard angled posts (Anguled abutment)

-Angulated antirotation post screwed from the lingual (Angulated antirotation post)

2.3.3 Spherical Posts

-Posts used in bar construction

-Posts used as precision connection (O-Ring / Ball head)

2.4. Impression Transfer and Laboratory Posts and Heads Posts

That are used to transfer the position and internal structure of the implants placed on the jaw bones to the plaster model are called impression post. They are made of titanium or aluminum in order to eliminate the difficulties during the transfer process due to the structure of the posts used in the prosthesis construction. We can examine impression posts and laboratory pieces prepared according to their intended use in 4 groups:

a.Original Posts: In some implant types, it is possible to use prosthetic posts prepared for superstructure prosthesis as impression and transfer posts.

b.Plastic impression Posts: Posts prepared from plastic can be used in impression transfer and laboratory studies. The advantage of this is that the original hides reduce the risk of deterioration and are inexpensive.

c.Metal impression posts: Upon the deterioration of plastic impression posts after a few uses, metal impression and transfer posts have been developed. It is used as a single or double piece according to the superstructure prostheses to be made.

d.Implant Die: These parts, also called "Dowel pin abutment-analogue", are metal replicas containing the position of the implants in the jawbone and the internal screwing system.

When working with the impression at the implant level, "replicas" allow to replicate the diameter of the placed implant.

Purpose of usage; is to transfer the appropriate implant to the model. Its features and benefits are; - To ensure exact copying of the implant placed in the mouth

- To provide fixed positioning in the plaster

Prosthetic posts are placed on them and the laboratory stages of the implant-supported prosthesis are completed. At least 2 weeks after the gingiva former is placed, the impression process is started for the prosthesis on the implant. After the "gingiva former" is removed, the "transfer coping" is placed in accordance with the implant diameter and fixed with a screw. Then, "transfer caps" that increase the impression sensitivity are placed on the copings and the impression is taken. (Ring, 1995)

After the measuring spoon is removed, the caps remain in the impression. Impression posts are removed from the patient's mouth and replaced with gingiva former. The existing impression posts are hand-joined with "implant analogues" and a plaster model is prepared by placing them in the impression.

3.Impression Techniques in Implant Supported Fixed Prostheses

3.1. Conventional Impression Techniques

There are 3 basic impression techniques used to transfer the implant positions to the main model. (Rashidan, 2012, ss. 218-225)

- Direct impression technique (Open spoon / Pick-up technique)

- Indirect impression technique (Closed spoon / Transfer technique)

- Snap-on (Pres-fit) technique

3.1.1. Direct Impression Technique (Open Spoon / Pick-Up Technique)

The first of the impression techniques is the direct impression technique, also called the open spoon or pick-up technique. In this technique, first impressions are taken to prepare a personal spoon. After the spoons are prepared, the impression caps are attached to the implants and the corresponding places on the spoon are pierced. Here, the feature of the measuring head is that it has a long screw on the body part. After the impression material is loaded on the spoon and pressed into the mouth, it

is expected to harden, and then the screws are loosened while the spoon is in the mouth and the caps are removed from the mouth with the spoon so that they remain in the impression material. Then, analogs are attached to the caps inside the impression material with the help of an implant key, and the modeling process is completed by pouring plaster. (Humphries et al., 1990, ss. 331-336; Spector et al., 1990, ss. 444-447; Chee et al., 2006, ss. 429-432; Ongül et al., 2012, ss. 184-189)

The advantages of this technique are that, by removing the impression caps from the mouth with the impression, errors caused by implant angles are prevented, the risk of deformation of the impression material is reduced and the error margin is minimized by eliminating the process of re-inserting the impression cap into the impression. The disadvantages are that the technique is sensitive and complicated, as well as rotational movements that may occur in the impression head. (Humphries et al., 1990, ss. 331-336)

3.1.2. Indirect Impression Technique (Closed Spoon / Transfer Technique)

The second impression technique is also called the indirect technique, the closed spoon or the transfer technique. In this technique, an impression is taken with a ready-made spoon or a personal spoon over the impression piece attached to the implant. Impression caps are not removed with the impression material, they remain attached to the implant. Afterwards, the impression caps are separated from the implant and connected to the analog and placed in the correct position in the negative space formed in the impression material. (Conrad et al., 2007, ss. 349-356)

The indirect technique is especially indicated in cases where the interarch distance is limited, there is difficulty in accessing the implants in the posterior region, or the patient has a nauseous reflex. (Conrad et al., 2007, ss. 349-356)

The advantages of this technique include visual comfort during the attachment of impression caps to implant analogues, more precise application, and less working time. However, it has been supported by studies that, as a disadvantage, the analogue combined with the impression cap cannot be placed in the impression material in the same way as in the mouth. (Liou et al., 1993, ss. 377-383) In addition, in cases where there are unparalleled implants and the number of implants is high, the problems to be encountered increase and the impression precision decreases.

3.1.3. Snap-On (Pres-Fit) Technique

Snap-on or press-fit technique, which has come to the fore in recent years, is a method in which plastic impression caps are used. It is similar

to the indirect technique in terms of taking impression with ready-made spoons and the direct technique in terms of keeping the plastic impression pieces inside the impression material. Therefore, the snap-on impression technique, which differs from these two techniques, is an approach to increase the impression precision. (Lee et al., 2008, ss. 285-291)

However, the micromovements that can be caused by the stretching of the plastic part in the impression material during the removal of the impression from the mouth can be shown as the disadvantage of the technique.

Impression at Implant or Abutment Level

Implant dimensions can also be classified as implant-level and abutment-level impression. In the impression technique at the implant level, after the healing caps are removed, the impression pieces are attached to the implant, and then the impression is taken with the open or closed spoon method.

Its advantages can be cited as facilitating temporary restoration preparation, improving aesthetics, easier abutment selection in the laboratory environment, and solving the position of angled implants with the help of angled abutments. In the impression technique at the abutment level, the abutment, which is selected in accordance with the angle, diameter and length, is connected directly to the implant by torque and the impression is taken by using plastic parts, similar to the snap-on impression technique. In this impression technique, the sensitivity of the sense of touch and the proper placement of the locking mechanism of the plastic part are of great importance. (Alikhasi et al., 2015, ss. 822-829)

Impression Material

The materials used for impression taking have different properties. These materials are important in terms of hydrophilic properties, that is, wettability with gypsum, sensitivity, clarity and ease of preparation. The most important features of the impression material are clinical sensitivity, adequate tear and deformation resistance, appropriate elastic properties and dimensional stability. (Zaimoğlu et al., 1993)

The ideal impression materials for fixed prostheses are elastomeric impression materials. Suggested materials for dental implant dimensions; polyethers, polysulfides, condensation silicones (type C silicones), polyvinylsiloxanes (type A silicones) and vinylsiloxane ethers. The impression material to be used plays an important role in the success of the treatment, especially in cases where the number of implants is high or there are deviations from the implant position.

Elastomers are synthetic polymer group impression materials that chemically cross-link when cured. These materials can flex under load and quickly return to their original dimensions when the force is removed. Chemically, there are three elastomers based on polymer chains: polysulfide, silicone (condensation and addition) and polyether. Elastomeric impression materials; It is available in low (syringe or wash material), medium or monophasic (medium), high (spoon or heavy body) and higher (putty) viscosities. Viscosity, that is, the internal resistance of the impression material to flow, increases in direct proportion to the filler content.

Polysulfide

As a result of the polysulfide reaction, water is released as a by-product. The water molecule leaving the cured material has a significant effect on the dimensional stability of the impression material.2 The cost of the polysulfide impression material is low and the working time is sufficient (4-6 min). It also has high tear strength and flexibility. This impression material should be poured between half an hour and an hour. The lead dioxide in it can cause toxic effect. Polysulfide impression material hardens over 10 minutes. It has an unpleasant taste and smell of mercaptan. Its susceptibility to plastic deformation, the need for personal spoons and adhesives can also be counted among its disadvantages.

Silicones with condensation reaction

These are called C-type silicon.

When they are mixed, there is a shrinkage due to the volatilization of the alcohol in them.

Therefore, their dimensional stability is not very good and they must be poured immediately after the impression is taken.

Additive-reactive silicones

Vinyl polysiloxane materials of the type mixed with the help of automatic devices, an ideal mixing ratio. Such as obtaining a completely homogeneous mixture and avoiding air bubbles.

For these reasons, it is more suitable for prosthetic work on implants. They are materials with good dimensional stability.

Polyether-based rubber-based impression materials

It has excellent dimensional stability.

For this reason, the pouring of the impression can be delayed.

Its working time is shorter than the other 3 rubber-based gauges.

3.2. Digital Impression Technique

The rapid development of computer aided design (Computer Aided Design / CAD) and computer aided manufacturing (CAM) technology in dentistry since the 1980s paved the way for computer aided impression (CAI) techniques. (Leinfelder et al., 1939, ss.703-707)

The most important purpose of the development of these systems can be shown as eliminating the mechanical and functional disadvantages of the materials used, increasing the production speed and reducing the cost, and providing standardization. (Strub et al., 2006, ss. 1289-1296)

The digital impression method is the first step of a digitally designed prosthetic restoration. Digital impression methods have advantages such as being a more acceptable method for the patient than conventional impression methods, eliminating negative aspects such as distortion of the impression material, creating a three-dimensional image, performing the procedure in a shorter time, and being more economical. In addition, taking impression without contacting the tissues in the early stages of osteointegration is also shown as an important advantage. (Christensen, 2009, ss. 1301-1304)

The use of digital impressions in the fabrication of implant-supported prostheses allows better evaluation of the prosthetic space around the implant, the depth of the interface to be restored, and the design and output profile configuration of the abutment. Implant supported prostheses can be produced by combining digital impression scans and CAD/CAM technology. (Patel et al., 2010, ss. 20S-4S)

Digital impression methods have been developed in order to eliminate the negative features such as the time consuming of conventional impression methods and the possibility of making mistakes during the placement of the impression heads in the impression. Digital impression technique is divided into two as direct and indirect.

3.2.1. Indirect technique

In this system, measurements are taken with conventional methods without using an intraoral scanner. The model obtained with the measurement taken is scanned with the optical or mechanical systems of the CAD / CAM system. In some systems, a virtual model can be obtained by scanning the impression surface taken without using a plaster model. The desired restoration can be applied on the virtual model. (Güth et al., 2013, ss. 1201-1208)

3.2.2. Direct technique

Conventional measurement methods have completely disappeared in

this technique. Accordingly, for the desired restoration, digital impression caps are attached to the implants and scanned with the help of intraoral imaging systems and transferred to the computer environment. In terms of sensitivity, the indirect technique differs from the direct technique as it includes conventional impression materials and measurement techniques. Because; There may be differences in the dimensional stability of the impression materials, storage conditions, distortions during disinfection, separation and incompatibility from the impression tray, and the stage during transfer to the laboratory.

4.DISCUSSION

Ensuring passive fit in implant-supported fixed partial dentures is directly related to the correct three-dimensional transfer of implant positions to the impression model. Obtaining a precise model is related to many factors such as impression technique, splinting methods, impression material, number of implants and their angles. (Jo et al., 2010, ss. 128-133)

Gimenez et al., on the other hand, used both parallel and 30 degree inclined angled implants in their models to evaluate whether they made a significant difference in their study using digital impression methods and reported that angled placement did not make a significant difference in the digital dimension. (Gimenez et al., 2014, ss. 853-862)

The features that should be in the impression materials used in implant measurements; It can be listed as giving an accurate and clear measurement, being resistant enough not to tear during removal from the mouth, dimensional stability and showing elasticity so that no permanent deformation occurs when exposed to tension. (Kempler J, 2011)

Although many impression materials have been used in the production of implant supported prostheses, including condensation silicone, polysulfide, irreversible hydrocolloid and impression plaster; It has been reported that more successful results are obtained with polyether and polyvinyl-siloxane. (Barrett et al., 1993, ss. 75-82)

There are many studies comparing the accuracy of polyether and polyvinyl-siloxane impression materials.

While no difference could be found between polyether and polyvinyl-siloxane impression materials in the majority of these studies (Bhakta et al., 2011, ss. 361-367), in 3 studies it was suggested that polyvinyl-siloxane gave a more accurate measurement compared to polyether. (Buzayan et al., 2013, ss. 1512-1520)

In our study, polyvinyl-siloxane was preferred as the sole impression material, due to its high dimensional stability and being a widely used

material, in order to provide standardization during the comparison of the open spoon and snap-on impression technique.

In order to increase the accuracy of implant measurements, impression caps with different designs and produced from different materials have been developed. In addition, it is aimed to increase the connection between the impression material and the coping and to minimize the movement of the coping in the impression material by techniques such as roughening the outer surface of the impression cap or applying adhesive. (Kempler J, 2011)

In another study comparing metal and plastic impression copings, it was concluded that the tendency of plastic copings to deformation negatively affects the accuracy of the impression. [(Walker et al., 2008, ss. 669-674)

In a study comparing square and conical shaped impression caps used in open tray and closed tray impression techniques of two different implant systems (Dentium and Nobel Biocare), more accurate measurements were obtained in the open and closed tray method with the copings of the Nobel Biocare system, and therefore the impressions were measured. It has been reported that the design of the coping has an effect on the accuracy of the measurement. (Rashidan et al., 2012, ss. 218-225)

Snap-on technique has become popular in recent years due to its clinical ease of application, providing comfort to the patient and physician, and saving time. Although this technique appears to be a reliable technique, more research is needed. (Nissan et al., 2009, ss. 413-414)

Digital scanning technology has paved the way for innovative dental treatments such as computer-guided implantation and the digital impression system for the fabrication of CAD/CAM-based restorations. Obtaining an accurate impression and uniformly transferring the intraoral state to the model is critical to producing a precise restoration. (Güth et al., 2013, ss. 1201-1208) Due to the elastic properties of impression materials, indirect digitization of the impression is not recommended. (Delong et al., 2003, ss. 438-442)

5.CONCLUSIONS

As a result of this study, in which the impression obtained from parallel and angled implants were evaluated according to digital and conventional impression techniques:

1) The effect of angled placement of the implant on the precision of the implant size is not found in digital impression, it exists in conventional sizes.

2) In conventional impression, the amount of positional and angular deviation of the distal implant was found to be higher than the implant in the mesial.

3) None of the parallel and angled implants could be transferred to the one-to-one impression model in 3D with any measurement technique used, the closest results were obtained with the digital impression method.

4) The digital impression method gave more precise results than conventional open spoon and snap-on impression methods in both parallel and angled implants.

5) When conventional impression techniques are compared among themselves, the open spoon impression technique gave better results than the snap-on technique, regardless of the angulation of the implant.

6) When the parallel and angled implant models are compared, the digital impression method, the open spoon impression method and the snap-on impression method gave the closest results to the main model, respectively.

7) It is thought that the widespread use of digital impression methods will provide higher sensitivity in terms of prosthetic superstructure and increase the success of dental implant treatment.

In the light of these results, it can be recommended to use three impression methods, firstly the digital impression method and then the conventional open spoon and snap-on in clinical applications. Among the limitations of the study, the use of a single digital impression method, the number of implants placed in a model is 2, and the implant angulation is planned as only 15°. In future studies, the study can be repeated both by increasing the number of implants and angle values and by using different digital impression systems.

6.REFERENCES

Alikhasi, M., Siadat, H., & Rahimian, S. (2015). The Effect of Implant Angulation on the Transfer Accuracy of External-Connection Implants. *Clinical implant dentistry and related research*, *17*(4), 822–829.

Anthogyr İmplants: the anthogyr guide, delivering implant solutions to you, 2007.

Astra Tech Dental: Simante üst yapılar, Protez ve laboratuar prosedürü, 2007.

Barrett, M. G., de Rijk, W. G., & Burgess, J. O. (1993). The accuracy of six impression techniques for osseointegrated implants. *Journal of prosthodontics : official journal of the American College of Prosthodontists*, *2*(2), 75–82.

Bhakta, S., Vere, J., Calder, I., & Patel, R. (2011). Impressions in implant dentistry. *British dental journal*, *211*(8), 361–367.

Burns, J., Palmer, R., Howe, L., & Wilson, R. (2003). Accuracy of open tray implant impressions: an in vitro comparison of stock versus custom trays. *The Journal of prosthetic dentistry*, *89*(3), 250–255.

Buzayan, M., Baig, M. R., & Yunus, N. (2013). Evaluation of accuracy of complete-arch multiple-unit abutment-level dental implant impressions using different impression and splinting materials. *The International journal of oral & maxillofacial implants*, *28*(6), 1512–1520.

Carr A. B. (1991). Comparison of impression techniques for a five-implant mandibular model. *The International journal of oral & maxillofacial implants*, *6*(4), 448–455.

Chee, W., & Jivraj, S. (2006). Impression techniques for implant dentistry. *British dental journal*, *201*(7), 429–432.

Christensen G. J. (2009). Impressions are changing: deciding on conventional, digital or digital plus in-office milling. *Journal of the American Dental Association (1939)*, *140*(10), 1301–1304.

Conrad, H. J., Pesun, I. J., DeLong, R., & Hodges, J. S. (2007). Accuracy of two impression techniques with angulated implants. *The Journal of prosthetic dentistry*, *97*(6), 349–356.

Del'Acqua, M. A., Arioli-Filho, J. N., Compagnoni, M. A., & Mollo, F., Jr (2008). Accuracy of impression and pouring techniques for an implant-supported prosthesis. *The International journal of oral & maxillofacial implants*, *23*(2), 226–236.

Del'Acqua, M. A., Chávez, A. M., Compagnoni, M. A., & Molo, F., Jr (2010). Accuracy of impression techniques for an implant-supported prosthesis. *The International journal of oral & maxillofacial implants*, *25*(4), 715–721.

De La Cruz, J. E., Funkenbusch, P. D., Ercoli, C., Moss, M. E., Graser, G. N., & Tallents, R. H. (2002). Verification jig for implant-supported prostheses: A comparison of standard impressions with verification jigs made of different materials. *The Journal of prosthetic dentistry*, *88*(3), 329–336.

DeLong, R., Heinzen, M., Hodges, J. S., Ko, C. C., & Douglas, W. H. (2003). Accuracy of a system for creating 3D computer models of dental arches. *Journal of dental research*, *82*(6), 438–442.

Friadent: Dentsply implant kataloğu, 2007.

Gallucci, G. O., Benic, G. I., Eckert, S. E., Papaspyridakos, P., Schimmel, M., Schrott, A., & Weber, H. P. (2014). Consensus statements and clinical recommendations for implant loading protocols. *The International journal of oral & maxillofacial implants*, *29 Suppl*, 287–290.

Giménez, B., Özcan, M., Martínez-Rus, F., & Pradíes, G. (2014). Accuracy of a digital impression system based on parallel confocal laser technology for implants with consideration of operator experience and implant angulation and depth. *The International journal of oral & maxillofacial implants*, *29*(4), 853–862.

Güth, J. F., Keul, C., Stimmelmayr, M., Beuer, F., & Edelhoff, D. (2013). Accuracy of digital models obtained by direct and indirect data capturing. *Clinical oral investigations*, *17*(4), 1201–1208.

Holst, S., Blatz, M. B., Bergler, M., Goellner, M., & Wichmann, M. (2007). Influence of impression material and time on the 3-dimensional accuracy of implant impressions. *Quintessence international (Berlin, Germany : 1985)*, *38*(1), 67–73.

Humphries, R. M., Yaman, P., & Bloem, T. J. (1990). The accuracy of implant master casts constructed from transfer impressions. *The International journal of oral & maxillofacial implants*, *5*(4), 331–336.

Jo, S. H., Kim, K. I., Seo, J. M., Song, K. Y., Park, J. M., & Ahn, S. G. (2010). Effect of impression coping and implant angulation on the accuracy of implant impressions: an in vitro study. *The journal of advanced prosthodontics*, *2*(4), 128–133.

Kempler J, 2011. The effect of impression technique, connection type and implant angulation on impression accuracy.

Lee, H., So, J. S., Hochstedler, J. L., & Ercoli, C. (2008). The accuracy of implant impressions: a systematic review. *The Journal of prosthetic dentistry*, *100*(4), 285–291.

Leinfelder, K. F., Isenberg, B. P., & Essig, M. E. (1989). A new method for generating ceramic restorations: a CAD-CAM system. *Journal of the American Dental Association (1939)*, *118*(6), 703–707.

Liou, A. D., Nicholls, J. I., Yuodelis, R. A., & Brudvik, J. S. (1993). Accuracy of replacing three tapered transfer impression copings in two elastomeric impression materials. *The International journal of prosthodontics*, *6*(4), 377–383.

Lorenzoni, M., Pertl, C., Penkner, K., Polansky, R., Sedaj, B., & Wegscheider, W. A. (2000). Comparison of the transfer precision of three different im-

pression materials in combination with transfer caps for the Frialit-2 system. *Journal of oral rehabilitation*, *27*(7), 629–638.

Ma, J., & Rubenstein, J. E. (2012). Complete arch implant impression technique. *The Journal of prosthetic dentistry*, *107*(6), 405–410.

Nissan, J., & Ghelfan, O. (2009). The press-fit implant impression coping technique. *The Journal of prosthetic dentistry*, *101*(6), 413–414.

Ongül, D., Gökçen-Röhlig, B., Şermet, B., & Keskin, H. (2012). A comparative analysis of the accuracy of different direct impression techniques for multiple implants. *Australian dental journal*, *57*(2), 184–189.

Patel N. (2010). Integrating three-dimensional digital technologies for comprehensive implant dentistry. *Journal of the American Dental Association (1939)*, *141 Suppl 2*, 20S–4S.

Phillips KM, 1994. The accuracy of three implant impression techniques: A three- dimensional analysis. Int J Oral Maxillofac Implants, 9, 533-40.

Rashidan, N., Alikhasi, M., Samadizadeh, S., Beyabanaki, E., & Kharazifard, M. J. (2012). Accuracy of implant impressions with different impression coping types and shapes. *Clinical implant dentistry and related research*, *14*(2), 218–225.

Ring M. E. (1995). A thousand years of dental implants: a definitive history--part 1. *Compendium of continuing education in dentistry (Jamesburg, N.J. : 1995)*, *16*(10), .

Samet, N., Shohat, M., Livny, A., & Weiss, E. I. (2005). A clinical evaluation of fixed partial denture impressions. *The Journal of prosthetic dentistry*, *94*(2), 112–117.

Sandallı P. : Oral İmplantoloji Kitabı, İstanbul, 2000.

Skalak R. (1983). Biomechanical considerations in osseointegrated prostheses. *The Journal of prosthetic dentistry*, *49*(6), 843–848.

Spector, M. R., Donovan, T. E., & Nicholls, J. I. (1990). An evaluation of impression techniques for osseointegrated implants. *The Journal of prosthetic dentistry*, *63*(4), 444–447.

Strub, J. R., Rekow, E. D., & Witkowski, S. (2006). Computer-aided design and fabrication of dental restorations: current systems and future possibilities. *Journal of the American Dental Association (1939)*, *137*(9), 1289–1296.

Yuzbasioglu, E., Kurt, H., Turunc, R., & Bilir, H. (2014). Comparison of digital and conventional impression techniques: evaluation of patients' perception, treatment comfort, effectiveness and clinical outcomes. *BMC oral health*, *14*, 10.

Zaimoğlu A, Can G, Ersoy E, Aksu L, 1993. Diş hekimliğinde maddeler bilgisi. AÜ Basımevi, Ankara, 515.

Walker, M. P., Ries, D., & Borello, B. (2008). Implant cast accuracy as a function of impression techniques and impression material viscosity. *The International journal of oral & maxillofacial implants*, *23*(4), 669–674.

Wee A. G. (2000). Comparison of impression materials for direct multi-implant impressions. *The Journal of prosthetic dentistry*, *83*(3), 323–331.

Wismeijer, D., Mans, R., van Genuchten, M., & Reijers, H. A. (2014). Patients' preferences when comparing analogue implant impressions using a polyether impression material versus digital impressions (Intraoral Scan) of dental implants. *Clinical oral implants research*, *25*(10), 1113–1118.

CHAPTER 4

MICROGLIAL ACTIVATION AND EFFECTS ON NEUROINFLAMMATION IN PARKINSON'S DISEASE

Hüseyin Avni EROĞLU[1]

Cemre AYDEĞER[2]

1 Associate Professor , Corresponsible author Çanakkale Onsekiz Mart University, Medicine Faculty, Department of Physiology Email: haeroglu@comu.edu.tr ORCID: 0000-0002-1040-3255

2 Research Assistant, Çanakkale Onsekiz Mart University, Medicine Faculty, Department of Physiology Email: cemre.ayd@hotmail.com.tr ORCID: 0000-0003-1654-6406

1. Parkinson's Disease

Parkinson's disease (PD) was as a neurodegenerative disease which is first identified by James Parkinson. Also, it is one the most common secondary neurodegenerative diseases mostly effecting %2-3 of 65+ aged people (Poewe et al., 2017). The disease becomes more common and more prevalent as people age. The higher incidence is between 70-79 ages. Prevalence riches highest level at between 85-89 ages. Recent studies indicated that number of sufferers from PD would be doubled in 2030 (Armstrong & Okun, 2020; Balestrino & Schapira, 2020; Zesiewicz, 2019).

Parkinson's disease is characterized with neuron injury in pars compacta area of Substantia Nigra and Lewy bodies released from alfa-synuclein proteins. Symptoms originate from dysfunction of extrapyramidal system due to lack of dopamine (Le et al., 2016; Makav & Eroğlu, 2021). One third of people don't have any symptoms in whole life but substantia nigra examination could indicate %50 neuron loss at the same time. Degeneration of substantia nigra based on a wide range diseases like vascular diseases, previous brain infections, some drugs, atherosclerosis, trauma, toxicities, tumours and loss of neuron, neurotransmitters, and synapse due to increase of erythrocytes. Independently from the causative reason, neuron loss results in dopamine deficiency and generates various symptoms (Akbayır et al., 2017; Armstrong & Okun, 2020). Those symptoms could be divided into two titles: motor and none motor symptoms. Main motor symptoms of the PD are tremor, rigidity, bradykinesia/akinesia, postural instability. Also, secondary motor dysfunctional symptoms are hypomimia and hypophonia could be counted in. None-motor symptoms commonly are neuropsychiatric, cognitive, autonomic, sleep disorders and sleep abnormalities. (Zesiewicz, 2019). The whole forementioned symptoms decrease the sufferer's life quality (Duncan et al., 2014). In addition, due to specific treatment methods mortality still increases (Herlofson et al., 2004).

PD has environmental and genetic originated multifactorial aetiology. Environmental factors are age, male gender, pesticides, rural life, brain traumas. PD occurs with combined effects of those reasons (Balestrino & Schapira, 2020; Simon et al., 2020). Most of the studies revealed that neuroinflammation by microglia are one of the reasons for PD (Doorn et al., 2012; Joers et al., 2017).

2. Microglial Activation

Microglia are yolk sac derived one of phagocytic brain immune system cells. Microglia enhance homeostasis of CNS, improvement of tissue, limit the damage and increase survival of neurons (Joers et al., 2017). Microglia are %10-15 of whole brain cells and have different distribution (Subramaniam & Federoff, 2017). Microglia have little cell bodies that

they don't phagocyte in physiologic conditions. In diseases microglia get activate and begin phagocytosis and release podocytes for moving and cytokines for killing (Zhang et al., 2016).

Microglia in fact get activate mainly in two phenotypes and depends on microglia polarisation phenotype (Zhang et al., 2016). M1 microglia are ameboid large, bodied cells. Phenotype of polarisation occurs in M1 phenotype composes a neuro-inflammatory response. This type of activation called classical activation arises from lipopolysaccharide and interferon gamma (INF-γ) stimulation and release proinflammatory cytokines like as Tumour Necrosis Factor (TNF)-α, (IL) -6, IL-lβ and redox molecules like NADPH oxidase (NOX), phagocyte oxidase (PHOX), inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1). As a result, microglia increase blood-brain barrier permeability, which leads to leucocyte infiltration.

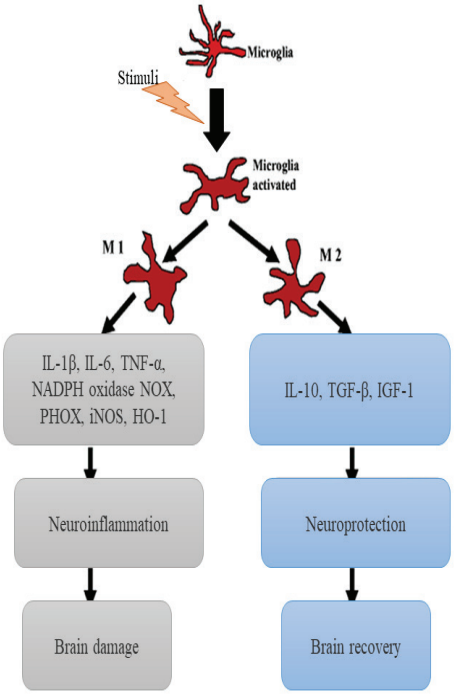


Figure 1. Representative scheme of microglial polarisation and mediators (adapted from Zhang et al., 2016).

Second type is M2 phenotype which has a large cell body. This type of polarisation is called alternative activation. Inflammatory stimulators like IL-4, IL-3, IL-13 activates M2 cells. The activation forces the release of IL-10, Transforming growth factor (TGF)-β, Insulin-like Growth Factor (IGF)-1 and improves neuroprotective effects. M2s also decrease M1

activation in order to rapid recover of tissues. M2 activation is specified like M2a, M2b and M2c. M2 activation depends on IL-4 and IL-13 release related to tissue recovery and phagocytosis. M2b activation includes TLR and IL-1 activation and release of IL-10, CD86 and MHC-II. M2c activation is induced by IL-10 and glucocorticoids. This type is related with anti-inflammatory response and improvement. Both M1 and M2 are the first defence like M2 activation enhances tissue recovery and improvement and M1 activation increase cell survival (Pajares et al., 2020a; Subramaniam & Federoff, 2017; Yao & Zu, 2020; Zhang et al., 2016).

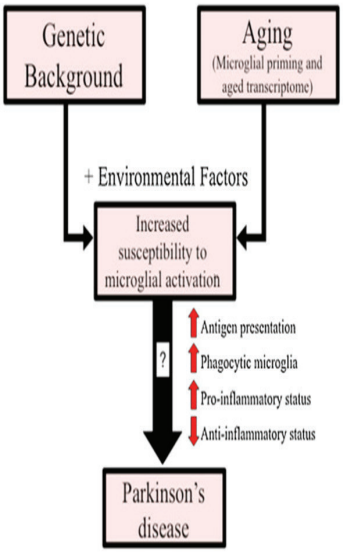


Figure 2. Inflammation's potential implications on Parkinson's disease (adapted from Joers et al., 2017) .

The imbalance between the two phenotypes is associated the onset and progression of PD (Le et al., 2016). M1 polarisation phenotype increase is one of common reason of PD. This such increase is based on the alpha-synuclein aggregates. Mass of alpha-synuclein aggregates cause microglial activation and induced to release of pro-inflammatory cytokines. The more aggregates adhesion causes the more microglial activation. This cycle at the end had a devastating effect on dopaminergic neurons. Previous studies indicated that centre of substantia nigra has a high density and is responsible of severe neurodegeneration by activated microglia. With this it is clear from the studies that dopaminergic neurons are more fragile to cytokines than other cells (Jin et al., 2019).

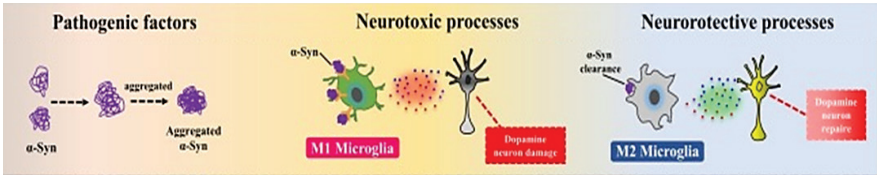


Figure 3. M1 and M2 Polarisation in Parkinson's Disease (Jin et al., 2019).

3. Inflammation in Parkinson's Disease

In PD, neuroinflammation has several causative reasons. One of the first related reasons is microglial activation. Unless the unclarified mechanisms which indicates the relationship with microglial activation and dopaminergic neuron loss, it is mainly an important factor of the disease pathology (Dzamko et al., 2017; Edison et al., 2012; Gerhard et al., 2006; Subramaniam & Federoff, 2017). Large amounts of microglia also have cytokines and mediators with that is associated with the disease underlying mechanism. Therefore, it is clear from the recent studies that there is a correlation between microglial activation, PD progression and dopaminergic neuron loss. As a result, microglial activation enhances pro-inflammatory and anti-inflammatory cytokines release and those increase the number of microglia cells (Ho, 2019; Subramaniam & Federoff, 2017).

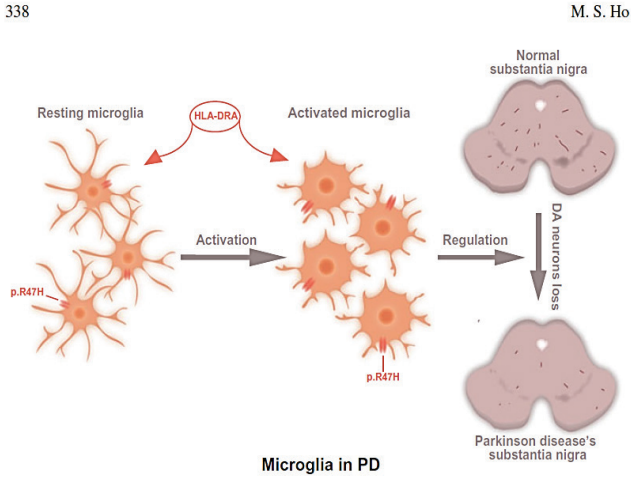


Figure 4. Microglial scheme in PD (Ho, 2019).

One of other reason of PD is peripheral inflammation. PD both involves peripheral and central inflammation. Previous studies observed that biochemical analysis of PD patients' serum and cerebrospinal fluid samples include pro-inflammatory cytokines and $CD4^{+}$ cells (Brodacki et al., 2008; Pajares et al., 2020b; Reale et al., 2009). Those changings are

based on two different theories: one of them is a neurotrophic pathogen is transmitted to brain. Both theories have the same basement that inflammation begins from brain-intestine axis. Also aggregates of alfa-synuclein is related to this type of inflammation (Holmqvist et al., 2014; Pajares et al., 2020b; Pan-Montojo et al., 2012; Subramaniam & Federoff, 2017; Ulusoy et al., 2013). Peripheral immune cells also contribute the microglial activation through increase of pro-inflammatory cytokines, that those cytokines enhance microglial activation so peripheral inflammation is also important in PD pathogenesis. All those findings suggest that there is a strong relationship between PD disease prognosis and peripheral inflammation (Subramaniam & Federoff, 2017) .

One of other inflammation reasons in PD are genetic factors. Pathogen related molecular patterns (PAMP) and Danger associated molecular pattern (DAMP) stimulates microglial activation and inflammation in PD. Studies claimed that genetic deficiencies devastating the injury and cause dopaminergic neuron loss. Novel studies revealed that there are 17 genes related to PD. PARK1, SNCA, LRK2 and PARK8 alpha-sinuclein related genes are associated with inflammation in PD (Dzamko et al., 2015; Pajares et al., 2020b; Subramaniam & Federoff, 2017).

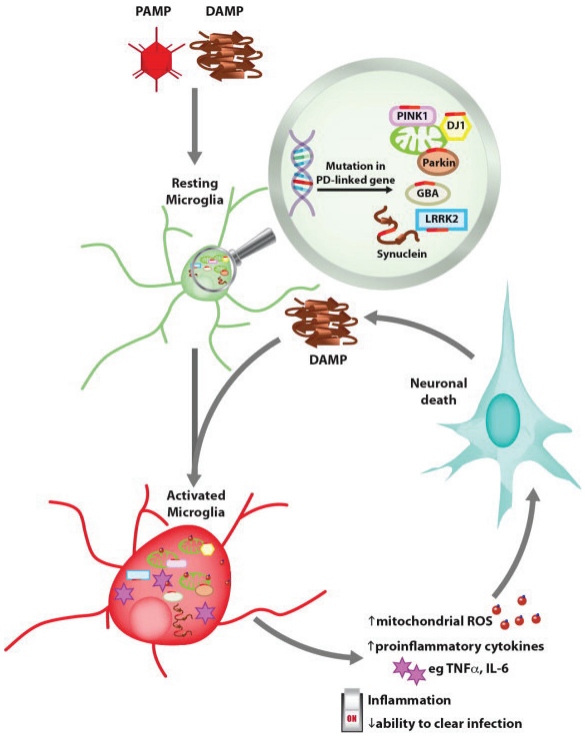


Figure 5. Genetic inflammation related genes in PD (Dzamko et al., 2015).

Even though vulnerability of dopaminergic neurons and activated inflammation mechanisms there are defensive mechanisms too. The main aim of those mechanisms is activation of M2s. These mechanisms could be specified as follows:

1- Anti-inflammation

2- Transrepression pathways

3- Neuron-microglia cross talk

4- Histone modification

5- MicroRNA regulation.

The initial part of alternative pathway begins with cytokine release in order to decrease inflammation and improve tissue damage. Also, there are substantial evidence that cytokines also trigger microglia polarisation through the alternative pathway. IL-4 is one of those suppliers (Le et al., 2016).

Secondly, transrepression pathway indicates M2 polarisation, and these activated mechanisms act in 4 different ways. Related receptors are glucocorticoid receptors, peroxisome receptors, peroxisome proliferator-activated receptor (PPAR), oestrogen receptors, and NURR1 receptors. Those receptors all inhibit nuclear factor (NF)-κB pathway and cytokines that are stimulating inflammation also activate microglia.

Third factor of alternative pathway activation is neuron-microglia cross talk. This type of cross talk is enhanced by CD200 gene and CD200R, Fraktakine (CX3CL1) and receptor CX3CR1. These molecules are placed on neurons and receptors are placed on microglia. This interaction inhibits pro-inflammatory cytokines and activate alternative pathway. In addition, CD200 and its receptor CD200R opens adenosine triphosphate sensitive K^+ channels, inhibit ATP release and decrease the activation of iNOS. CX3CL1 molecule release help signals over the neurons. CX3CR1 interaction increase the release of pro-inflammatory cytokines and decrease of NOS2 expression (Le et al., 2016; Pajares et al., 2020a).

The other factor for alternative pathway activation is histone modification. Epigenetic effective gene H3K27me3 demethylase JMJD3 mainly plays an important role. One of the studies claimed that silencing of JMJD3 increased the pro-inflammatory cytokines release and NO production. Also, JMJD3 regulates Agr1 which is the regulator of classical pathway. Those pathways both protect dopaminergic neurons via histone modification (Le et al., 2016; Tang et al., 2013).

The last and activating M2 phenotype and alternative pathway microglial activation agents are microRNAs too. Previous studies revealed

that miR-689, miR-124, miR-155, miR-21 and miR-181c are effective in PD mechanism (Le et al., 2016).

4. **Therapeutic Approaches for Neuroinflammation in Parkinson's Disease**

One of main therapeutic agent for PD is L-DOPA which is a precursor of dopamine. In addition, carbidopa, a decarboxylase inhibitor of DOPA, degrades dopamine in peripheral sideis also one of treatment agent. Providing the treatment agent is lacking, deeply brain stimulation as an operational intervention is applied. However, these types of management only improve symptoms but not the inflammation in PD. Deleteriously L-DOPA and deeply brain stimulation worsen the inflammation. Vesicles saturation gets higher with L-DOPA treatment, and it causes increase of free cytosolic dopamine level. In the end monoaminoxidase degrades dopamine. Herewith enhancement of reactive oxygen specifies augment neuroinflammation (Gordon et al., 2022). Novel studies focused on decreasing inflammation approaches (Pajares et al., 2020c).

In an experimental study, dexamethasone one of non-steroid anti-inflammatory (NSAI) was prevented degeneration in LPS induced neuro-inflammation model (Castaño et al., 2002) Ibuprofen and piroxicam both had a protective effect on dopaminergic neurons, enhanced motor functions and delayed dyskinesia caused by L-DOPA2 in Rotenone induced PD model (Teema et al., 2016). Minocycline one of half synthetic tetracycline indicated neuroprotective effects both in vitro and in vivo on PH (Cankaya et al., 2019). Similarly, ibuprofen had a reducing effect in PD risk in one of NSAI treated study, but main responsible agent couldn't be clarified (Samii et al., 2009). The reason of this uncertainty was considered as differences of drug use methods (Pajares et al., 2020c). COX inhibitors, PPAR-α agonists, adenosine antagonists, PPARγ agonists, Angiotensin II AT1 receptor inhibitors were also used for inflammation (Barbiero et al., 2014; Grammatopoulos et al., 2007; Hodgson et al., 2010; Hunter et al., 2008; Pourcher et al., 2012; Quinn et al., 2008; Reksidler et al., 2007; Rey et al., 2007; Teismann & Ferger, 2001). In addition to these studies immunomodulatory agents are also in use for inflammation in PD. Main target of these studies are stimulation of microglia via specific antibodies, reduce of cumulative alpha-synuclein and blocking alpha-synuclein transfer through intracellular area (Marogianni et al., 2020).

As a result, inflammation and microglia polarisation are important in PD. In addition to common PD management, prevention of neuroinflammation is also indispensable. Polarisation of M2 phenotype is one of neuroprotection ways. Further studies are required for the investigation of inflammation in PD and microglia polarisation.

References

Akbayır, E., Şen, M., Ay, U., Şenyer, S., Tüzün, E., & İsmail Küçükali, C. (2017). Parkinson Hastalığının Etyopatogenezi. *Deneysel Tıp Dergisi*, *7*(13), 1–23.

Armstrong, M. J., & Okun, M. S. (2020). Time for a New Image of Parkinson Disease. *JAMA Neurology*, *77*(11), 1345–1346. https://doi.org/10.1001/JAMANEUROL.2020.2412

Balestrino, R., & Schapira, A. H. V. (2020). Parkinson disease. *European Journal of Neurology*, *27*(1), 27–42. https://doi.org/10.1111/ENE.14108

Barbiero, J. K., Santiago, R., Tonin, F. S., Boschen, S., da Silva, L. M., de Paula Werner, M. F., da Cunha, C., Lima, M. M. S., & Vital, M. A. B. F. (2014). PPAR-α agonist fenofibrate protects against the damaging effects of MPTP in a rat model of Parkinson's disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *53*, 35–44. https://doi.org/10.1016/J.PNPBP.2014.02.009

Brodacki, B., Staszewski, J., Toczyłowska, B., Kozłowska, E., Drela, N., Chalimoniuk, M., & Stepien, A. (2008). Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFα, and INFγ concentrations are elevated in patients with atypical and idiopathic parkinsonism. *Neuroscience Letters*, *441*(2), 158–162. https://doi.org/10.1016/J.NEULET.2008.06.040

Cankaya, S., Cankaya, B., Kilic, U., Kilic, E., & Yulug, B. (2019). The therapeutic role of minocycline in Parkinson's disease. *Drugs in Context*, *8*. https://doi.org/10.7573/DIC.212553

Castaño, A., Herrera, A. J., Cano, J., & Machado, A. (2002). The degenerative effect of a single intranigral injection of LPS on the dopaminergic system is prevented by dexamethasone, and not mimicked by rh-TNF-α, IL-1β and IFN-γ. *Journal of Neurochemistry*, *81*(1), 150–157. https://doi.org/10.1046/J.1471-4159.2002.00799.X

Doorn, K. J., Lucassen, P. J., Boddeke, H. W., Prins, M., Berendse, H. W., Drukarch, B., & van Dam, A. M. (2012). Emerging roles of microglial activation and non-motor symptoms in Parkinson's disease. *Progress in Neurobiology*, *98*(2), 222–238. https://doi.org/10.1016/J.PNEUROBIO.2012.06.005

Duncan, G. W., Khoo, T. K., Yarnall, A. J., O'Brien, J. T., Coleman, S. Y., Brooks, D. J., Barker, R. A., & Burn, D. J. (2014). Health-related quality of life in early Parkinson's disease: The impact of nonmotor symptoms. *Movement Disorders*, *29*(2), 195–202. https://doi.org/10.1002/MDS.25664

Dzamko, N., Geczy, C. L., & Halliday, G. M. (2015). Inflammation is genetically implicated in Parkinson's disease. *Neuroscience*, *302*, 89–102. https://doi.org/10.1016/J.NEUROSCIENCE.2014.10.028

Dzamko, N., Gysbers, A., Perera, G., Bahar, A., Shankar, A., Gao, J., Fu, Y. H., & Halliday, G. M. (2017). Toll-like receptor 2 is increased in neurons in

Parkinson's disease brain and may contribute to alpha-synuclein pathology. *Acta Neuropathologica*, *133*(2), 303–319. https://doi.org/10.1007/S00401-016-1648-8/FIGURES/7

Edison, P., Ahmed, I., Fan, Z., Hinz, R., Gelosa, G., Ray Chaudhuri, K., Walker, Z., Turkheimer, F. E., & Brooks, D. J. (2012). Microglia, Amyloid, and Glucose Metabolism in Parkinson's Disease with and without Dementia. *Neuropsychopharmacology 2013 38:6*, *38*(6), 938–949. https://doi.org/10.1038/npp.2012.255

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., Eggert, K., Oertel, W., Banati, R. B., & Brooks, D. J. (2006). In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiology of Disease*, *21*(2), 404–412. https://doi.org/10.1016/J.NBD.2005.08.002

Gordon, J., Lockard, G., Monsour, M., Alayli, A., Choudhary, H., & Borlongan, C. v. (2022). Sequestration of Inflammation in Parkinson’s Disease via Stem Cell Therapy. *International Journal of Molecular Sciences 2022, Vol. 23, Page 10138*, *23*(17), 10138. https://doi.org/10.3390/IJMS231710138

Grammatopoulos, T. N., Jones, S. M., Ahmadi, F. A., Hoover, B. R., Snell, L. D., Skoch, J., Jhaveri, V. v., Poczobutt, A. M., Weyhenmeyer, J. A., & Zawada, W. M. (2007). Angiotensin type I receptor antagonist losartan, reduces MPTP-induced degeneration of dopaminergic neurons in substantia nigra. *Molecular Neurodegeneration*, *2*(1), 1–17. https://doi.org/10.1186/1750-1326-2-1/TABLES/4

Herlofson, K., Lie, S. A., Årsland, D., & Larsen, J. P. (2004). Mortality and Parkinson disease. *Neurology*, *62*(6), 937–942. https://doi.org/10.1212/01.WNL.0000115116.56955.50

Ho, M. S. (2019). Microglia in parkinson's disease. In *Advances in Experimental Medicine and Biology* (Vol. 1175, pp. 335–353). Springer New York LLC. https://doi.org/10.1007/978-981-13-9913-8_13/COVER

Hodgson, R. A., Bedard, P. J., Varty, G. B., Kazdoba, T. M., di Paolo, T., Grzelak, M. E., Pond, A. J., HadjTahar, A., Belanger, N., Gregoire, L., Dare, A., Neustadt, B. R., Stamford, A. W., & Hunter, J. C. (2010). Preladenant, a selective A2A receptor antagonist, is active in primate models of movement disorders. *Experimental Neurology*, *225*(2), 384–390. https://doi.org/10.1016/J.EXPNEUROL.2010.07.011

Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z. Y., Roybon, L., Melki, R., & Li, J. Y. (2014). Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathologica*, *128*(6), 805–820. https://doi.org/10.1007/S00401-014-1343-6/FIGURES/7

Hunter, R. L., Choi, D. Y., Ross, S. A., & Bing, G. (2008). Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague–Dawley rats.

Neuroscience Letters, *432*(3), 198–201. https://doi.org/10.1016/J.NEULET.2007.12.019

Jin, X., Liu, M. Y., Zhang, D. F., Zhong, X., Du, K., Qian, P., Gao, H., & Wei, M. J. (2019). Natural products as a potential modulator of microglial polarization in neurodegenerative diseases. *Pharmacological Research*, *145*, 104253. https://doi.org/10.1016/J.PHRS.2019.104253

Joers, V., Tansey, M. G., Mulas, G., & Carta, A. R. (2017). Microglial phenotypes in Parkinson's disease and animal models of the disease. *Progress in Neurobiology*, *155*, 57–75. https://doi.org/10.1016/J.PNEUROBIO.2016.04.006

Le, W., Wu, J., & Tang, Y. (2016). Protective microglia and their regulation in Parkinson's disease. *Frontiers in Molecular Neuroscience*, *9*(SEP2016), 89. https://doi.org/10.3389/FNMOL.2016.00089/BIBTEX

Makav, M., & Eroğlu, H. A. (2021). Recuperative effect of estrogen on rotenone-induced experimental model of Parkinson's disease in rats. *Environmental Science and Pollution Research*, *28*(17), 21266–21275. https://doi.org/10.1007/S11356-020-11985-5/FIGURES/4

Marogianni, C., Sokratous, M., Dardiotis, E., Hadjigeorgiou, G. M., Bogdanos, D., & Xiromerisiou, G. (2020). Neurodegeneration and Inflammation—An Interesting Interplay in Parkinson's Disease. *International Journal of Molecular Sciences 2020, Vol. 21, Page 8421*, *21*(22), 8421. https://doi.org/10.3390/IJMS21228421

Pajares, M., I Rojo, A., Manda, G., Boscá, L., & Cuadrado, A. (2020a). Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. *Cells*, *9*(7). https://doi.org/10.3390/CELLS9071687

Pajares, M., I Rojo, A., Manda, G., Boscá, L., & Cuadrado, A. (2020b). Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. *Cells 2020, Vol. 9, Page 1687*, *9*(7), 1687. https://doi.org/10.3390/CELLS9071687

Pajares, M., I Rojo, A., Manda, G., Boscá, L., & Cuadrado, A. (2020c). Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. *Cells 2020, Vol. 9, Page 1687*, *9*(7), 1687. https://doi.org/10.3390/CELLS9071687

Pan-Montojo, F., Schwarz, M., Winkler, C., Arnhold, M., O'Sullivan, G. A., Pal, A., Said, J., Marsico, G., Verbavatz, J. M., Rodrigo-Angulo, M., Gille, G., Funk, R. H. W., & Reichmann, H. (2012). Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. *Scientific Reports 2012 2:1*, *2*(1), 1–12. https://doi.org/10.1038/srep00898

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A. E., & Lang, A. E. (2017). Parkinson disease. *Nature Re-*

views Disease Primers 2017 3:1, *3*(1), 1–21. https://doi.org/10.1038/nrdp.2017.13

Pourcher, E., Fernandez, H. H., Stacy, M., Mori, A., Ballerini, R., & Chaikin, P. (2012). Istradefylline for Parkinson's disease patients experiencing motor fluctuations: Results of the KW-6002-US-018 study. *Parkinsonism & Related Disorders*, *18*(2), 178–184. https://doi.org/10.1016/J.PARKRELDIS.2011.09.023

Quinn, L. P., Crook, B., Hows, M. E., Vidgeon-Hart, M., Chapman, H., Upton, N., Medhurst, A. D., & Virley, D. J. (2008). The PPARγ agonist pioglitazone is effective in the MPTP mouse model of Parkinson's disease through inhibition of monoamine oxidase B. *British Journal of Pharmacology*, *154*(1), 226–233. https://doi.org/10.1038/BJP.2008.78

Reale, M., Iarlori, C., Thomas, A., Gambi, D., Perfetti, B., di Nicola, M., & Onofrj, M. (2009). Peripheral cytokines profile in Parkinson's disease. *Brain, Behavior, and Immunity*, *23*(1), 55–63. https://doi.org/10.1016/J.BBI.2008.07.003

Reksidler, A. B., Lima, M. M. S., Zanata, S. M., Machado, H. B., da Cunha, C., Andreatini, R., Tufik, S., & Vital, M. A. B. F. (2007). The COX-2 inhibitor parecoxib produces neuroprotective effects in MPTP-lesioned rats. *European Journal of Pharmacology*, *560*(2–3), 163–175. https://doi.org/10.1016/J.EJPHAR.2006.12.032

Rey, P., Lopez-Real, A., Sanchez-Iglesias, S., Muñoz, A., Soto-Otero, R., & Labandeira-Garcia, J. L. (2007). Angiotensin type-1-receptor antagonists reduce 6-hydroxydopamine toxicity for dopaminergic neurons. *Neurobiology of Aging*, *28*(4), 555–567. https://doi.org/10.1016/J.NEUROBIOLAGING.2006.02.018

Samii, A., Etminan, M., Wiens, M. O., & Jafari, S. (2009). NSAID use and the risk of parkinsons disease: Systematic review and meta-analysis of observational studies. *Drugs and Aging*, *26*(9), 769–779. https://doi.org/10.2165/11316780-000000000-00000/FIGURES/5

Simon, D. K., Tanner, C. M., & Brundin, P. (2020). Parkinson Disease Epidemiology, Pathology, Genetics, and Pathophysiology. *Clinics in Geriatric Medicine*, *36*(1), 1–12. https://doi.org/10.1016/J.CGER.2019.08.002

Subramaniam, S. R., & Federoff, H. J. (2017). Targeting microglial activation states as a Therapeutic Avenue in Parkinson's disease. *Frontiers in Aging Neuroscience*, *9*(JUN), 176. https://doi.org/10.3389/FNAGI.2017.00176/BIBTEX

Tang, Y., Li, T., Li, J., Yang, J., Liu, H., Zhang, X. J., & Le, W. (2013). Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease. *Cell Death & Differentiation 2014 21:3*, *21*(3), 369–380. https://doi.org/10.1038/cdd.2013.159

Teema, A. M., Zaitone, S. A., & Moustafa, Y. M. (2016). Ibuprofen or piroxicam protects nigral neurons and delays the development of l-dopa induced dyskinesia in rats with experimental Parkinsonism: Influence on angiogenesis. *Neuropharmacology*, *107*, 432–450. https://doi.org/10.1016/J.NEUROPHARM.2016.03.034

Teismann, P., & Ferger, B. (2001). Inhibition of the Cyclooxygenase Isoenzymes COX-1 and COX-2 Provide Neuroprotection in the MPTP-Mouse Model of Parkinson's Disease. *Synapse*, *39*, 167–174. https://doi.org/10.1002/1098-2396

Ulusoy, A., Rusconi, R., Pérez-Revuelta, B. I., Musgrove, R. E., Helwig, M., Winzen-Reichert, B., & di Monte, D. A. (2013). Caudo-rostral brain spreading of α-synuclein through vagal connections. *EMBO Molecular Medicine*, *5*(7), 1119–1127. https://doi.org/10.1002/EMMM.201302475

Yao, K., & Zu, H. bing. (2020). Microglial polarization: novel therapeutic mechanism against Alzheimer's disease. *Inflammopharmacology*, *28*(1), 95–110. https://doi.org/10.1007/S10787-019-00613-5/FIGURES/1

Zesiewicz, T. A. (2019). Parkinson Disease. *CONTINUUM Lifelong Learning in Neurology*, *25*(4), 896–918. https://doi.org/10.1212/CON.0000000000000764

Zhang, Z., Zhang, Z., Lu, H., Yang, Q., Wu, H., & Wang, J. (2016). Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. *Molecular Neurobiology 2016 54:3*, *54*(3), 1874–1886. https://doi.org/10.1007/S12035-016-9785-6

CHAPTER 5

A NEW APPROACH FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA: PIPERINE

Ozge CAGLAR[1]
Mustafa Baris KOCER[2]
Elif OZYILMAZ[3]

1 Selçuk University, Institute of Science, Department of Chemistry, Konya/Turkey,E-mail: ozgecaglar07@gmail.com ORCID ID: https://orcid.org/0000-0002-6595-1582
2 Selçuk University, Faculty of Science, Department of Chemistry, Konya/Turkey, E-mail: mbariskocer@gmail.com ORCID ID: https://orcid.org/0000-0001-8439-1966
3 Corresponding Author: Selçuk University, Faculty of Science, Department of Biochemistry, Konya/Turkey, E-mail: eyilmaz80@gmail.com ORCID ID: https://orcid.org/0000-0003-4360-4165

Introduction

Cancer is a systematic disease (Inamura et al. 2022). Liver cancer is only one of the five most common cancer types with an annual increase of 5%, causing approximately 1.8 million deaths worldwide in 2020 (Tseng et al. 2020; Anwanwan et al. 2020; Hiam-Galvez et al. 2021). Considering the case-death rates, it ranks second among all cancer types (Serper et al. 2022; Sia et al. 2017; Morrissey et al. 2016). It is estimated that the number of liver cancer patients will be over 1 million by 2030 (Caglar & Ozyilmaz 2021). There are several different types of liver cancer. Hepatocellular carcinoma (HCC) is the predominant type of liver cancer that is most common in adults and accounts for approximately 75%-85% of all liver cancers (Shehata et al. 2022, Petrick et al. 2020). HCC develops due to many factors, especially hepatitis C virus (HCV), abuse of alcohol, non-alcoholic fatty liver disease (NAFLD), and hepatitis B virus (HBV) (Fujiwara et al. 2018; McGlym et al. 2021; Topal et al. 2021; Guzel et al. 2019; Aksoy et al. 2014). The factors causing the disease and the incidence of the disease vary among societies. The highest rates of HCC in the world are seen in Africa and Asia (McGlym et al. 2021).

There are three groups of disease prevention techniques. Primary prevention focuses on preventing exposure to cancer-causing factors and eliminating disease at an early stage through lifestyle changes or vaccination or environmental interventions in an etiology-specific manner. Secondary or tertiary prevention includes early detection of HCC occurrence or recurrence in patients exposed to etiologic agents, and chemo-prevention, respectively. The figure shows the types of chemo-prevention **(Figure 1)** (Fujiwara et al. 2018).

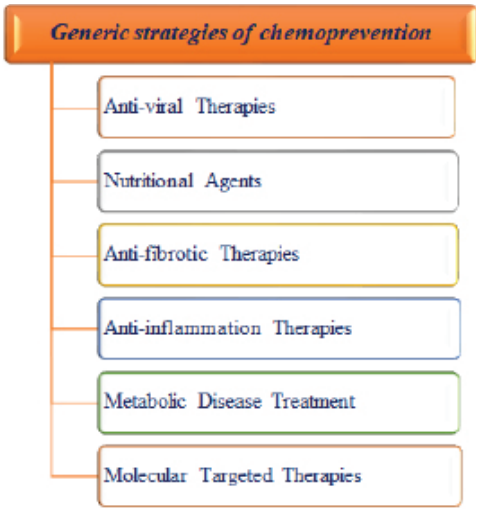


Figure 1 *Generic strategies of chemoprevention (*Fujiwara et al. 2018)

Tertiary prevention after radical HCC treatment in the patient aims to reduce de novo carcinogenesis in diffuse recurrence or residual fibrotic/ cirrhotic livers following the dissemination of residual tumor cells (Fujiwara et al. 2018).

Three methods are used in cancer treatment strategies: surgical removal of the tumor, chemotherapy, and radiotherapy (Jain et al. 2021; Ewesuedo & Ratain). Although chemotherapy is a widely used method in cancer treatments, it can cause various problems such as impairing the immune system (Jain et al. 2021; Hiam-Galvez et al. 2021). In understanding the effect on the immune system, it is significant to determine the optimum timing, effective and appropriate dose level, or to design strategies that increase anti-tumor immune responses, which may include drug combinations, rather than inhibit them (Hiam-Galvez et al. 2021). In the treatment of individual patients, standard chemotherapy combination management is determined only depending on the tumor histology and the extent of the disease (Morrissey et al. 2016).

Studies have shown that anti-cancer agents that have been traditionally applied to date often bind to serum proteins and body tissues in a rather irregular manner, and therefore only a small portion of the total drug administered reaches the tumor site. In this case, it has been observed that therapeutic efficacy is generally reduced and systemic drug toxicity is increased (Jain et al. 2021). Many patients either relapse or do not respond to treatment (Ewesuedo & Ratain). Considering that chemotherapy impairs the immune system, its undesirable effects on the body, the resistance of disease-causing pathogens to traditional antibiotics, and the side effects of drugs are considered, the development of new strategies in the treatment of HCC becomes extremely important (Jain et al. 2021; Ewesuedo & Ratain; Caglar & Ozyilmaz 2021; Das et al. 2020). In recent years, it has been explained that functional compounds obtained from food sources provide benefits in preventing diseases and improving health. Therefore, these compounds are of great interest.

Piperine

Piperine has been isolated from several species of the Piperaceae family including *Piper sarmentosum* (Hussain K et al. 2009), *Piper chaba* (Khan 2015; Rameshkumar et al. 2011), *Piper nigrum* (Kanaki et al. 2008), *Piper longum* (Mohapatara & Basak 2015), *Piper guineense* (ACS sempozyum 2013). Black pepper (Piper nigrum) is the most widely used spice worldwide with its bitter and pungent taste, and it is attracting great attention thanks to its rich chemical content and bioactivity (Haq, IU et al. 2021; Zadorozhna et al. 2021; Gupta et al. 2015). Plants of this family are leading sources of other bioactive compounds such as chalcones, lignans,

phenolics, steroids, terpenes, amides, flavonoids, neolignans, alkaloids, and various other phytochemicals. These plants appear to exhibit numerous pharmacological activities due to the presence of phytochemicals. In addition, studies have found the existence of three geometric isomers of piperine (isopiperine, isocavicin, and chavisin) **(Figure 2)** (Haq, IU et al. 2021; Ahmad et al. 2012).

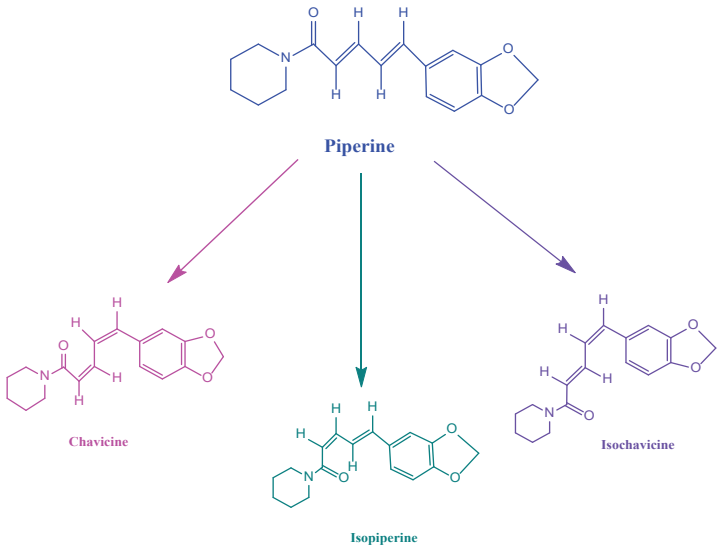


Figure 2. *Piperine and its isomers' structures* (Haq, IU et al. 2021)

Thanks to its important properties such as having numerous bioactivity and pharmacological effects, piperine acts as an antiseptic, digestive, diuretic, antibacterial, and insecticidal in traditional medicines and has been used to alleviate various diseases (Meghwal & Goswami, 2013). It has many properties such as antifungal, antiapoptotic, antimutagenic, immunomodulatory, antithyroid, antispermatogenic, and antimetastatic, and it is used in the treatment of gastrointestinal disorders such as anti-asthmatic, antioxidant, anti-inflammatory, antitumor, antihypertensive, antispasmodic, analgesic, antipyretic, anti-diarrhea, anxiolytic, hepatoprotective, antidepressant, antibacterial, dyspepsia, flatulence, constipation and hemorrhoids (Haq, IU et al. 2021; Gupta et al. 2015; Smilkov et al. 2019). Piperine has also been found to increase the oral bioavailability of multiple drugs, nutrients, and vaccines by inhibiting various metabolizing enzymes. It has also been noted to facilitate the digestive process through the stimulation of intestinal and pancreatic enzymes (Haq, IU et al. 2021; Ahmad et al., 2012). It has also been proven to increase cognitive actions and fertility (Wattanathorn et al. 2022). Based on studies of piperine's nutraceutical properties, several mechanisms have been suggested to understand its therapeutic effects (Haq, IU 2021, Hu et

al. 2022). Apoptosis is programmed cell death (Ozcan et al. 2013). It is recognized as a potential candidate in cancer research and chemotherapeutic research. Although piperine is known to induce ROS-mediated apoptosis in rectal (Yaffe et al. 2013), prostate (Ouyang et al. 2013), and colon cancer cells (Yaffe et al. 2014), it seems that the excellent scheme behind the pro-oxidant property and the effect of piperine against HCC has not yet been explained (Gunasekaran et al. 2017). Therefore, the specific focus of this study is to explain its potential in the treatment of hepatocellular carcinoma, a type of liver cancer.

With its unique properties, piperine has attracted the attention of researchers in both medicine and biochemistry in recent years (Haq, IU et al. 2021). Piperine, which is called the "king of spices", is one of the Indian medicines, and has an effect on digestive and respiratory medicines. Today, it contributes to the treatment of many important diseases (Song et al. 2020; Sun et al. 2019; Kashat et al. 2010). Piperine has poor water solubility, so dose planning becomes important in regulating its biological activities (Gorgani et al. 2017).

In patients diagnosed with colorectal cancer, the primary cause of death is cancer metastasis. Piperine has important therapeutic effects with its non-toxic, anti-inflammatory, and anticancer properties. In a study conducted by Song et al. in 2020, the role of piperine in the treatment of colorectal cancer was examined and it was determined that the migration and invasion of colorectal cancer cells were inhibited. In addition, piperine was found to reverse the epithelial-mesenchymal transition, downregulating the signal transducers and activators of transcription 3 (STAT3). Considering all the results, it has been explained that piperine can be applied as an alternative therapeutic treatment in the prevention of colorectal cancer metastasis. (Song et al. 2020).

Apart from the studies conducted by considering the unique properties of piperine, there are also studies in which it is used as a combined treatment. It is known that curcumin has a suppressive effect on induced tumor types formed by different clinical and experimental models, but its bioavailability is low due to its less absorption and rapid metabolic effect (Patial et al. 2015). Piperine increases the bioavailability of curcumin (Hashemian et al. 2019). Patial et al. investigated the synergistic effect of combined treatment with piperine in suppressing HCC by constructing a diethylnitrosamine (DENA)-induced HCC model in rats. In the results obtained, it was observed that the combined treatment significantly reduced the biochemical, apoptotic, morphological, proliferative, and histopathological changes in the liver and serum compared to curcumin alone and the control group. It has been determined that the combination of curcumin and piperine suppresses DENA-induced HCC better than

curcumin alone (Patial et al. 2015). For example, with regard to papillary thyroid carcinoma, TPC-1 cells are the most common cell line used in research because they present genetic characteristics similar to papillary thyroid cancer *in vivo* (Kashat et al. 2010). There are studies showing that cell metabolic activity can affect viability in human papillary thyroid carcinoma TPC-1 cells. Esposito et al. Considering these studies, in their study in 2019, it was investigated how the combination of curcumin and curcumin with piperine affects cell metabolic activity viability in human papillary thyroid carcinoma TPC-1 cells (Esposito et al. 2019).

Abdul Manap et al. (2019) investigated the effects of curcumin, piperine, bacoside A, and chebulinic acid in the treatment of Alzheimer's disease (AD). Of these four compounds, only curcumin and piperine have been found to have favorable affinity and interactions against acetylcholinesterase (AChE). It was determined that the combination therapy showed greater inhibition of AChE. In addition, combined curcumin and piperine were found to reverse the Aβ-induced upregulation of neuronal oxidative stress. It has been stated that the effective results of curcumin and piperine are promising in the treatment of AD and can be progressed with other studies (Abdul Manap et al. 2019; Ozyilmaz et al. 2021).

In the study conducted by Hatab et al. in 2019 and examining the new treatment approach to HCC treatment, curcumin, taurine, and piperine were administered to 20 patients at certain daily doses, and the patients were followed for 24 months. It was observed that the combined treatment caused a significant decrease in serum interleukin-10 and miR-21 levels, while the serum miR-141 expression level resulted in a nonsignificant upregulation (Hatab et al. 2019). Bolat et al. reported that combined therapy came to the fore in the follow-up and treatment of the disease. Considering that piperine increases the therapeutic effect of curcumin in their research, they examined the effect of piperine isolated from black pepper and curcumin isolated from turmeric on colorectal cancer and various cancer types. When the effect of curcumin and piperine emulsomal nanoformulations was examined, it was determined that piperine treatment alone had no effect on both free and emulzonanoforms of HCT116 cells, and it was found that anticancer activities increased when combined treatment with curcumin was applied (Bolat et al. 2020).

In a study by Zhu et al. in 2020, it was observed that natural products piperlongumine and piperine block cancer cell proliferation through the upregulation of reactive oxidative species (ROS) and eventually cell death, but have only moderate cytotoxic potential (Zhu et al. 2020). Ding et al. developed mixed micelles of Soluplus ® and TPGS in the study in which they examined the synergistic effect of docetaxel and piperine.

It was determined that cytotoxicity increased and anticancer activity was improved in HepG2 cell lines compared to free cells (Ding et al. 2020). Cancer stem cells (CSCs) contain recurrent HCC units, lacking in effective therapy targeting these CSCs, and $CD44^+$ and $CD133^+$ CSCs are prominently expressed in HepG2 cells. In the study, fluorescently activated cells were isolated and characterized using classification (FACS) analysis. Considering that piperine has a significant effect against metastasis, the effect of piperine against $CD44^+/CD133^+$ CSCs was investigated. When the data were examined, it was explained that piperine may be an effective treatment method for recurrent hepatocarcinogenesis (Tiwari et al. 2021).

Biswas et al. investigated the hepatoprotective effect by using ursolic acid (UA) for combined treatment with piperine (PIP). The bioavailability of UA alone is low. It has been tried to increase its bioavailability with the strong effect of piperine. The hepatoprotective activity of UA and PIP was evaluated by measuring the level of hepatic marker enzymes, and pharmacokinetic analyzes were also performed to determine the improvement of bioavailability. Combinations have been observed to significantly reduce enzyme levels, which show much better hepatoprotective activity than single drugs. When the findings were evaluated, it was observed that the combination of PIP and UA was an effective strategy to increase the bioavailability and hepatoprotective potential of UA (Biswas et al. 2021).

Role of Piperine in Hepatic Disorders:

It decreases alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and total protein levels (Sethiya et al. 2015). It significantly increases plasma adiponectin levels (Choi et al. 2013; Sehgal et al. 2013). It is used in the treatment of gastrointestinal disorders such as anti-asthmatic, antioxidant, anti-inflammatory, antitumor, antihypertensive, antispasmodic, analgesic, antipyretic, anti-diarrhea, anxiolytic, hepatoprotective, antibacterial, dyspepsia, flatulence, constipation, and hemorrhoids (Haq, IU et al. 2021; Gupta et al. 2015). It inhibits tyrosine kinase and catalase. Causes induction of peroxy-induced, mitochondria-mediated apoptosis. Suppressing the bioactivation of mycotoxin mediated by cytochrome P450, it prevented the toxicity of aflatoxin B1 and the formation of micronuclei in the hepatoma cells of rats (Anwanwan et al. 2020). It decreases the expression of lipogenic target genes and increases the expression of the carnitine palmitoyl transferase-1 (CPT1) gene. Reduces phosphorylation of insulin receptor substrate-1 (IRS-1) (Haq, UI et al.) Piperine also reversed insulin resistance and hepatic steatosis through activation of adiponectin-AMPK signaling (Haq, IU et al. 2021; Choi et al. 2013; Sehgal et al. 2013). Treatment resulted in a decrease in lipid peroxidation, frequency of polychromatic erythrocytes with bone marrow micronuclei, and protein

carbonyl content along with an increase in endogenous antioxidants such as glutathione peroxidase, SOD, catalase, and reductase in the liver (Haq, IU et al. 2021; Makhov et al. 2012; Sehgal et al. 2013).

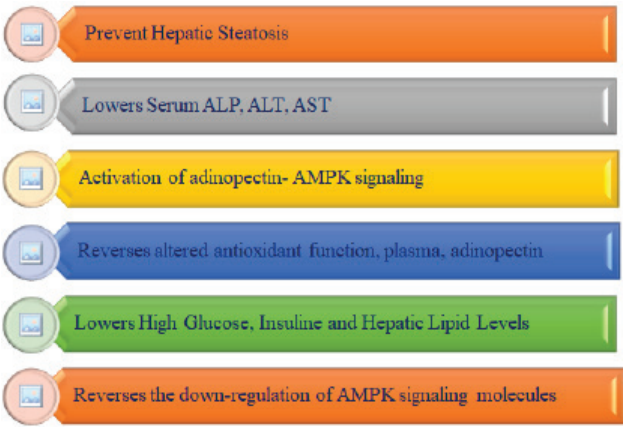


Figure 3. *Role of Piperine in Hepatic Disorders*

Conclusion and Suggestions:

HCC is an important disease that is very common not only in Turkey, but also in the world and is at the forefront of the mortality rate. Disease-causing factors differ between societies and regions. There are different stages for the treatment process of the disease. As in all diseases, early diagnosis and a good definition of the stage of the disease are very important in HCC.

Treatments with medicinal plants have been used as alternative treatment methods in the treatment of diseases for centuries. Piperine is also an important biocompatible flavonoid in the alkaloid class with numerous beneficial properties. It is thought that it will contribute to the reduction of liver damage in the treatment of HCC, reducing the course of the disease and its treatment. In this review, it is aimed to explain how piperine, which has many important properties, will contribute to the treatment of HCC.

References

Abdul Manap, A. S., Wei Tan, A. C., Leong, W. H., Yin Chia, A. Y., Vijayabalan, S., Arya, A., Wong, E. H., Rizwan, F., Bindal, U., Koshy, S., Madhavan, P. (2019). Synergistic effects of curcumin and piperine as potent acetylcholine and amyloidogenic inhibitors with significant neuroprotective activity in SH-SY5Y cells via computational molecular modeling and in vitro assay. *Front Aging Neurosci. 11*, 206.

Ahmad, N., Fazal, H., Abbasi, B. H., Farooq, S., Ali, M., Khan, M. A. (2012). Biological role of Piper nigrum L. (Black pepper): A review. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), S1945-S1953.

Aksoy, D., Solmaz, V., Taskiran, D., Erbas, O. (2014). The association between seizure predisposition and inflammation in a rat model of fatty liver disease. Neurological Sci., *35*, 1441-1446.

American Chemical Society. African Natural Plant Products Volume II: Discoveries and Challenges in Chemistry, Health, and Nutrition. *Amer. Chem. Soc*. *ACS Symposium Series*Vol. 2013, 1127.

Anwanwan, D., Singh, S. K., Singh, S., Saikam, V., Singh, R. (2020). Challenges in liver cancer and possible treatment approaches. Biochim Biophys Acta Rev Cancer. *1873*, 188314.

Biswas, S., Kar, A., Sharma, N., Haldar, P. K., Mukherjee, P. K. (2021). Synergistic effect of ursolic acid and piperine in CCl4 induced hepatotoxicity. *Ann Med. 53*, 2009-2017.

Bolat, Z. B., Islek, Z., Demir, B. N., Yilmaz, E. N., Sahin, F., Ucisik, M. H. (2020). Curcumin- and Piperine-Loaded Emulsomes as Combinational Treatment Approach Enhance the Anticancer Activity of Curcumin on HCT116 Colorectal Cancer Model. *Front Bioeng Biotechnol. 11*, 50.

Choi, S., Choi, Y., Choi, Y., Kim, S., Jang, J., Park, T. (2013). Piperine reverses high fat diet-induced hepatic steatosis and insulin resistance in mice. Food Chem. 15;141(4):3627-35.

Caglar, O., Ozyilmaz, E. Effect of Quercetin in Hepatocellular Carcinoma. In: Gece Kitaplığı, Research & Revıews In Health Scıences. Editor: Cem Evereklioğlu. Turkey; 2021. pp:311-329.

Das, S., Bera, D., Pal, K., Mondal, D., Karmakar, P., Das, S., Dey, A. (2020). Guar gum micro-vehicle mediated delivery strategy and synergistic activity of thymoquinone and piperine: An in vitro study on bacterial and hepatocellular carcinoma cells. J Drug Deliv Sci Technol., *60*, 101994.

Ding, Y., Ding, Y., Wang, Y., Wang, C., Gao, M., Xu, Y., (2020). Soluplus®/ TPGS mixed micelles for co-delivery of docetaxel and piperine for combination cancer therapy. *Pharm Dev Technol. 25*, 107-115.

Esposito, T., Lucariello, A., Hay, E., Contieri, M., Tammaro, P., Varriale, B., Guerra, G., De Luca, A. Perna, A. (2019). Effects of curcumin and its adjuvant on TPC1 thyroid cell line. *Chem Biol Interact., 305*, 112-118.

Ewesuedo, R. B., Ratain, M. J. (2003). Principles of cancer chemotherapy. In *Oncologic therapies* (pp. 19-66). Springer, Berlin, Heidelberg.

Fujiwara, N., Friedman, S. L., Goossens, N., Hoshida, Y. (2018). Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. *68*, 526-549.

Gorgani, L., Mohammadi, M., Najafpour, G. D., Nikzad, M. (2017). Piperine-the bioactive compound of black pepper: from isolation to medicinal formulations. Compr Rev Food Sci Food Saf., *16*, 124-140.

Gunasekaran V, Elangovan K, Niranjali Devaraj S. (2017). Targeting hepatocellular carcinoma with piperine by radical-mediated mitochondrial pathway of apoptosis: An in vitro and in vivo study. *Food Chem Toxicol. 105*, 106-118.

Gupta, R. A., Motiwala, M. N., Dumore, N. G., Danao, K. R., Ganjare, A. B. (2015). Effect of piperine on inhibition of FFA induced TLR4 mediated inflammation and amelioration of acetic acid induced ulcerative colitis in mice. *J. Ethnopharmacol*, *164*, 239-246.

Guzel, S., Sahinogulları, Z. U., Canacankatan, N., Antmen, S. E., Kibar, D., Bayrak, G. (2019). The ameliorating effect of silymarin against vancomycin-induced apoptosis and inflammation in rat liver. J Res Pharm, *23*, 719-728.

Haq, I. U., Imran, M., Nadeem, M., Tufail, T., Gondal, T. A., Mubarak, M. S. (2021). Piperine: A review of its biological effects. *Phytother Res. 35*, 680-700.

Hashemian, M., Poustchi, H., Murphy, G., Etemadi, A., Kamangar, F., Pourshams, A., Khoshnia, M., Gharavi, A., Brennan, P. J., Boffetta, P., Dawsey, S. M., Abnet, C. C., Malekzadeh, R. (2019). Turmeric, pepper, cinnamon, and saffron consumption and mortality. *Journal of the American Heart Association*, *8*, e012240.

Hatab, H. M., Hamid, F. F. A., Soliman, A. F., Al-Shafie, T. A., Ismail, Y. M., El-Houseini, M. E. (2019). A combined treatment of curcumin, piperine, and taurine alters the circulating levels of IL-10 and miR-21 in hepatocellular carcinoma patients: a pilot study. *J Gastrointest Oncol. 10*, 766.

Hiam-Galvez KJ, Allen BM, Spitzer MH. (2021). Systemic immunity in cancer. Nat Rev Cancer. *21*, 345-359.

Hu, X., Wu, D., Tang, L., Zhang, J., Zeng, Z., Geng, F., Li, H. (2022). Binding mechanism and antioxidant activity of piperine to hemoglobin. *Food Chem.*, *394*, 133558.

Hussain, K., Ismail, Z., Sadikun, A., Ibrahim, P. (2009). Antioxidant, anti-TB activities, phenolic and amide contents of standardised extracts of Piper sarmentosum Roxb. *Nat. Product Res.*, *23*, 238-249.

Inamura, K., Hamada, T., Bullman, S., Ugai, T., Yachida, S., Ogino, S. (2022). Cancer as microenvironmental, systemic and environmental diseases: opportunity for transdisciplinary microbiomics science. *Gut*, *71*, 2107-2122.

Jain, S., Deore, S. V., Ghadi, R., Chaudhari, D., Kuche, K., Katiyar, S. S. (2021). Tumor microenvironment responsive VEGF-antibody functionalized pH sensitive liposomes of docetaxel for augmented breast cancer therapy. Mater. Sci. Eng.: C. *121*, 111832.

Kanaki, N., Dave, M., Padh, H., Rajani, M. (2008). A rapid method for isolation of piperine from the fruits of Piper nigrum Linn. *J Nat. Med., 62*, 281-283.

Kashat, L., So, A. K. C., Masui, O., Wang, X. S., Cao, J., Meng, X., Macmillan, C., Ailles, L. E., Siu, K. W., Ralhan, R., Walfish, P. G. (2010). Secretome-based identification and characterization of potential biomarkers in thyroid cancer. J Proteome Res. *9*, 5757-5769.

Khan, M. (2015). Comparative Physicochemical Evaluation of Fruits and Antidepressant Potential of volatile oils of fruits of Local Piper Species. Oriental Journal of Chemistry, *31*, 541-545.

Makhov, P., Golovine, K., Canter, D., Kutikov, A., Simhan, J., Corlew, M. M., Uzzo, R. G., Kolenko, V. M. (2012). Co-administration of piperine and docetaxel results in improved anti-tumor efficacy via inhibition of CYP3A4 activity. Prostate. *72*, 661-7.

McGlynn, K. A., Petrick, J. L., El-Serag, H. B. (2021). Epidemiology of hepatocellular carcinoma. *Hepatol.*, *73*, 4-13.

Meghwal M, Goswami TK. (2013). Piper nigrum and piperine: an update. *Phytother Res. 27*, 1121-30.

Mohapatra, M., Basak, U. (2015). Evaluation of piperine content from roots of Piper longum Linn., originated from different sources with comparison of zonal variation in Odisha, India. *Int. J. Pharma Res. Rev, 4*, 1-8.

Morrissey, K. M., Yuraszeck, T. M., Li, C. C., Zhang, Y., Kasichayanula, S. (2016). Immunotherapy and novel combinations in oncology: current landscape, challenges, and opportunities. *Clin. Transl. Sci.*, *9*, 89.

Ouyang, D. Y., Zeng, L. H., Pan, H., Xu, L. H., Wang, Y., Liu, K. P., He, X. H. (2013). Piperine inhibits the proliferation of human prostate cancer cells via induction of cell cycle arrest and autophagy. *Food Chem. Toxicol.*, *60*, 424-430.

Ozcan, K., Satar, M., Canacankatan, N., Taskin, E., Daglioglu, K. (2013). Allopurinol's effect on caspase-3 and caspase-8 activity in hypoxic-ischemic newborn rats. *Turkish Pediatrics Archive*, *48*, 48-53.

Ozyılmaz, E., Caglar, O., Ascioglu, S., Bezgin, M., Saklan, M., Saglam, H. Erbas, O. (2021). Curcumin extraction from turmeric plant using magnetic Fe_3O_4 nanoparticles. D J Med Sci *7*, 240-247.

Patial, V., Mahesh, S., Sharma, S., Pratap, K., Singh, D., Padwad, Y. S. (2015). Synergistic effect of curcumin and piperine in suppression of DENA-induced hepatocellular carcinoma in rats. Environ Toxicol Pharmacol. *40*, 445-452.

Petrick, J. L., Florio, A. A., Znaor, A., Ruggieri, D., Laversanne, M., Alvarez, C. S., Ferlay, J., Valery, P. C., Bray, F., McGlynn, K. A. (2020). International trends in hepatocellular carcinoma incidence, 1978–2012. Int J Cancer. *147*, 317-330.

Rameshkumar, K. B., Aravind, A. A., Mathew, P. J. (2011). Comparative phytochemical evaluation and antioxidant assay of Piper longum L. and Piper chaba hunter used in Indian traditional systems of medicine. Journal of herbs, spices & medicinal plants, *17*, 351-360.

Sehgal, A., Kumar, M., Jain, M., Dhawan, D. K. (2012). Piperine as an adjuvant increases the efficacy of curcumin in mitigating benzo(a)pyrene toxicity. *Hum Exp Toxicol. 31*, 473-482.

Sehgal, A., Kumar, M., Jain, M., Dhawan, D. K. (2013). Modulatory effects of curcumin in conjunction with piperine on benzo(a)pyrene-mediated DNA adducts and biotransformation enzymes. *Nutr Cancer. 65*, 885-90.

Serper, M., Parikh, N. D., Thiele, G., Ovchinsky, N., Mehta, S., Kuo, A., Ho, C., Kanwal, F., Volk, M., Asrani, S. K., Ghabril, M. S., Lake, J. R., Merriman, R. B., Morgan, T. R., Tapper, E. B. (2022). Patient-reported outcomes in HCC: A scoping review by the Practice Metrics Committee of the American Association for the Study of Liver Diseases. *Hepatol. 76*, 251-274.

Sethiya, N. K., Shah, P., Rajpara, A., Nagar, P. A., Mishra, S. H. (2015). Antioxidant and hepatoprotective effects of mixed micellar lipid formulation of phyllanthin and piperine in carbon tetrachloride-induced liver injury in rodents. *Food Funct. 6*, 3593-603.

Shehata, E. M., Gowayed, M. A., El-Ganainy, S. O., Sheta, E., Elnaggar, Y. S., Abdallah, O. Y. (2022). Pectin coated nanostructured lipid carriers for targeted piperine delivery to hepatocellular carcinoma. Int J Pharm., *619*, 121712.

Sia, D., Villanueva, A., Friedman, S. L., Llovet, J. M. (2017). Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterol.*, *152*, 745-761.

Smilkov, K., Ackova, D. G., Cvetkovski, A., Ruskovska, T., Vidovic, B., Atalay, M. (2019). Piperine: old spice and new nutraceutical? *Curr Pharm Des., 25*, 1729-1739.

Song, L., Wang, Y., Zhen, Y., Li, D., He, X., Yang, H., Zhang, H., Liu, Q. (2020). Piperine inhibits colorectal cancer migration and invasion by regulating

STAT3/Snail-mediated epithelial–mesenchymal transition. *Biotechnol Lett. 42*, 2049-2058.

Sun, X., Veeraraghavan, V. P., Surapaneni, K. M., Hussain, S., Mathanmohun, M., Alharbi, S. A., Aladresi, A., Chinnathambi, A. (2021). Eugenol–piperine loaded polyhydroxy butyrate/polyethylene glycol nanocomposite-induced apoptosis and cell death in nasopharyngeal cancer (C666-1) cells through the inhibition of the PI3K/AKT/mTOR signaling pathway. J Biochem Mol Toxicol. *35*, e22700.

Tiwari, A., Modi, S. J., Gabhe, S. Y., Kulkarni, V. M. (2021). Evaluation of piperine against cancer stem cells (CSCs) of hepatocellular carcinoma: Insights into epithelial-mesenchymal transition (EMT). *Bioorg Chem. 110*:104776.

Topal, E., Aydemir, K., Caglar, O., Arda, B., Kayabas*ı, O., Y*ildiz, M, Ozyilmaz, E., Erbas, O. (2021). Fatty Liver Disease: Diagnosis and Treatment. JEB Med Sci. *2*, 343-357.

Tseng, H. C., Xiong, W., Badeti, S., Yang, Y., Ma, M., Liu, T., Ramos, C. A., Dotti, G., Fritzky, L., Jiang, J. G., Yi, Q., Guarrera, J., Zong, W. X., Liu, C., Liu, D. (2020). Efficacy of anti-CD147 chimeric antigen receptors targeting hepatocellular carcinoma. Nat Commun. *11*, 1-15.

Wattanathorn, J., Chonpathompikunlert, P., Muchimapura, S., Priprem, A., Tankamnerdthai, O. (2008). Piperine, the potential functional food for mood and cognitive disorders. *Food Chem. Toxicol.*, *46*, 3106-3110.

Yaffe, P. B., Doucette, C. D., Walsh, M., Hoskin, D. W. (2013). Piperine impairs cell cycle progression and causes reactive oxygen species-dependent apoptosis in rectal cancer cells. Exp Mol Pathol. *94*, 109-114.

Zadorozhna, M., Tataranni, T., Mangieri, D. (2019). Piperine: role in prevention and progression of cancer. *Mol Biol Rep. 46*, 5617-5629.

Zhu, P., Qian, J., Xu, Z., Meng, C., Liu, J., Shan, W. (2020). Piperlonguminine and Piperine Analogues as TrxR Inhibitors that Promote ROS and Autophagy and Regulate p38 and Akt/mTOR Signaling. *J Nat Prod. 83*, 3041-3049.

CHAPTER 6

PHYSIOLOGICAL ROLES OF NESFATIN-1 AND ASPROSIN IN MECHANISMS CONTROLLING APPETITE

Gözde ATİLA USLU[1]
Hamit USLU[2]

1 Assoc. Prof. Gözde ATİLA USLU, Erzincan Binali Yıldırım University, gzd.gozde@hotmail.com, Orcid id: 0000-0002-2328-9164
2 Assoc. Prof. Hamit USLU, Erzincan Binali Yıldırım University, hamit_uslu@hotmail.com, Orcid id: 0000-0002-3974-5814

1. Introduction

Appetite is the desire to eat food, caused by the feeling of hunger. Appetite, which has a very complex mechanism, is regulated by the coordinated functioning of several tissues, organs, hormones and nerve cells between central and peripheral pathways. It is among the main factors related to the regulation of energy metabolism. Therefore, it is an undeniable fact that the regulation of appetite is highly related to a healthy life. Nesfatin-1 and asprosin are known to be involved in numerous mechanisms, particularly those of energy metabolism and appetite. In this review, the physiological roles of nesfatin-1 and asprosin in the arrangement of mechanisms controlling appetite are discussed.

Nesfatin-1

Nesfatin-1 is an anorexigenic peptide consist of non-esterified fatty acid (NEFA)/nucleobindin2 (NUCB2) that is expressed in various areas, including arcuate nuclei (ARC), supraoptic nuclei (SON) paraventricular nuclei (PVN), lateral hypothalamic area (LHA) and nucleus tractus solitaries (NTS) (Oh et al., 2006; Kohno et al., 2008). Post-translational processing of NUCB2 by prohormone convertase produces 3 cleavage products called nesfatin (nesfatin-1, 2 and 3) (Oh et al., 2006; Ramanjaneya et al., 2010). It has been expressed that NUCB2, the precursor of this anorexigenic peptide, is highly expressed in two endocrine cell pools in rat oxinic gastric glands (Stengel et al., 2009) and that another resource of nesfatin-1 is the adipose tissue an endocrine organ involved in the peripheral regulation of satiety (Senin et al., 2015). Research has shown the presence of nesfatin-1 in both mouse and human adipocytes. Moreover, it was determined that the levels of nesfatin-1 determined in the circulatory system of mice on a high-fat diet were higher than those on a normal diet (Ramanjaneya et al., 2010). In another study on mouse, it was shown that nesfatin-1 is expressed in interstitial cells and Leydig cells of the pancreas, columnar epithelium of the epididymis, theca and interstitial cells of the ovary, and epithelial cells of the endometrium in the uterus and uterine glands (Kim et al., 2014). Zhang et al. (2010) reported that nesfatin-1/NUCB2 is expressed in the stomach and duodenum, pancreas, but not in the oesophagus, liver and colon of rodents.

Investigation of the mechanisms of action of nesfatin-1 disclosed that nesfatin-1 interacting with G protein-coupled receptors and stimulates Ca^{2+} flow in most cell types, thereby causing both neuronal depolarisation and intracellular Ca^{2+} increase (Brailoiu et al., 2007).

1.1. Physiological Functions

➢ Intracerebroventricular application of nesfatin-1 demonstrated that cause a dose-dependently reduce in food intake, while infusion of an

antibody neutralising nesfatin-1 has been reported to stimulate appetite. In addition, it was determined that nesfatin-1, which was applied chronically to rats, caused a decrease in body weight (Oh et al., 2006).

➢ Because nesfatin-1 was detected that expressed in the pancreas, some studies have examined its potential role in insulin- and glucagon-mediated glucose metabolism. In a study performed with hyperglycemic db/db mouse, it was shown that nesfatin-1 application caused an anti-hyperglycemic effect that was time- and dose-dependent. In addition, the same study also found that nesfatin-1 did not exhibit a blood glucose-lowering effect in normoglycemic animals. Anti-hyperglycemic activity of nesfatin-1 is insulin dependent has been demonstrated by the ineffectiveness of intravenous administration of nesfatin in lowering blood sugar in mouse with experimentally induced type I diabetes (Su, Zhang, Tang, Bi & Liu, 2010). Li et al. (2013) have reported nesfatin-1 influenced the arrangement of glucose metabolism by altering GLUT4 translocation and AKT phosphorylation in the liver, fat and skeletal muscles, consequently increasing insulin secretion and sensitivity. In another study, it has been revealed that nesfatin-1 perform an insulinotropic effect by affecting β cells in the pancreas. Considering the changes in pancreatic nesfatin-1 production in type I and II diabetes, this suggests that nesfatin-1 might have a concidderable role of the pathophysiology of both diabetes and obesity (Gonzalez et al., 2011). Moreover, glucose and insulin were found to interact directly with nesfatin-1 neurons in the PVN and increase the Ca^{2+} levels (Gantulga, Maejima, Nakata & Yada, 2012).

➢ Nesfatin-1 affects cardiovascular regulation, and when administered intracerebroventricularly, it exerts a hypertensive effect by acting on the melanocortin-3/4 receptors in the hypothalamus (Osaki & Shimizu, 2014). Further, nesfatin-1 is not only involved in central mechanisms but also inhibits NO-mediated smooth muscle relaxation by impairing peripheral arterial cGMP production and affecting peripheral arterial resistance (Yamawaki et al., 2012). In another research, it was stated that intracerebroventricularly administered nesfatin-1 stimulates renal sympathetic nerve activity and increases blood pressure in a dose-dependent manner (Tanida & Mori, 2011).

➢ The expression of NUCB2 mRNA and nesfatin-1 protein in the adipose tissue is suggested to be regulated by cytokines, insulin and corticosteroids, and theirs secretion is affected by metabolic factors involved in energy balance; it has also been suggested that nesfatin-1 may be a novel adipokine (Ramanjaneya et al., 2010). In another study, nesfatin-1 protein was defined in brown adipocytes, which plays a significant role in thermoregulation. Nesfatin-1 reportedly affects the differentiation of primary brown adipocytes through an mTOR-dependent mechanism, and its level

decreases significantly during this differentiation (Wang et al., 2016). Further, nesfatin-1 application to the lateral parabrachial nucleus (PBN) of rats decreases body-weight gain and increases thermogenin expression in brown adipocytes. Nesfatin-1 exerts a highly significant effect on energy balance by reducing appetite, altering glucose-sensitive neurons, and increasing the expression of thermogenin in brown adipocytes (Yuan et al., 2017).

➢ Nazarnezhad et al. (2019) statement that nesfatin-1 exerts a cytoprotective effect by reducing the excessive production of reactive oxygen radicals and also inhibits mitochondrial dysfunction-induced apoptosis. In lipopolysaccharide-induced acute lung injury in mice, exogenously administered nesfatin-1 decreased inflammation and oxidative stress, and similar findings were revealed in an alveolar epithelial cell line, BEAS-2B (Wang et al., 2020). Further, intraperitoneal administration of nesfatin-1 significantly improves histological lesions in renal ischaemia-reperfusion injury induced in rats and reduces oxidative stress by significantly decreasing malondialdehyde levels and significantly increasing superoxide dismutase and catalase activities (Jiang et al., 2015). The researchers indicated that plasma nesfatin-1 level was important reduced in rats exposed to mobile phone electromagnetic radiation, and there was a negative relationship between the decrease in nesfatin-1 levels and thyroid dysfunction, apoptosis, and oxidative stress (Hussien, Mousa & Shoman, 2020).

➢ When administered centrally, Nesfatin-1 has been shown to be effective in producing anxiogenic and fear-enhancing effects in the assessment of anxiety and/or fear in various animal models. For instance, a study reported that the duration spent in the arms of the raised + shaped maze and the consumption of delicious snacks in the anxiogenic environment decreased, whereas in the conditioned emotional response test, there was an increase in the startle response that reinforces fear (Merali, Cayer, Kent & Anisman, 2008). In another human study, it was suggested that major depressive disorder is associated with increased plasma nesfatin-1 levels (Ari, Ozturk, Bez, Oktar & Erduran, 2011). In another research, it was found that both saliva and serum nesfatin-1 levels in individuals with epilepsy were excessively elevated compared to healthy individuals (Aydin et al., 2009).

1.2. Asprosin

Asprosin is a glucogenic protein structure hormone encoded by exons 65-66 of the fibrillin 1 (*FBN1*) gene. Asprosin was discovered by Romere et al. (2016) during their investigation of patients with neonatal progeroid syndrome (NPS). Despite the low plasma insulin level in these patients, normoglycemia is observed. In In addition, patients with NPS have

increased anorexia, decreased calorie intake, and consequently extreme weakness. Therewithal, in these patients, energy spending decreased and in part lipodystrophy was formed in the face and extremities. These abnormalities may result from possible effects of asprosin on energy metabolism (Romere et al., 2016). FBN1 contains 66 exons 235 kb wide, encoding a 2871 amino acid long proprotein, and is located on chromosome 15q21.1. (Sakai, Keene, Renard & Backer, 2016; Lönnqvist, Reinhardt, Sakai & Peltonen, 1998). This translated proprotein is then divide into the active protease furin into mature fibrillin-1 and asprosin (140 aa in length) (Lönnqvist et al., 1998).

Asprosin, a hormone secreted by white adipose tissues (WAT), is transported from the adipose tissues to the liver, where it allows glucose to pass into the circulation rapidly (Romere et al., 2016). Kocaman and Kuloğlu (2020) reported that in a diabetic rat model, asprosin is expressed in the stomach, kidney, testis, brain, heart, and liver tissues. The finding that FBN1 is secreted from many tissues in humans showed that although WAT is the main source of plasma asprosin production, it is not the only source (Hoffmann, Xie & Chopra, 2020). Harmoniously with the hepatic glucose secretion requirement during fasting, the circulating asprosin levels increases in the fasting state and decreases quickly with refeeding. In short, asprosin exhibits a circadian rhythm in coordination with the nutritional status. In a recent study, it was reported that the interlobular ducts and striated ducts of the submandibular and parotid salivary glands expressed asprosin (Ugur & Aydin, 2019).

1.2.1. Physiological functions

➢ Romere et al. (2016) stated that asprosin has no direct effect on insulin level and/or sensitivity, and its effect on glucose homeostasis is restricted only to production of glucose in the liver. Asprosin uses the cAMP second messenger system to activate the PKA signal and thus exerts its glucogenic effect. And hepatocyte glucose release induced by asprosin was determined to be blocked when G protein function or cAMP-PKA signals were pharmacologically hindered. In a study in mice with type I diabetes, increased liver asprosin levels were reported (Ko et al., 2019). In addition, in a study involving 170 adults with type II diabetes, serum asprosin levels were shown to increase (Zhang, Chen, Zhou, Fu & Cheng, 2019). Ko et al. (2019) expressed that exercise decreased hepatic asprosin levels in mice with chemical agent (streptozotocin)-induced type I diabetes, while improving diabetes-related parameters. Individuals with type II diabetes with and without nephropathy were determined that have higher asprosin levels according to individuals with normal glucose tolerance. This increase in asprosin levels seen in type II diabetes has suggested that it may be related to the early stages of diabetic nephropathy (Zhang, Hu & Zhang, 2020).

In fact, how asprosin acts has not been fully elucidated today. However, preliminary studies have shown that the asprosin-specific antibody could possibly be used to treat type II diabetes and obesity (Atila Uslu, 2019).

➢ Duerrschmid et al. (2017) showed that asprosin administered intravenously from the periphery helped maintain adiposity by stimulating appetite in mutant mice. In the same study, it was reported that asprosin can exceed the blood-brain barrier, and recombinant asprosin injection (i.c.v) triggers appetite in mice. Another study found that asprosin level, increasing in response to a rising in BMI, decreases when the BMI decreases (Ugur & Aydin, 2019). Many researchers have stated that obese boys have lower asprosin levels compared to obese girls, and there is a negative correlation between the male sex and asprosin levels. This is possibly due to well-known sex-related differences such as thermogenesis, fat oxidation and lipid metabolism in obesity (Corica et al., 2021; Long et al., 2019). A positive correlation has been reported between asprosin and polycystic ovary syndrome (PCOS), which involves impaired hormonal balance and affects women of reproductive age (Alan et al., 2019). In a study of 41 people with PCOS, it was found that the level of asprosin increased compared to healthy individuals. Besides, a positive relationship was demonstrated between plasma asprosin levels and testosterone, LDL-C, HbA1c and ApoB levels (Li et al., 2018).

➢ A study on 120 untreated patients with pathologically approved lung or gastrointestinal cancer and 14 patients with mild gastritis found not significant differences in asprosin levels by cancer type or stage, sex and age. Moreover, asprosin levels are positively related with body fat mass, but not with C-reactive protein, blood platelet count, haemoglobin, leukocyte count, body mass index, albumin, glucose, LDL, HDL, triglyceride, cholesterol, muscle mass, skeletal muscle, protein, body fat percentage, basal metabolic rate and lean body mass. The same study also determined that no difference in asprosin levels between patients with and without cachexia and patients with gastritis and gastric cancer. In addition, it was determined that asprosin levels of anorexia patients were lower than those without anorexia (Du et al., 2021).

➢ Wen et al. (2020) in their in vitro study using cardiomyoblasts, reported that asprosin prevents hypoxia-related cell death, as well as increases proton leakage and mitochondrial respiration in hypoxia.

1.3. Overview of Central and Peripheral Mechanisms Involved in the Regulation of Appetite

Appetite is regulated by central and peripheral mechanisms. Centrally, the neural pathways connect the nuclei of the hypothalamus to the higher centres and the brain stem. These centres control sensory pleasure,

reward-based food consumption, gastric motility, and gastric emptying and calorie consumption. The ARC, ventromedial hypothalamic nucleus (VMH), dorsomedial hypothalamic nucleus (DMH), PVN and LHA in the hypothalamus play a significant role in the central control of hunger / satiety. In ARC, one group of neurons is co-localised with the orexigenic neuropeptides NPY and agouti-related peptide (AgRP), whereas another group of neurons is co-localised with the anorexigenic neuropeptides proopiomelanocortin (POMC), leptin receptors, cocaine and amphetamine-regulated transcript (CART) and α-melanin stimulating hormone (α-MSH) (Elias et al., 1998; Harrold, Dovey, Blundell & Halford, 2012). The PVN and PBN are anorexigenic centres in addition to POMC neurons and central melanocortin pathways, and an important population of neurons in the PVN suppress food intake. However, Sim1 neurons in the PVN expressing TRH or pituitary adenylate cyclase-activating peptide are also associated with orexigenic NPY/AgRP neurons in the ARC, i.e. they are not only responsible for anorexigenic effects (Sohn, 2015). LHA has been found to be associated with brain regions such as PVN and ARC, which have an impact on energy metabolism and regulation of food consumption. In fact, electrical excitation of the LHA results in increased food consumption (Delgado & Anand, 1952; Hsu et al., 2015). The VMH is the most abundant region of the subpopulation of neurons concerned in the regulation of food consumption. It also contains leptin, insulin, MCH and orexin receptors, which are involved in energy metabolism (Unger, Calderon, Bradley, Sena-Esteves & Rios, 2007). In a study on rats, it was found that bilateral lesions in the VMC region triggered hyperphagia and obesity (Anand & Brobeck, 1951). Consistent with this information, it has been stated that the DMC contains high levels of NPY terminals and α-MSH terminals originating from the ARC, and DMC damage results in hyperphagia and obesity (Bernardis & Bellinger, 1987; Yu & Kim, 2012). Another part of the brain involved in the central regulation of appetite is the brain stem. The satiety signals stem from the gastrointestinal system are transmitted to the nucleus tractus solitarius (NTS) in the brain stem via the vagus nerve. Transmissions from the NTS and PBN innervate the PVN, DMH, and ARC of the hypothalamus, the LHA, and the central nucleus of the amygdala (Ahima & Antwi, 2008). NTS has a high density of NPY binding sites, including Y1 and Y5 receptors (Wynne, Stanley, McGowan & Bloom, 2005). The brain stem is also dense in melanocortin-4 receptors (MC4Rs), which play a significantly role in regulating appetite, body weight, energy consumption and locomotor activity (Fang et al., 2016). In another study, it was suggested that the application of MC3R/MC4R agonist to the dorsal motor nucleus (DMN) of the vagus nerve decreased food intake, while the application of MC3R/MC4R antagonists increased food intake (Williams, Kaplan & Grill, 2000).

Peripheral signals born of the oral cavity, oropharynx and gastrointestinal tract are highly effective in regulating appetite. A number of circulatory factors like insulin and leptin are associated with the hindbrain and hypothalamus and are effective in the regulation of nutrition. Moreover, vagal afferent neurons originating from the gut transmit enteric information to the NTS through the nodose ganglia, which is the lower ganglion of the vagus nerve (Kim, Seeley & Sandoval, 2018). The NTS is a sensorial complex that connects to the DMN of the vagus located in the brainstem for the activation of digestive processes. The NTS exerts this effect through neurons that secrete neuropeptide Y, acetylcholine (ACh) and TRH. The nervus vagus is the main mediator of gastric motility and the cephalic phase of digestion, and its major neurotransmitter ACh. Additionally, gastrin and cholecystokinin are among the factors that regulate pepsin, gastrin and gastric acid secretions and pancreaticobiliary secretions. In this way, they act on gastric motility and gastric emptying. With food intake, gastrointestinal hormones are secreted, thereby activating stomach - bowel movements and gastric/pancreaticobiliary secretions and absorptions (Camilleri, 2006). Incretins, such as Glucagon-Like Peptide, peptide YY and oxyntomodulin, released from the proximal/distal part of the small intestine, inhibit the cephalic phase of digestion, which is usually mediated by the vagus nerve. CCK, gastrin, glucose-dependent insulinotropic peptide (GIP), gastric leptin, ghrelin and pancreatic polypeptide are secreted from the upper part of the small intestine. Upper intestinal hormones like GIP and CCK suppress gastric motility to prevent gastric emptying. To achieve this, they either relax the fundus and antrum or stimulate pyloric contractions. As a result, food digestion slows down and calorie intake is reduced, consequently inducing satiety (Camilleri, 2015).

Leptin, which is secreted in small amounts by the gastric epithelium, is mostly secreted by WAT. There are abundant leptin receptors in the hypothalamus neurons, especially in the ARC. Leptin connect to its long form receptor on the ARC. In this way, inhibition of JAK2-STAT3 pathway and AMPK activity occurs (Minokoshi et al., 2004). Activation of leptin signalling in the hypothalamus causes an increase in the activity of POMC/CART neurons and a decrease in activity in NPY/AgRP neurons (Sahu, 2003). In this way, while food intake is reduced, energy expenditure is increased (Yu & Kim, 2012). Insulin is one of the hormones that binds to its receptors on ARC neurons, causing activation of POMC neurons and inhibition of NPY/AgRP neurons via IRS-2 and PI3K-Akt-FoxO1. Thus, insulin transmits an appetite suppressing stimulus to the brain (Taniguchi, Emanuelli & Kahn, 2006).

In various studies, it has been observed that peptide $YY_{3–36}$ inhibits

hypothalamic NPY-expressing neurons and AgRP-expressing neurons via inhibitory neuropeptide Y2 receptors, thereby inhibiting neighboring proopiomelanocortin-expressing neurons and consequently reducing food consumption. Ghrelin, an acylated peptide with 28 amino acids synthesized in oxyntic cells take place in the fundus of the stomach, has a considerable role in energy balance and especially in pre-meal fasting and meal initiation. It is known that circulating ghrelin levels increase before food consumption and reduce after food consumption. Ghrelin increases the feeling of feeding by binding to receptors on hypothalamic NPY-expressing neurons and AgRP-expressing neurons (Korner & Leibel, 2003).

1.4. Physiological Effects of Nesfatin-1 and Asprosin on the Regulation of Mechanisms Controlling Appetite

A study on male rats found a dose-dependent decreased in feeding 6 hours after intracerebroventricular injection of nesfatin/NUCB2. Moreover, chronic injection of nesfatin-1 in rats demonstrated to decrease body-weight gain and adipose tissue weight, including mesenteric WAT weight, but not gastrocnemius muscle weight (Shimizu, Ohsaki, Oh, Okada & Mori, 2009). Another study investigated the effects of peripheral application of three separate segments of nesfatin-1 (N23-M30-C29) on food intake and reported that the middle segment consisting of only 30 amino acids (M30) causes anorexia by activating POMC and CART neurons in the NTS, possibly through a leptin-independent mechanism. Although the intracellular mechanism induced by nesfatin-1 is not fully known, nesfatin-1 interacts with G protein-coupled receptors and causes an elevated in Ca^{2+} owing to PKA activation (Shimizu et al., 2009). Since the anorexigenic function of NUCB2/nesfatin-1 is modulated in a non-leptin-dependent manner, unlike most hormones involved in the appetite mechanism, the idea that it can be used in drug treatment of metabolic diseases such as leptin resistance and obesity gains importance (Stengel & Taché, 2013). Nesfatin-1 acutely injected into the brain reduces food intake in the dark phase by 13%, 27%, and 46% in a dose-dependent manner. However, it was stated that a statistically significant decrease occurred only in the group that received a dose of 3 μg/mouse. Further, Nesfatin-1 perform its anorexigenic effect via central mechanisms, and a peripherally injected dose (intraperitoneal or subcutaneous) 23 times higher than the central dose does not affect dark-phase food consumption or meal pattern (Goebel, Stengel, Wang & Taché, 2011). Another study in mice showed that centrally application nesfatin-1 reduced food consumption and suppressed gastroduodenal motility (Atsuchi et al., 2010). Nesfatin/NUCB2 is expressed in endocrine cell types of gastric oxyntic mucosa of rats, and NUCB2 mRNA is expressed approximately 10 times more in the

gastric mucosa than in brain and heart tissues. Because NUCB2 expression was found to reduce in oxyntic mucosal small endocrine cells after 24 hours of fasting, it has been suggested that it may be involved in peripheral satiety signalling (Stengel et al., 2009). In a research investigating whether the level of nesfatin-1 has an effect on regulating circadian nutrition, it was found that NUCB2 mRNA levels in the PVN increased in the early light phase when rats showed minimal food consumption and reduced during the dark phase when rats showed higher food consumption (Sedbazar, Maejima, Nakata, Mori & Yada, 2013). In another study investigating the effects of nesfatin-1 on membrane excitability, nesfatin-1 application was shown to hyperpolarise the orexigenic NPY neurons through the activation of K_{ATP} channels, thereby contributing to its anorexigenic effect (Price, Samson & Ferguson, 2008). It has been found that Nesfatin-1 has anorexic effects in newborn chicken and histamine H1 and H3 receptors and corticotropin CRF1/CRF2 have a share in this effect (Heidarzadeh, Zendehdel, Babapour & Gilanpour, 2018). Activated nesfatin-1 neurons stimulate oxytocin neurons in the PVN, resulting in the transmission of oxytocinergic signals to the NTS POMC neurons, consequently causing melanocortin-induced anorexia (Maejima et al., 2009). It has been shown in confocal immunohistochemical images of the general of nesfatin-1/NUCB2 neurons in the PVN respond to NPY and/or α-MSH, and that both NPY and α-MSH neuronal terminals contact nesfatin-1/NUCB2 neurons in the PVN. This information has shown that NPY inhibits PVN nesfatin-1/NUCB2 neurons and activates α-MSH, making important contributions to the balanced regulation of nutrition (Sedbazar, Ayush, Maejima & Yada, 2014). Another physiological mechanism by which nesfatin-1 is involved in regulating food consumption is its role in causing changes in the excitability of glucose-sensitive neurons. For instance, it has been expressed that nesfatin-1 modulates food consumption by inhibiting most glucose-inhibited neurons in the LHA, stimulating most glucose-stimulated neurons in the VMN, and modulating the activity of glucose-sensitive neurons in the PVN and the excitability of glucose-sensitive neurons and gastric distension-sensitive neurons in the dorsal vagal complex (Chen, Dong & Jiang, 2012; Dong, Guan, Jiang & Chen, 2014).

Asprosin perform a glucogenic effect on the liver through the olfactory G protein-coupled receptor OR4M1. Asprosin activates orexigenic AgRP neurons in the brain via an as yet unidentified receptor. As a result, it indirectly blocks anorexigenic POMC neurons and also stimulates appetite (Hoffmann et al., 2020). Li et al. (2019) revealed that the olfactory receptor Olfr734 acts like an asprosin receptor to maintain glucose balance not only in fasting but also in obesity. Asprosin also activates the hypothalamic feeding circuit in the fasting state or as a result of its intravenous administration,

consequently stimulating appetite in mice. According to the data obtained, asprosin can increase food affinity and fat mass without changing energy expenditure. It has been determined that asprosin shows its orexigenic effect by increasing AgRP + neuron activation via Gαs-cAMP-protein kinase A, and changes in resting membrane potential and firing frequency having a role in the emergence of these effects. Researchers demonstrated that in rodents asprosin significantly increases food affinity, whereas in obese experimental animals administered an asprosin-specific antibody there is a reduction in body weight (Duerrschmid et al., 2017). Similarly, Wang (2019) reported that asprosin may have a complicated interaction with intestinal microbiota changes or gastrointestinal hormones. In another research, it was specified increased appetite and food intake during pregnancy were positively correlated with an increase in asprosin levels. It brings to mind the idea that this is an extremely important mechanism that can have an impact on the physiology of pregnancy. Similarly, rats with gestational diabetes have been reported to have important higher levels of asprosin than normal pregnant (Mohamad, Hany & Rania, 2020).

2. Conclusion

Expanding our knowledge and experience about the physiological mechanisms of nesfatin-1 and asprosin, which have very important roles in the regulation of appetite, would be very useful in overcoming and/or controlling metabolic diseases, which have become a growing social health problem in the world.

References

Ahima, R. S., & Antwi, D. A. (2008). Brain regulation of appetite and satiety. *Endocrinology and metabolism clinics of North America, 37* (4), 811-823.

Alan, M., Gurlek, B., Yilmaz, A., Aksit, M., Aslanipour, B., Gulhan, I., ... & Taner, C. E. (2019). Asprosin: a novel peptide hormone related to insulin resistance in women with polycystic ovary syndrome. *Gynecological Endocrinology, 35* (3), 220-223.

Anand, B. K., & Brobeck, J. R. (1951). Localization of a "feeding center" in the hypothalamus of the rat. *Proceedings of the society for experimental biology and Medicine, 77* (2), 323-325.

Ari, M., Ozturk, O. H., Bez, Y., Oktar, S., & Erduran, D. (2011). High plasma nesfatin-1 level in patients with major depressive disorder. *Progress in neuro-psychopharmacology and biological psychiatry, 35* (2), 497-500.

Atila Uslu G. (2019). Yeni Bir Adipokin; Asprosin. M. Kalafat & M. Akbaş (ed.), Sağlık Bilimleri Alanında Araştırma ve Değerlendirmeler (First edition), (151-156). Ankara, Türkiye: Gece kitaplığı.

Atsuchi, K., Asakawa, A., Ushikai, M., Ataka, K., Tsai, M., Koyama, K., ... & Inui, A. (2010). Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. *Neuroreport, 21* (15), 1008-1011.

Aydin, S., Dag, E., Ozkan, Y., Erman, F., Dagli, A. F., Kilic, N., ... & Kendir, Y. (2009). Nesfatin-1 and ghrelin levels in serum and saliva of epileptic patients: hormonal changes can have a major effect on seizure disorders. *Molecular and cellular biochemistry, 328* (1), 49-56.

Bernardis, L. L., & Bellinger, L. L. (1987). The dorsomedial hypothalamic nucleus revisited: 1986 update. *Brain Research Reviews, 12* (3), 321-381.

Brailoiu, G. C., Dun, S. L., Brailoiu, E., Inan, S., Yang, J., Chang, J. K., & Dun, N. J. (2007). Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain. *Endocrinology, 148* (10), 5088-5094.

Camilleri, M. (2006). Integrated upper gastrointestinal response to food intake. *Gastroenterology, 131* (2), 640-658.

Camilleri, M. (2015). Peripheral mechanisms in appetite regulation. *Gastroenterology, 148* (6), 1219-1233.

Chen, X., Dong, J., & Jiang, Z. Y. (2012). Nesfatin-1 influences the excitability of glucosensing neurons in the hypothalamic nuclei and inhibits the food intake. *Regulatory peptides, 177* (1-3), 21-26.

Corica, D., Aversa, T., Curro, M., Tropeano, A., Pepe, G., Alibrandi, A., ... & Wasniewska, M. (2021). Asprosin serum levels and glucose homeostasis in children with obesity. *Cytokine, 142*, 155477.

Delgado, J. M., & Anand, B. K. (1952). Increase of food intake induced by electrical stimulation of the lateral hypothalamus. *American Journal of Physiology-Legacy Content, 172* (1), 162-168.

Dong, J., Guan, H. Z., Jiang, Z. Y., & Chen, X. (2014). Nesfatin-1 influences the excitability of glucosensing neurons in the dorsal vagal complex and inhibits food intake. *PLoS One, 9* (6), e98967.

Du, C., Wang, C., Guan, X., Li, J., Du, X., Xu, Z., ... & Zheng, Z. (2021). Asprosin is associated with anorexia and body fat mass in cancer patients. *Supportive Care in Cancer, 29* (3), 1369-1375.

Duerrschmid, C., He, Y., Wang, C., Li, C., Bournat, J. C., Romere, C., ... & Chopra, A. R. (2017). Asprosin is a centrally acting orexigenic hormone. *Nature medicine, 23* (12), 1444-1453.

Elias, C. F., Saper, C. B., Maratos-Flier, E., Tritos, N. A., Lee, C., Kelly, J., ... & Elmquist, J. K. (1998). Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *Journal of comparative neurology, 402* (4), 442-459.

Fang, T., do Carmo, J. M., Wang, Z., Aberdein, N., de Lara Rodriguez, C. P., & Hall, J. E. (2016). Role of brainstem melanocortin-4 receptor (MC4R) in regulating glucose, energy balance and body fat in female mice. *The FASEB Journal, 30*, 750-752.

Gantulga, D., Maejima, Y., Nakata, M., & Yada, T. (2012). Glucose and insulin induce Ca2+ signaling in nesfatin-1 neurons in the hypothalamic paraventricular nucleus. *Biochemical and biophysical research communications, 420* (4), 811-815.

Goebel, M., Stengel, A., Wang, L., & Taché, Y. (2011). Central nesfatin-1 reduces the nocturnal food intake in mice by reducing meal size and increasing inter-meal intervals. *Peptides, 32* (1), 36-43.

Gonzalez, R., Reingold, B. K., Gao, X., Gaidhu, M. P., Tsushima, R. G., & Unniappan, S. (2011). Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet β-and MIN6 cells. *Journal of Endocrinology, 208* (3), R9-R16.

Harrold, J. A., Dovey, T. M., Blundell, J. E., & Halford, J. C. (2012). CNS regulation of appetite. *Neuropharmacology, 63* (1), 3-17.

Heidarzadeh, H., Zendehdel, M., Babapour, V., & Gilanpour, H. (2018). The effect of Nesfatin-1 on food intake in neonatal chicks: role of CRF1/CRF2 and H1/H3 receptors. *Veterinary research communications, 42* (1), 39-47.

Hoffmann, J. G., Xie, W., & Chopra, A. R. (2020). Energy regulation mechanism and therapeutic potential of asprosin. *Diabetes, 69* (4), 559-566.

Hsu, T. M., Hahn, J. D., Konanur, V. R., Noble, E. E., Suarez, A. N., Thai, J., ... & Kanoski, S. E. (2015). Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. *Elife, 4*, e11190.

Hussien, N. I., Mousa, A. M., & Shoman, A. A. (2020). Decreased level of plasma nesfatin-1 in rats exposed to cell phone radiation is correlated with thyroid

dysfunction, oxidative stress, and apoptosis. *Archives of Physiology and Biochemistry*, 1-7.

Jiang, G., Wang, M., Wang, L., Chen, H., Chen, Z., Guo, J., ... & Liu, X. (2015). The protective effect of nesfatin-1 against renal ischemia–reperfusion injury in rats. *Renal failure, 37* (5), 882-889.

Kim, J., Chung, Y., Kim, H., Im, E., Lee, H., & Yang, H. (2014). The tissue distribution of nesfatin-1/NUCB2 in mouse. *Development & reproduction, 18* (4), 301.

Kim, K. S., Seeley, R. J., & Sandoval, D. A. (2018). Signalling from the periphery to the brain that regulates energy homeostasis. *Nature Reviews Neuroscience, 19* (4), 185-196.

Ko, J. R., Seo, D. Y., Kim, T. N., Park, S. H., Kwak, H. B., Ko, K. S., ... & Han, J. (2019). Aerobic exercise training decreases hepatic asprosin in diabetic rats. *Journal of clinical medicine, 8* (5), 666.

Kocaman, N., & Kuloğlu, T. (2020). Expression of asprosin in rat hepatic, renal, heart, gastric, testicular and brain tissues and its changes in a streptozotocin-induced diabetes mellitus model. *Tissue and Cell, 66*, 101397.

Kohno, D., Nakata, M., Maejima, Y., Shimizu, H., Sedbazar, U., Yoshida, N., … & Yada, T. (2008). Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding. *Endocrinology, 149* (3), 1295-1301.

Korner, J., & Leibel, R. L. (2003). To eat or not to eat-how the gut talks to the brain. *New England Journal of Medicine, 349* (10), 926-927.

Li, E., Shan, H., Chen, L., Long, A., Zhang, Y., Liu, Y., ... & Wang, Y. (2019). OLFR734 mediates glucose metabolism as a receptor of asprosin. *Cell metabolism, 30* (2), 319-328.

Li, X., Liao, M., Shen, R., Zhang, L., Hu, H., Wu, J., ... & Zheng, H. (2018). Plasma asprosin levels are associated with glucose metabolism, lipid, and sex hormone profiles in females with metabolic-related diseases. *Mediators of inflammation,*7375294.

Li, Z., Gao, L., Tang, H., Yin, Y., Xiang, X., Li, Y., ... & Zhang, W. (2013). Peripheral effects of nesfatin-1 on glucose homeostasis. *PloS one, 8* (8), e71513.

Long, W., Xie, X., Du, C., Zhao, Y., Zhang, C., Zhan, D., ... & Luo, X. (2019). Decreased circulating levels of asprosin in obese children. *Hormone research in paediatrics, 91* (4), 271-277.

Lönnqvist, L., Reinhardt, D., Sakai, L., & Peltonen, L. (1998). Evidence for furin-type activity-mediated C-terminal processing of profibrillin-1 and interference in the processing by certain mutations. *Human molecular genetics, 7* (13), 2039-2044.

Maejima, Y., Sedbazar, U., Suyama, S., Kohno, D., Onaka, T., Takano, E., ... & Yada, T. (2009). Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. *Cell metabolism, 10* (5), 355-365.

Merali, Z., Cayer, C., Kent, P., & Anisman, H. (2008). Nesfatin-1 increases anxiety-and fear-related behaviors in the rat. *Psychopharmacology, 201* (1), 115-123.

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., ... & Kahn, B. B. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature, 428* (6982), 569-574.

Mohamad, Y. R., Hany, A. E., & Rania, A. F. (2020). Plasma asprosin levels changes in pregnant and non-pregnant rats with and without gestational diabetes. *International Journal of Medical Research & Health Sciences, 9* (3), 54-63.

Nazarnezhad, S., Rahmati, M., Shayannia, A., Abbasi, Z., Salehi, M., & Khaksari, M. (2019). Nesfatin-1 protects PC12 cells against high glucose-induced cytotoxicity via inhibiting oxidative stress, autophagy and apoptosis. *Neurotoxicology, 74*, 196-202.

Oh, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoque, K., ... & Mori, M. (2006). Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature, 443,* (7112), 709-712.

Osaki, A., & Shimizu, H. (2014). Peripheral administration of nesfatin-1 increases blood pressure in mice. *Hypertension Research, 37*(2), 185-186.

Price, C. J., Samson, W. K., & Ferguson, A. V. (2008). Nesfatin-1 inhibits NPY neurons in the arcuate nucleus. *Brain research, 1230*, 99-106.

Ramanjaneya, M., Chen, J., Brown, J. E., Tripathi, G., Hallschmid, M., Patel, S., ... & Randeva, H. S. (2010). Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. *Endocrinology, 151* (7), 3169-3180.

Romere, C., Duerrschmid, C., Bournat, J., Constable, P., Jain, M., Xia, F., ... & Chopra, A. R. (2016). Asprosin, a fasting-induced glucogenic protein hormone. *Cell, 165* (3), 566-579.

Sahu, A. (2003). Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Frontiers in neuroendocrinology, 24* (4), 225-253.

Sakai, L. Y., Keene, D. R., Renard, M., & De Backer, J. (2016). FBN1: The disease-causing gene for Marfan syndrome and other genetic disorders. *Gene, 591* (1), 279-291.

Sedbazar, U., Ayush, E. A., Maejima, Y., & Yada, T. (2014). Neuropeptide Y and α-melanocyte-stimulating hormone reciprocally regulate nesfatin-1

neurons in the paraventricular nucleus of the hypothalamus. *Neuroreport, 25*(18), 1453-1458.

Sedbazar, U., Maejima, Y., Nakata, M., Mori, M., & Yada, T. (2013). Paraventricular NUCB2/nesfatin-1 rises in synchrony with feeding suppression during early light phase in rats. *Biochemical and Biophysical Research Communications, 434* (3), 434-438.

Senin, L. L., Al-Massadi, O., Barja-Fernandez, S., Folgueira, C., Castelao, C., Tovar, S. A., ... & Seoane, L. M. (2015). Regulation of NUCB2/nesfatin-1 production in rat's stomach and adipose tissue is dependent on age, testosterone levels and lactating status. Molecular and Cellular *Endocrinology, 411*, 105-112.

Shimizu, H., Oh-i, S., Hashimoto, K., Nakata, M., Yamamoto, S., Yoshida, N., ... & Mori, M. (2009). Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism. *Endocrinology, 150* (2), 662-671.

Shimizu, H., Ohsaki, A., Oh, S., Okada, S., & Mori, M. (2009). A new anorexigenic protein, nesfatin-1. *Peptides, 30* (5), 995-998.

Sohn, J. W. (2015). Network of hypothalamic neurons that control appetite. *BMB reports, 48* (4), 229.

Stengel, A., & Taché, Y. (2013). Role of NUCB2/Nesfatin-1 in the hypothalamic control of energy homeostasis. *Hormone and Metabolic Research, 45* (13), 975-979.

Stengel, A., Goebel, M., Yakubov, I., Wang, L., Witcher, D., Coskun, T., ... & Lambrecht, N. W. (2009). Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. *Endocrinology, 150* (1), 232-238.

Su, Y., Zhang, J., Tang, Y., Bi, F., & Liu, J. N. (2010). The novel function of nesfatin-1: anti-hyperglycemia. *Biochemical and biophysical research communications, 391* (1), 1039-1042.

Tanida, M., & Mori, M. (2011). Nesfatin-1 stimulates renal sympathetic nerve activity in rats. *Neuroreport, 22* (6), 309-312.

Taniguchi, C. M., Emanuelli, B., & Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nature reviews Molecular cell biology, 7* (2), 85-96.

Ugur, K., & Aydin, S. (2019). Saliva and blood asprosin hormone concentration associated with obesity. *International journal of endocrinology,* 2521096.

Unger, T. J., Calderon, G. A., Bradley, L. C., Sena-Esteves, M., & Rios, M. (2007). Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *Journal of Neuroscience, 27* (52), 14265-14274.

Wang, C. Y., Lin, T. A., Liu, K. H., Liao, C. H., Liu, Y. Y., Wu, V. C. C., ... & Yeh, T. S. (2019). Serum asprosin levels and bariatric surgery outcomes in obese adults. *International journal of obesity, 43* (5), 1019-1025.

Wang, Y., Li, Z., Zhang, X., Xiang, X., Li, Y., Mulholland, M. W., & Zhang, W. (2016). Nesfatin-1 promotes brown adipocyte phenotype. *Scientific reports, 6* (1), 1-10.

Wang, Z. Z., Chen, S. C., Zou, X. B., Tian, L. L., Sui, S. H., & Liu, N. Z. (2020). Nesfatin-1 alleviates acute lung injury through reducing inflammation and oxidative stress via the regulation of HMGB1. *Eur Rev Med Pharmacol Sci, 24* (9), 5071-5081.

Wen, M. S., Wang, C. Y., Yeh, J. K., Chen, C. C., Tsai, M. L., Ho, M. Y., ... & Hsieh, I. (2020). The role of Asprosin in patients with dilated cardiomyopathy. *BMC Cardiovascular Disorders, 20* (1), 1-8.

Williams, D. L., Kaplan, J. M., & Grill, H. J. (2000). The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation. *Endocrinology, 141* (4), 1332-1337.

Wynne, K., Stanley, S., McGowan, B., & Bloom, S. (2005). Appetite control. *Journal of Endocrinology, 184* (2), 291-318.

Yamawaki, H., Takahashi, M., Mukohda, M., Morita, T., Okada, M., & Hara, Y. (2012). A novel adipocytokine, nesfatin-1 modulates peripheral arterial contractility and blood pressure in rats. *Biochemical and biophysical research communications, 418* (4), 676-681.

Yu, J. H., & Kim, M. S. (2012). Molecular mechanisms of appetite regulation. *Diabetes & metabolism journal, 36* (6), 391-398.

Yuan, J. H., Chen, X., Dong, J., Zhang, D., Song, K., Zhang, Y., ... & Chen, P. (2017). Nesfatin-1 in the lateral parabrachial nucleus inhibits food intake, modulates excitability of glucosensing neurons, and enhances Uncoupling Protein 1 expression in brown adipose tissue. *Frontiers in physiology, 8*, 235.

Zhang, A. Q., Li, X. L., Jiang, C. Y., Lin, L., Shi, R. H., Chen, J. D., & Oomura, Y. (2010). Expression of nesfatin-1/NUCB2 in rodent digestive system. W*orld journal of gastroenterology: WJG, 16* (14), 1735.

Zhang, H., Hu, W., & Zhang, G. (2020). Circulating asprosin levels are increased in patients with type 2 diabetes and associated with early-stage diabetic kidney disease. *International Urology and Nephrology, 52* (8), 1517-1522.

Zhang, L., Chen, C., Zhou, N., Fu, Y., & Cheng, X. (2019). Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triglyceride. *Clinica chimica acta, 489*, 183-188.

CHAPTER 7

USAGE AREAS AND PROPERTIES OF CALCIUM SILICATE-BASED MATERIALS IN PEDIATRIC DENTISTRY FROM PAST TO PRESENT[1]

Hanife Betül KARAASLAN[2]

Emin Caner TÜMEN[3]

1 Bu çalışma, 16.05.2022 tarihinde "Trikalsiyum Silikat Esaslı Biyomateryallerin Farklı İçerikteki Cam İyonomer Esaslı Restoratif Simanlara Bağlanım Dayanımının Değerlendirilmesi" adlı uzmanlık bitirme tezi olarak, Dicle Üniversitesi Çocuk Diş Hekimliği Anabilim Dalı'nda sunulmuştur. Tezi hazırlayan; Dt. Hanife Betül Karaaslan, tezin danışmanı Prof. Dr. Emin Caner Tümen'dir.

2 Arş. Gör. Dicle Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği A.D./ Diyarbakır/Türkiye/dentistbetulokumus@gmail.com (Orcid ID: 0000-0002-9732-177X)

3 Prof. Dr. Dicle Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği A.D./ Diyarbakır/Türkiye /ect1976@gmail.com (Orcid ID: 0000-0002-0905-1096)

INTRODUCTION

Calcium silicate-based materials are biomaterials used in indirect and direct pulp coatings, pulpotomy apexification and root perforation treatments (Gandolfi et al., 2015). These materials are self-hardening cements with appropriate physicochemical content for pulp treatments in children (Martens, & Rajasekharan, 2021).

Biomaterials containing calcium silicate used in vital and nonvital pulp treatments from past to present; Portland cement, MTA, Calcium Enriched Cement (CEM), Biodentine, Bioaggreagate, TheraCal, MTA Plus, Neo MTA Plus and Neo MTA 2 are examples.

PORTLAND CEMENT (PC)

Portland cement was patented by "Joseph Aspin" in 1824 (Hall, 1976). Portland cement consists of tricalcium silicate, dicalcium silicate, tetracalcium aluminoferrite, and dehydrated calcium sulfate. It has clinical uses in dentistry as pulp capping, partial pulpotomy, cervical pulpotomy, root and furcation perforation repair, and retrograde filling material (Tenório De França, da Silva, Sedycias De Queiroz, & Aguiar, 2010). Although Portland Cement (PC) is seen as a different material from MTA because it contains potassium ions and not bismuth ions, they have a very similar structure in terms of content (Song, Mante, Romanow, & Kim, 2006; Parirokh, & Torabinejad, 2010). Therefore, it was observed that the results of antimicrobial efficacy tests were similar (Estrela et al., 2000). It has been proven that Portland cement contributes to bone healing by secreting cytokines (IL-1ß and IL-6), which are responsible for bone remodeling, within the first 12 hours and initiating the activation phase (Abdullah et al., 2002). The long-term use of this material is questioned due to the heavy metals in its content and the release of arsenic to the surrounding tissues (Monteiro Bramante et al., 2008).

MINERAL TRIOXIDE AGGREGATE (MTA)

MTA was first introduced in 1993 at Loma Linda University in California by Torabinejad et al. It is a calcium silicate cement developed by the American Food and Drug Administration (FDA) in 1998 and was put into use (Parirokh, & Torabinejad, 2010).

It has been proven that this material is biocompatible in cell culture and animal experiments (Koh, Mcdonald, Pitt Ford, & Torabinejad, 1998; Camilleri, 2005).

It has superior properties such as preventing bacterial transmission, stimulating hard tissue formation and at the same time initiating tissue regeneration with its good sealing capacity (Torabinejad et al., 1995a). The powder part of MTA contains 75% Portland cement, 20% bismuth

oxide and 5% gypsum by weight. It also contains trace amounts of silicon dioxide (SiO_2), calcium oxide (CaO), magnesium oxide (MgO), potassium sulfate (K_2SO_4) and sodium sulfate (Na2SO4) (Sarkar et al., 2005).

In the hydrophilic part of MTA powder, there are tricalcium silicate ($3CaO.SiO_2$. C_3S, Alite), tricalcium aluminate ($3CaO.Al_2O_3$, C_3A, Aluminite), tricalcium oxide (Ca_2O_3) and silicate oxide (SiO_2) as the main components. In addition, traces of mineral oxides are also present. Bismuth oxide has been added to the material to give it radiopacity and improve its quality (Torabinejad et al., 1995b).

The gray MTA was first introduced to the market. In order to prevent discoloration of the teeth, white MTA was obtained by removing the tetracalcium alumina ferrite component from this material (Ferris, & Craig Baumgartner, 2004).

Asgary et al., in a study on the differences in the chemical content of gray and white MTA, stated that aluminum oxide (Al_2O_3), magnesium oxide (MgO) and especially iron oxide (FeO) concentrations were lower in white MTA. (Asgary, Parirokh, Eghbal, & Brink, 2005).

Preparation of MTA

The powder should be mixed with sterile water at a ratio of 3:1 on a glass plate or paper using a metal or plastic spatula. While preparing the mixture, moisture should be avoided in the first place. When the material is first mixed, a colloidal gel-like layer is formed. Afterwards, the mixture begins to harden to form a more solid structure. Calling the patient for the second session by placing a moist cotton pellet on the MTA contributes positively to the setting reaction. Plugger, gutta percha, ultrasonic tip or special MTA carriers can be used to transport the prepared MTA mixture to the desired area (Torabinejad, 1999; Macwan, 2014).

Hardening Mechanism and Duration

When hydrophilic particles in MTA powder combined with water, colloidal gel formation was observed in less than 4 hours. The structural feature of the mixture may vary depending on the powder/liquid ratio, particle size, ambient temperature, presence of moisture and air bubbles that may form in the mixture (Lee, Monsef, & Torabinejad, 1993).

The chemical reaction that causes the material to harden is called the “hydration reaction”. The hydrophilic particles in MTA dissolve in water at various speeds and heat is released as a result of the reaction. The hydration reaction consists of different stages, such as the mixing process, the dormant period (induction), the hardening period, the cooling process and the condensation process. During the mixing process, aluminate and gypsum dissolve in water within minutes, forming a gel-like layer. This

layer prevents the aluminates from participating in the reaction quickly and prevents the cement from hardening quickly. In the induction phase, the reaction still continues and the cement dissolves in water and begins to become saturated. In the hardening phase, as a result of the temperature increase as a result of the reaction, new products are released and the cement begins to solidify. During the cooling process, the cement became saturated and its durability began in this period. In the condensation phase, the reaction slows down and the heat production is significantly reduced. As a result, the cement has reached its most rigid state (Camilleri, 2007).

The time required for MTA to harden varies as a result of the studies conducted by the researchers. Torabinejad et al. According to this, this period is 2 hours and 45 minutes (Torabinejad et al., 1995b).

Since the long curing time gives the material a negative feature, the researchers aimed to shorten this time by adding various substances to the MTA material. Ber et al., in their study, observed that the setting time was shortened by adding methylcellulose and calcium chloride ($CaCl_2$) to the content of MTA (Ber, Hatton, & Stewart, 2007).

Physical, Mechanical and Biological Properties

MTA shows physical, mechanical and biological properties such as compressive strength, marginal adaptation and impermeability, radiopacity, solubility and pH value, biocompatibility and antibacterial properties.

Compressive Strength: In a study comparing the compressive strength of MTA and Biodentine, it was found that although Biodentine seemed to have higher compressive strength at the end of the first day, there was no significant difference between them at the end of twenty-one days (Alzraikat, Taha, & Salameh, 2016).

Marginal Adaptation and Sealing: Recently, the use of MTA in perforation repairs has become very popular. In a study using MTA, Biodentine and TheraCal LC, in the treatment of furcation perforation repair, MTA and Biodentine showed positive marginal adaptability, while TheraCal LC showed poor biocompatibility and marginal sealing. (Alazrag, Abu-Seida, El-Batouty, & el Ashry, 2020).

Radioopacity: Bismuth oxide component has been added to this material to provide radioopacity. Its radiopacity is equivalent to the aluminum thickness of 7.17 mm on average, providing sufficient visibility in radiography. Compared to IRM and Super-EBA, it is more radiopaque than them. When the root tip is used as a filling material, it can be easily distinguished on radiographs as it is more radiopaque than dentin and traditional guttapercha (Torabinejad et al., 1995b).

Solubility and pH value: MTA exhibits low solubility properties (Torabinejad et al., 1995b). It was determined that the solubility and porosity increased as the water ratio in the MTA mixture increased. Therefore, the amount of water in the mixture is important (Fridland, Rosado, & Eng, 2003). After the MTA material is mixed with water, its pH is 10.2, and after it hardens, it rises to 12.5 within 3 hours. This pH value is close to calcium hydroxide. The close alkaline pH of MTA and calcium hydroxide gives these materials antibacterial properties (Torabinejad et al., 1995b; Schwartz, Mauger, Clement, & Walker, 1999).

Biocompatibility: The biocompatibility and cytotoxicity of a dental material becomes more important when used in the treatment of furcal perforation or as a retrograde filling material at the root tip. Shahi et al. evaluated their biocompatibility by using white MTA, gray MTA and amalgam as root end filling material in their animal experiments. They found that white MTA is more biocompatible than the others (Shahi et al., 2006). Mitchell et al., in their in vivo study using different types of MTA, stated that MTA can be used safely in vital tissues as a biocompatible dental material (Mitchell, Ford, Torabinejad, & Mcdonald, 1999).

Antibacterial Properties: The antibacterial and antifungal properties of MTA are related to the fact that it is a dental material with a high pH value. The alkaline environment has an inhibitory effect on the growth of microorganisms (Kaur et al., 2017). Hiremath et al., in a study evaluating the antimicrobial activity of Biodentine, MTA and MTA Plus, stated that MTA and Biodentine showed significant antimicrobial activity against *E. faecalis*. According to the results of this study, they found that MTA Plus is a good antifungal agent against *Candida albicans* (Hiremath, Kulkarni, & Naik, 2015).

Clinical Uses of MTA

Although MTA was initially used as a root-end filling material, its current clinical use has expanded due to its biocompatibility and regeneration ability, its almost non-existent solubility in tissue fluids, its high degree of impermeability, and its resistance to pressure.

Treatments using MTA;

- Pulp coating of primary and permanent teeth
- Primary tooth amputation
- Repair of furcations and root perforations
- Apexification
- Regenerative endodontic treatments

• Internal and external root resorption

• Root canal treatment of primary teeth and permanent teeth that do not have permanent teeth underneath (Tawil, Duggan, & Galicia, 2015; Rodd et al., 2006).

In studies on amputation treatments applied to primary teeth, it has been stated that MTA is more advantageous because it is applied in a shorter time compared to formocresol and shortens the treatment period (Farsi, Alamoudi, Balto, & Mushayt, 2005; Godhi, Sood, & Sharma, 2011).

In addition, apexification treatments in young permanent teeth with MTA are preferred more frequently because they eliminate the prolongation of the process and the number of multiple sessions (Hachmeister, Schindler, Walker, & Denee Thomas, 2002).

The advantage of using MTA in regenerative endodontics is the continuation of root development after treatment. When used in immature permanent teeth with necrotic pulp, it creates a hard barrier tissue and shortens the treatment period (Chueh et al., 2009).

Disadvantages of MTA

In addition to many positive features that support the clinical use of MTA, there are also some disadvantages. There may be a long curing time, difficulty in application, and discoloration of the treated tooth (Kumar, Gupta, Singh, & Resident, 2018). The presence of infection in the area where MTA is applied prevents the hardening process of the material due to the acidic environment. Its consistency is difficult to adjust and its price is expensive, which also limits its clinical use (Kadali et al., 2020).

CALCIUM ENRICHED MATERIAL (CEM)

Calcium-enriched material was first introduced in 2006 as a new endodontic cement (Asgary, Jafar Eghbal, Parirokh, & Torabzadeh, 2006). Thanks to the different calcium compounds such as calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide, calcium chloride in the material and the calcium and phosphorus ions in its structure, it plays a role in the formation of hydroxyapatite crystals. Calcium hydroxide component stimulates dentin bridge formation. Unlike MTA and Portland cement, the powder part of CEM contains calcium oxide (CaO), sulfur trioxide (SO_3), phosphorus pentoxide (P_2O_5) and silicon dioxide (SiO_2) (Asgary et al., 2008; Heithersay, Rcs, 1975). The clinical uses of CEM and MTA are similar. CEM is a moisture resistant, biocompatible material with good sealing properties (Asgary et al., 2008).

The particle size of the calcium-enriched material is smaller than that of MTA (Soheilipour et al., 2009). Altunsoy et al., in their study comparing the shear bond strength of MTA, CEM and Biodentine to the composite material, stated that MTA and CEM could be preferred as a pulp coating agent in vital pulp treatments since they showed higher shear bond values compared to Biodentine (Altunsoy, Tanriver, Ok, & Kucukyilmaz, 2015). In pulpal treatment applied to canines using MTA and CEM, both materials showed biocompatibility by supporting dentin bridge formation (Asgary et al., 2008).

In a study evaluating the antibacterial properties of MTA, Portland cement and CEM, the antibacterial activity of CEM and MTA was stated to be superior to Portland cement (Zarrabi, Javidi, Naderinasab, & Gharechahi, 2009).

BIODENTINE

Biodentine (Septodont, St Maur des Fosses, France) is a new cement containing tricalcium silicate developed based on the superior biological properties of Portland cement (Laurent, Camps, & About, 2012).

It was first introduced to the market in 2009 (Vidal et al., 2016). It has been developed as a material that can replace dentin (Camilleri, 2013).

In the studies, it has been proven that the Biodentine material has a biocompatible feature considering that it allows the proliferation of odontoblasts and the healing pulpal cells, and no cytotoxic effect has been observed (Laurent et al., 2008; Pérard et al., 2013).

Content, Preparation and Hardening Mechanism of Biodentine

The powder part contains tricalcium silicate as the main main component, dicalcium silicate and calcium oxide as the second main components, calcium carbonate as a filler, zirconium oxide providing radiopacity and trace amounts of iron oxide. Thanks to the calcium chloride in its liquid, the setting time is shortened (Camilleri, 2013; Shayegan et al., 2012; Shen et al., 2015). In the hardening reaction, tricalcium silicate reacts to form a calcium silicate-based gel and calcium hydroxide. After the interaction of these products with phosphate ions, a structure similar to hydroxyapatite crystals is formed (Poggio et al., 2014).

Biodentine consists of a powder in a capsule and a liquid part in a tube. The mixture formed by adding 5 drops of liquid to the powder content is mixed for 30 seconds in the amalgamator and is made ready for use. The hardening time of the mixture, which is prepared in accordance with the manufacturer's instructions, is between 9-12 minutes. After mixing, it has a consistency that allows manipulation with a spatula, and it can be placed in the desired area with special carriers used to carry amalgam carriers or endodontic cements (BiodentineTM - Scientific File, 2020).

Physical, Mechanical and Biological Properties of Biodentine

Compressive strength (Compressive strength): Compressive strength is a property used to evaluate the mechanical properties of dental materials. The manufacturer stated that the compressive strength value of Biodentine reached approximately 300 MPa in one month. The compressive strength of natural dentin is in the range of 275-300 MPa. Biodentine is similar to natural dentine in terms of compressive strength values (Alzraikat, Taha, & Salameh, 2016).

In a study where Lucas et al. evaluated the compressive strengths of MTA and Biodentine, Biodentine exhibited higher compressive strength values at the end of 21 days. By adding water-soluble polycarboxylate-based polymers to the liquid of Biodentine, the water/powder ratio in the mixture decreased. Thus, the porosity in the material structure decreased and the compressive strength increased (de Paula Telles Pires Lucas et al., 2017).

In a study evaluating the physical properties of Biodentine, Bioaggregate and MTA biomaterials, it was reported that the compressive strength of Biodentine was superior to other materials used in the research (Jang et al., 2014).

Bond Strength: The bond strength between the biomaterial used as the pulp coating material and the permanent restoration placed on them is of great importance for the success of the treatment (Raina, Sawhny, Paul, & Nandamuri, 2020). The high bonding value ensures an equal distribution of the stress caused by the chewing forces in the mouth to the adhesion layer. Altunsoy et al., in a study examining the shear bond strength of Biodentine, MTA and calcium-enriched material (CEM) to flowable composites, stated that MTA and CEM showed higher bonding values to flowable composites than Biodentine (Altunsoy, Tanriver, Ok, & Kucukyilmaz, 2015).

In a study evaluating the bond strength of Biodentine to the composite using three-stage, two-stage and universal adhesives, it was observed that the highest bonding value was obtained with the use of a three-stage adhesive system (Carretero, Giner-Tarrida, Peñate, & Arregui, 2019).

El-Ma'aita et al. reported that the removal of the smear layer adversely affected the bond strength of calcium silicate-containing biomaterials such as Biodentine in their study (El-Ma'Aita, Qualtrough, & Watts, 2013).

Ph value: In a study where the pH values of Biodentine were analyzed, it was stated that the pH reached 11.16 ± 0.52 by showing the highest value on the 14th day (Yousef et al., 2015).

In a study evaluating the pH of calcium silicate-containing root repair materials such as Biodentine, Neo MTA Plus and MTA, it was stated that

all of them exhibited a high alkaline pH value and promoted tissue repair (Quintana et al., 2019).

Radioopacity: Zirconium oxide component has been added to the content of Biodentine in order to provide radioopacity. In addition, zirconium oxide made the material more biocompatible, resulting in corrosion resistance and a bioinert structure with improved mechanical properties (Piconi, Maccauro, 1999; Malkondu, Kazandağ, & Kazazoğlu, 2014).

Ochoa-Rodríguez et al., in their study, stated that adding 15% zirconium oxide to Biodentine without adversely affecting its physicochemical properties, increased the radiopacity of the biomaterial and improved radiographic visibility (Ochoa-Rodríguez et al., 2019).

Microleakage: Thanks to its improved physicochemical properties, Biodentine material can be used in root tip fillings for endodontic treatment. In a study examining the marginal sealing of MTA, Biodentine and glass ionomer cement as root tip filling material, Biodentine showed the lowest microleakage value of 0.13 mm (Kokate, & Pawar, 2012).

In a study using Biodentine, glass ionomer cement and MTA as root-end filling material and examining the microleakage values; Biodentine and MTA exhibited better sealing properties than glass ionomer cement (Nepal et al., 2020).

Biocompatibility: Biodentine is a highly biocompatible material without cytotoxic properties (Grech, Mallia, & Camilleri, 2013). Perard et al., in their study of the three-dimensional multicellular spheroid model in which Biodentine and MTA evaluated gene expression and biocompatibility, stated that both materials were similarly biocompatible (Pérard et al., 2013).

In a study evaluating the cytotoxicity of MTA and Biodentine on human osteoblast-like MG 63 cells, it was stated that both materials were similarly biocompatible with osteoconductive properties (Attik et al., 2014).

Antibacterial property: In order to be successful in endodontic treatments, bacteria and bacterial residues must be cleaned from the root canal system (Ramachandran Nair et al., 1990). The main factor in cases where endodontic treatment fails is the presence of resistant bacteria in the root canal system. The chance of success of treatment decreases in channels where E. faecalis, a type of opportunistic pathogen, is common (Evans, Davies, Sundqvist, & Figdor, 2002).

In a study investigating the antimicrobial activities of MTA and Biodentine materials, it was observed that Biodentine was more effective

by creating multiple areas of inhibition on opportunistic pathogenic microorganisms, S.mutans and E.faecalis (Vats, & Maheshwari, 2019).

Pulp coating agents used in vital pulp treatments should have antibacterial properties. The surface characteristics of these materials have been designed by the manufacturers to prevent bacterial adhesion, thus providing the material with antibacterial properties. In a study by Farrugia et al., The relationship between the surface characteristics of MTA, Theracal LC and Biodentine materials on their antimicrobial activity was investigated. In the research, it was observed that Biodentine surface exhibited effective antibacterial properties due to its hydrophobic and smoothness (Farrugia et al., 2018).

Clinical Uses of Biodentine

Biodentine has been the biomaterial of choice in many different clinical applications since its introduction in Europe in late 2010. Biodentine can be used as a replacement material for permanent dentin in vital pulp treatments, regenerative endodontics including cementation and dentin repair, teeth with dental trauma, restorative treatments, and many treatments in pediatric dentistry (Rajasekharan, Martens, Cauwels, & Verbeeck, 2014).

Treatments using Biodentine;

- Direct and indirect pulp coating
- Pulpotomy
- Furcation and root perforation repair
- Apexification
- Regenerative endodontic treatments
- Internal and external root resorption repair (Kaur et al., 2017).

In a clinical study by Brizuela et al., MTA, Biodentine and calcium hydroxide materials were preferred in the treatment of direct pulp coating applied to young permanent teeth. They concluded that calcium silicate-based biomaterials such as MTA and Biodentine, whose clinical success rates were compared after a 1-year follow-up period, could be preferred primarily because of their higher success in pulp coating treatments (Brizuela et al., 2017).

MTA and Biodentine were preferred as pulpotomy material in a clinical study on deciduous teeth whose pulp was exposed by caries. As a result of the 24-month follow-up, it was observed that both materials, which were evaluated clinically and radiographically, had similar success rates in primary tooth pulpotomy treatments. It has been stated that Biodentine

is a material that can be preferred over MTA due to its advantages such as short curing time and clinical ease of use (Çelik, Mutluay, Arıkan, & Sarı, 2019).

In a study by Mancino et al., it was reported that teeth with preformed root perforation had a high clinical success rate in the treatments in which this material was used, after completing the perforation repair treatments with Biodentine, after a 64-month follow-up (Mancino, Meyer, & Haikel, 2018).

As a result of a study in which a single-stage regeneration treatment was applied using Biodentine material to a devitalized young permanent tooth that has not completed root development, it was observed that Biodentine provides sufficient dentin bridge formation and helps to complete the root development of the related tooth (Aldakak, Capar, Rekab, & Abboud, 2016).

In addition to long-lasting traditional apexification applications in young permanent teeth that have not completed root development, one-stage treatments using Biodentine can be offered as an alternative. Thanks to the calcium and silicon components in Biodentine, it attaches to the dentinal tubules and forms crystal-like structures. This makes the treated tooth more durable physically and mechanically. In a study by Bajwa et al., it was stated that Biodentine, a bioactive cement, could be used as a substitute for dentin in apexification treatments (Bajwa, Jingarwar, & Pathak, 2015).

Disadvantages of Biodentine

Although biodentine has good physical and mechanical properties, it has been stated as a result of studies that it does not have sufficient radiopacity when used as a retrograde filling material in endodontic surgical treatments and this property should be improved (Caron et al., 2014; Tanalp,Karapinar-Kazandağ, Dölekoğlu, & Kayahan, 2013). The biomaterial, which does not have suitable radiopacity, affects the prognosis negatively because it cannot be distinguished from the surrounding tissues in post-treatment radiographs (Alhashimi, 2015).

Despite its successful clinical results, the expensive price of Biodentine is also seen as one of the disadvantages of this material (Caruso et al., 2018).

BioAggregate

BioAggregate, which consists of biocompatible, ceramic nanoparticles, is basically tricalcium silicate, dicalcium silicate, calcium phosphate and synthetic components; calcium silicate hydrate, calcium hydroxide,

hydroxyapatite, tantalum oxide and silicon dioxide in amorphous form. Unlike MTA, aluminum, which has harmful effects on the body, is not included in the content of this material. In addition, while bismuth oxide is the component that provides radiopacity in conventional MTA, it is tantalum oxide in Bioaggregate (Park et al., 2010).

In a study evaluating the antibacterial activity of MTA and BioAggregate, it was reported that both materials exhibited strong antimicrobial properties against a resistant microorganism, *E.faecalis* (Zhang, Pappen, & Haapasalo, 2009).

BioAggregate (Innovative Bioceramix, Vancouver, Canada, lotM72058) has received FDA product approval and has been developed for use in vital pulp treatments, perforation repair, apical resection as root tip retrograde filling material (Innovative Bioceramix, 2010).

In a study using BioAggregate, MTA and calcium hydroxide; The fracture resistances of immature young permanent teeth were compared by filling the canals with these materials. Teeth with root canals filled with BioAggregate were reported as the group with the highest fracture resistance after 1 year follow-up. According to this study, removing the pulp completely and filling the canals with BioAggeregate material in traumatized teeth with open apex reduces the risk of cervical root fracture and increases the chance of success in the treatment (Tuna, Dinçol, Gençay, & Aktören, 2011).

Yoldaş et al., in a study in which they evaluated the color change in cattle teeth using BioAggregate, Biodentine and white MTA, observed that all the materials they used caused discoloration. The biomaterial with the least color change was Biodentine. They stated that in endodontic treatments, especially in the anterior region, where aesthetics is important, attention should be paid to the selection of materials in terms of discoloration of the teeth (Yoldaş, Bani, Atabek, & Bodur, 2016).

TheraCal LC

TheraCal LC (Bisco, Schaumburg, IL, USA) was first introduced in 2011 (Voicu et al., 2019). TheraCal is a new light-cured resin-modified calcium silicate material. It consists of approximately 45% by weight mineral (Type 3 Portland Cement), 10% radiopaque component, 5% hydrophilic thickener (fumed silica) and the remaining 45% resin. The resin content consists of hydrophobic components such as bisphenol A-glycidyl dimethacrylate (BisGMA), urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA), and hydrophilic components such as hydroxyethyl methacrylate (HEMA), polyethylene glycol dimethacrylate (PEGDMA) (Suh, Yin, Cannon, & Martin, 2008).

TheraCal LC consists of tricalcium silicate particles in a hydrophilic monomer, making it a durable material that releases calcium as a base or base. The release of calcium ions stimulates hydroxyapatite crystals and secondary dentin bridge formation (Qureshi et al., 2014). It is used as a pulp coating agent in vital pulp treatments (Makkarr, Aggarwall, Vashisht, 2015). In a study conducted by Cannon et al., by applying direct pulp treatment to monkey teeth, they observed that the dentin bridge formation in the group with TheraCal material was more regular and tighter than the group with Dycal material (Cannon et al., 2014). In a study by Alazrag et al., the solubility, biocompatibility and marginal adaptability of these materials were compared using MTA, Biodentine and TheraCal LC in the treatment of perforation repair applied to canine teeth. It has been observed that TheraCal exhibits lower solubility properties due to its higher resin content. In the observations within 3 months following the treatment, moderate/severe signs of inflammation were observed in the cells in the group treated with Theracal LC. As a result, it was stated that this material is less biocompatible than MTA and Biodentine. Due to polymerization shrinkage, the lowest marginal adaptability among the materials compared was reported as "TheraCal LC" (Alazrag, Abu-Seida, El-Batouty, & el Ashry, 2020). In a study comparing the biological effects after differential gene expression change by applying MTA and TheraCal LC to the deciduous tooth pulp; It was observed that osteoclastic activity was indirectly initiated by differential gene expression in primary teeth treated with TheraCal LC. Therefore, care should be taken when placing TheraCal LC material that will be applied directly to the primary tooth pulp, which is an indication for direct pulp treatment. It is thought that if the resin monomers in its content are not sufficiently polymerized, it initiates the inflammation process by stimulating the pulp cells. For this reason, when the material is to be placed, it should be applied in 1 mm layers and polymerized (Nam, Kim, Choi, & Kim, 2020).

MTA Plus

MTA Plus is a calcium silicate-based biomaterial developed in 2011, consisting of a powder-liquid system. Contains tricalcium silicate and dicalcium silicate particles in the powder portion (Avalon Biomed Inc, Bradenton, Florida). The particle size is smaller than conventional MTAs available in the market. Its special liquid, which does not contain salt, provides resistance to the material (Neelakantan, Grotra, & Sharma, 2013; Qi et al., 2012). In a study, since the color stability provided by the material is adversely affected when the powder of MTA Plus is mixed with distilled water, color change should be prevented by mixing it with its own special liquid (Keskin, & Sarıyılmaz, 2018). MTA Plus is used as root tip filling material in teeth with pulp coating treatments, apexification, root perforation repair and apical resection.

MTA Plus begins to release calcium ions three hours after mixing and the ion release stops after one day. The high release of calcium ions and the rapid formation of apatite crystals represent epigenetic signals for pulp cells. Thus, repair dentin formation is stimulated and clinical improvement is achieved (Prati, & Gandolfi, 2015).

Rodrigues et al., in a study they conducted using MTA and MTA Plus materials, stated that high calcium ion release in MTA Plus contributed to healing at a higher rate by increasing mineral deposition by providing early maturation of osteoblasts (Rodrigues et al., 2017).

NeoMTA Plus

NeoMTA Plus is a new biomaterial containing MTA derivative calcium silicate produced in recent years. To provide radioopacity, tantalum oxide (Ta_2O_5) has been added to its content. Clinical usage areas; vital pulp treatments (pulp coating and pulpotomy) are apexification, root resorption and perforation repair as a retrograde filling material at the root tip (Avalon Biomed, Neo MTA Plus, 2017). In powder content; tricalcium silicate, dicalcium silicate, tantalum oxide, tricalcium aluminate, calcium sulfate and gypsum. In its liquid, there are water-based gels containing thickeners and water-soluble polymers. To prevent discoloration of the tooth after treatment, tantalum oxide was added instead of bismuth oxide (unlike MTA Plus) (Tanomaru-Filho, Viapiana, & Guerreiro-Tanomaru, 2016). The final hardening time of the material is between 55-315 minutes.

Siboni et al., in their study, stated that the Ca^{+2} ion release of NeoMTA Plus is higher than that of conventional MTA, and thus the amount of released calcium is an important factor in promoting endodontic and periodontal tissue regeneration (Siboni, Taddei, Prati, & Gandolfi, 2017).

In a study where MTA Plus, Biodentine and NeoMTA Plus were tested on dental pulp stem cells; It has been observed that these materials contribute to healing by providing fibroblastic cell adhesion to the region thanks to the calcium ions in their content (Tomás-Catalá et al., 2018).

Alsanouni et al. concluded that in pulpotomy treatment of primary molar teeth performed with ProRoot MTA and NeoMTA Plus, these materials were clinically similarly successful at the end of a 1-year follow-up (Alsanouni, & Bawazir, 2019).

In a study, it was stated that the antibacterial properties of MTA and NeoMTA Plus did not affect resistant microorganisms, *E. faecalis* and *C. albicans* (Jacob et al., 2020).

Jardine et al., in a study investigating the antibacterial activity of NeoMTA Plus, Biodentine and MTA Angelus biomaterials, observed that

these biomaterials were not effective against biofilms containing a large number of species (Jardine et al., 2019). In another study, it was reported that NeoMTA Plus is similar to Biodentine in terms of sealing, and that it has a more successful sealing feature than ProRoot MTA and glass ionomer (Bhavsar et al., 2020).

Compared to conventional MTA, NeoMTA Plus exhibits a high degree of bond strength by better penetrating the dentinal tubules due to its smaller particle size (Patil et al., 2019).

NeoMTA 2

NeoMTA 2 is a powder liquid system consisting of tricalcium and dicalcium silicate-containing fine particles and powder part containing inorganic components and water-based gel that initiates the curing reaction. The running time is fourteen minutes. When mixed with a less intense consistency, this time can be extended up to twenty-one minutes. In order to prolong the working time, the mixed material should be covered with a damp cloth or liquid should be added to the mixture again. The final hardening time of Neo MTA 2 is in the range of 14-70 minutes. It should be mixed with a metal or plastic spatula on a durable paper pad that will not absorb the glass plate or liquid. Plastic carrier, Hollenbach spatula, amalgam gun or MTA carriers can be used to transport the prepared mixture to the desired area. After the material is placed, it can be spread into the cavity with a moist cotton pellet or amalgam fulvar. In the treatment of apexification, inverted gutta percha or paper point can be used to deliver biomaterial to the root tip.

Clinical usage areas; pulp coating treatments (indirect and direct pulp coating), pulpotomy, microsurgical procedures in which the root tip is used as a retrograde filling material, and apexification treatments. When used in pulpotomy treatments or as a base, it should be placed in layers of 1.5 mm thickness. In the treatment of apexification, an apical plug should be created by placing 3-5 mm thick material at the root tip. It is a bioactive material that contributes to healing by releasing calcium and hydroxide ions and creating hydroxyapatite in the tooth to which it is applied. It has antibacterial properties thanks to its high alkaline pH value. It exhibits low resolution feature. Since it does not contain resin, it is dimensionally stable and is a shrink-resistant biomaterial. Tantalum oxide, added to its content in order to provide radioopacity, does not change the color of the teeth. Compared to conventional MTA, the component that provides radiopacity and replaces tantalum oxide in MTA is bismuth oxide, which causes discoloration in the teeth to which conventional MTA is applied.

An important feature that affects the clinical performance of MTA-like biomaterials is the stability of the material after it is placed in the tooth.

The stability of the biomaterial is determined by ‘wash tests’. It has been stated that Neo MTA 2 is a durable biomaterial due to the resistance it has shown in these tests.

The differences between Neo MTA 2 and Neo MTA Plus are:

• Neo MTA 2 is easier to prepare and mix.

• NeoMTA 2 is approximately 30% more radiopaque than NeoMTA Plus. (The radiopacity of Neo MTA 2 is equivalent to 6 mm thick aluminum, while that of Neo MTA Plus corresponds to 5 mm thick aluminum.)

• Neo MTA 2 has a brighter white color (Avalon Biomed, Neo MTA 2, 2020).

REFERENCES

Abdullah, D., Pitt Ford, T.R., Papaioannou, S., Nicholson, J., & Mcdonald, F. (2002). An evaluation of accelerated Portland cement as a restorative material. Biomaterials, 23:4001–10.

Alazrag, M.A., Abu-Seida, A.M., El-Batouty, K.M., & el Ashry, S.H. (2020). Marginal adaptation, solubility and biocompatibility of TheraCal LC compared with MTA-angelus and biodentine as a furcation perforation repair material. BMC Oral Health, 20(1):2–12.

Aldakak, M.M.N., Capar, I.D., Rekab, M.S., & Abboud, S. (2016). Single-visit pulp revascularization of a nonvital immature permanent tooth using biodentine. Iranian Endodontic Journal, 11(3):246–9.

Alhashimi, R.A. (2015). Assessing the radiopacity of new root end filling materials using digital radiography technique. MDJ, 12:10–5.

Alsanouni, M., & Bawazir, O.A. (2019). A randomized clinical trial of NeoMTA plus in primary molar pulpotomies. Pediatric Dentistry. 2019;41(2):107–11.

Altunsoy, M., Tanriver, M., Ok, E., & Kucukyilmaz, E. (2015). Shear bond strength of a self-adhering flowable composite and a flowable base composite to mineral trioxide aggregate, calcium-enriched mixture cement, and biodentine. Journal of Endodontics, 41(10):1691–5.

Alzraikat, H., Taha, N., & Salameh, A. (2016). A comparison of physical and mechanical properties of Biodentine and Mineral Trioxide Aggregate. Journal of Research in Medical and Dental Science, 4(2):121.

Asgary, S., Parirokh, M., Eghbal, M.J., &, Brink, F. (2005). Chemical Differences Between White and Gray Mineral Trioxide Aggregate. Journal of Endodontics., 31(2):101–3.

Asgary, S., Jafar Eghbal, M., Parirokh, M., & Torabzadeh, H. (2006). Sealing Ability of Three Commercial Mineral Trioxide Aggregates and an Experimental Root-End Filling Material. Iranian Endodontic Journal, 1(3):101–5.

Asgary, S., Eghbal, M.J., Parirokh, M., Ghanavati, F., & Rahimi, H.A. (2008). A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, 106(4):609–14.

Attik, G.N., Villat, C., Hallay, F., Pradelle-Plasse, N., Bonnet, H., & Moreau, K. (2014). In vitro biocompatibility of a dentine substitute cement on human MG63 osteoblasts cells: BiodentineTM versus MTA®. International Endodontic Journal, 47(12):1133–41.

Avalon Biomed, Neo MTA Plus. (2017). (erişim tarihi: 19.02.2022, https://www.avalonbiomed.com/wp-content/uploads/2019/10/IFU-37-NeoMTA-and-Grey-MTA-Plus-Rev-0.pdf).

Avalon Biomed, & NeoMTA 2. (2020). (erişim tarihi: 19.02.2022, https://www.avalonbiomed.com/wp-content/uploads/2020/07/IFU-62-Avalon-Biomed-NeoMTA-2-FAQ-Rev.-09.pdf).

Bajwa, N.K., Jingarwar, M.M., Pathak, A. (2015). Single Visit Apexification Procedure of a Traumatically Injured Tooth with a Novel Bioinductive Material (Biodentine). International Journal of Clinical Pediatric Dentistry, 8(1):58–61.

Ber, B.S., Hatton, J.F., & Stewart, G.P. (2007). Chemical Modification of ProRoot MTA to Improve Handling Characteristics and Decrease Setting Time. Journal of Endodontics, 33(10):1231–4.

Bhavsar, B., Ramchandran, R., Kharat, N., Singh, S., & Gaidhankar, S. (2020). A Comparative Stereomicroscopic Evaluation of Bioactivity of Different Biomimetic Root End Filling Materials-An In Vitro Study. || International Journal of Medical Science and Diagnosis Research (IJMSDR) Original Research Article, 4(6):6–11.

BiodentineTM - Scientific File, [Internet]., (2020). (erişim tarihi: 19.02.2022, https://biodentine.com/wp-content/uploads/2020/08/Biodentine-Scientific-File.pdf).

Brizuela, C., Ormeño, A., Cabrera, C., Cabezas, R., Silva, C.I., & Ramírez, V. (2017). Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. Journal of Endodontics, 43(11):1776–80.

Camilleri, J. (2005). The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. International Endodontic Journal, 38(11):834–42.

Camilleri, J. (2007). Hydration mechanisms of mineral trioxide aggregate. International Endodontic Journal, 40(6):462–70.

Camilleri, J. (2013). Investigation of Biodentine as dentine replacement material. Journal of Dentistry, 41(7):600–10.

Cannon, M., Gerodias, N., Vieira, A., Percinoto, C., & Jurado, R. (2014). Primate pulpal healing after exposure and TheraCal application. Journal of Clinical Pediatric Dentistry, 38(4):333–7.

Caron, G., Azérad, J., Faure, M.O., Machtou, P., & Boucheri, Y. (2014). Use of a new retrograde filling material (Biodentine) for endodontic surgery: Two case reports. International Journal of Oral Science, 6(4):250–3.

Carretero, V., Giner-Tarrida, L., Peñate, L., & Arregui, M. (2019). Shear bond strength of nanohybrid composite to biodentine with three different adhesives. Coatings, 9(12):783.

Caruso, S., Dinoi, T., Marzo, G., Campanella, V., Giuca, M.R., & Gatto, R. (2018). Clinical and radiographic evaluation of biodentine versus calcium

hydroxide in primary teeth pulpotomies: A retrospective study. BMC Oral Health, 18(1):1–7.

Chueh, L.H., Ho, Y.C., Kuo,T.C., Lai, W.H., Chen, Y.H.M., & Chiang, C.P. (2009). Regenerative Endodontic Treatment for Necrotic Immature Permanent Teeth. Journal of Endodontics, 35(2):160–4.

Çelik, B.N., Mutluay, M.S., Arıkan, V., & Sarı, Ş. (2019). The evaluation of MTA and Biodentine as a pulpotomy materials for carious exposures in primary teeth. Clinical Oral Investigations, 23(2):661–6.

de Paula Telles Pires Lucas, C., Viapiana, R., Bosso-Martelo, R., Guerreiro-Tanomaru, J.M., Camilleri, J., & Tanomaru-Filho, M. (2017). Physicochemical properties and dentin bond strength of a tricalcium silicate-based retrograde material. Brazilian Dental Journal, 28(1):51–6.

El-Ma'Aita, A.M., Qualtrough, A.J.E., Watts, D.C. (2013). The effect of smear layer on the push-out bond strength of root canal calcium silicate cements. Dental Materials,29(7):797–803.

Estrela, C., Luschke Bammann, L., Rodrigues, C., Estrela, A., Silva, R.S., & Pécora, J.D. (2000). Antimicrobial and Chemical Study of MTA, Portland Cement, Calcium Hydroxide Paste, Sealapex and Dycal. Brazilian Dental Journal, 11(1):3–9.

Evans, M., Davies, J.K., Sundqvist, G., & Figdor, D. (2002). Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. International Endodontic Journal, 35:221–8.

Evans, M., Davies, J.K., Sundqvist, G., & Figdor, D. (2002). Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. International Endodontic Journal, 35:221–8.

Farrugia, C., Lung, C.Y.K., Schembri Wismayer, P., Arias-Moliz, M.T., & Camilleri, J. (2018). The Relationship of Surface Characteristics and Antimicrobial Performance of Pulp Capping Materials. Journal of Endodontics, 44(7):1115–20.

Farsi, N., Alamoudi, N., Balto, K., & Mushayt, A. (2005). Success of mineral trioxide aggregate in pulpotomized primary molars. Journal of Clinical Pediatric Dentistry, 29(4):307–11.

Ferris, D.M., & Craig Baumgartner, J. (2004). Perforation Repair Comparing Two Types of Mineral Trioxide Aggregate. Journal of Endodontics, 30(6):422–4.

Asgary, S., Parirokh, M., Eghbal, M.J., Brink, F. (2005). Chemical Differences Between White and Gray Mineral Trioxide Aggregate. Journal of Endodontics, 31(2):101–3.

Fridland, M., Rosado, R., & Eng, C. (2003). Mineral Trioxide Aggregate (MTA) Solubility and Porosity with Different Water-to-Powder Ratios. Journal of Endodontics, 29(12):814–7.

Gandolfi, M.G., Siboni, F., Botero, T., Bossù, M., Riccitiello, F., & Prati, C. (2015). Calcium silicate and calcium hydroxide materials for pulp capping: Biointeractivity, porosity, solubility and bioactivity of current formulations. Journal of Applied Biomaterials and Functional Materials, 13(1):1–18.

Godhi, B., Sood, P., & Sharma, A. (2011). Effects of mineral trioxide aggregate and formocresol on vital pulp after pulpotomy of primary molars: An in vivo study. Contemporary Clinical Dentistry, 2(4):296.

Grech, L., Mallia, B., Camilleri, J. (2013). Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. Dental Materials, 29(2):e20–8.

Hachmeister, D.R., Schindler, W.G., & Walker, W.A. (2002). Denee Thomas D. The Sealing Ability and Retention Characteristics of Mineral Trioxide Aggregate in a Model of Apexification. Journal of Endodontics, 28(5):386–90.

Hall, C. (1976). On the history of Portland cement after 150 years. Journal of Chemical Education, 53(4):222.

Heithersay, G.S., & Rcs, F.D.S. (1975). Calcium hydroxide in the treatment of pulpless teeth with associated pathology. Journal of the British Endodontic Society, 8(2):74–93.

Hiremath, G.S., Kulkarni, R.D., & Naik, B.D. (2015). Evaluation of minimal inhibitory concentration of two new materials using tube dilution method: An in vitro study. Journal of Conservative Dentistry, 18:159–62.

Innovative Bioceramix [Internet]., (2010). (erişim tarihi: 19.02.2022, http://www.ibioceramix.com/index.html).

Jacob, V.P., Paião, L.I., da Silva, A.C.G., Magario, M.K.W., Kaneko, T.Y., & Martins, C.M. (2020). Antimicrobial action of NeoMTA Plus on mono- and dual-species biofilms of Enterococcus faecalis and Candida albicans: An in vitro study. Archives of Oral Biology, 120:1–7.

Jang, Y.E., Lee, B.N., Koh, J.T., Park, Y.J., Joo, N.E., & Chang, H.S. (2014). Cytotoxicity and physical properties of tricalcium silicate-based endodontic materials. Restorative Dentistry & Endodontics, 39(2):89.

Jardine, A.P., Montagner, F., Quintana, R.M., Zaccara, I.M., & Kopper, P.M.P. (2019). Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: a confocal laser microscopy study. Clinical Oral Investigations, 23(3):1367–72.

Kadali, N., Alla, R.K., Guduri, V., AV, R., MC, S.S., & Raju, R.V. (2020). Mineral Trioxide Aggregate: an overview of composition, properties and clinical applications. International Journal of Dental Materials, 02(01):11–8.

Kaur, M., Singh, H., Dhillon, J.S., Batra, M., & Saini, M. (2017). MTA versus biodentine: Review of literature with a comparative analysis. Journal of Clinical and Diagnostic Research, 11(8):1–5.

Keskin, C., & Sarıyılmaz, E. (2018). Color Stability of NeoMTA Plus and MTA Plus when Mixed with Anti-washout Gel or Distilled Water. Meandros Medical and Dental Journal, 19(4):296–301.

Kokate, S.R., & Pawar, A.M. (2012). An in vitro comparative stereomicroscopic evaluation of marginal seal between MTA, glass inomer cement and biodentine as root end filling materials using 1% methylene blue as tracer. Endodontology, 24(2):36–42.

Koh, E.T., Mcdonald, F., Pitt Ford, T.R., & Torabinejad, M. (1998). Cellular Response to Mineral Trioxide Aggregate. Journal of Endodontics, 24(8):543–7.

Kumar, D., Gupta, A.K., Singh, B.P., & Resident, J. (2018). Comparative Evaluation of Sealing Ability of white MTA, Biodentine, Calcium Phosphate Cement, and Glass Ionomer Cement as Furcation Repair Materials: An Ex Vivo Study. International Journal of Research in Health and Allied Sciences, 4(3):10–4.

Laurent, P., Camps, J., de Méo, M., Déjou, J., & About, I. (2008). Induction of specific cell responses to a Ca3SiO5-based posterior restorative material. Dental Materials, 24(11):1486–94.

Laurent, P., Camps, J., & About, I. (2012). Biodentine TM induces TGF-β1 release from human pulp cells and early dental pulp mineralization. International Endodontic Journal, 45(5):439–48.

Lee, S.J., Monsef, M., & Torabinejad, M. (1993). Sealing Ability of a Mineral Trioxide Aggregate for Repair of Lateral Root Perforations. Journal of Endodontics, 19(11):541–4.

Macwan, C., & Deshpande, A. (2014). Mineral trioxide aggregate (MTA) in dentistry: A review of literature. Journal of Oral Research and Review, 6(2):71.

Makkarr, S., Aggarwall, A., & Vashisht, R. (2015). A confocal laser scanning microscopic study evaluating the sealing ability of mineral trioxide aggregate, biodentine and anew pulp capping agent-theracal. Dental Journal of Advance Studies, 3(1):20–5.

Malkondu, Ö., Kazandağ, M.K., & Kazazoğlu, E. (2014). A review on biodentine, a contemporary dentine replacement and repair material. BioMed Research International, 2014:1–10.

Mancino, D., Meyer, F., Haikel, Y. (2018). Improved single visit management of old infected iatrogenic root perforations using Biodentine®. Giornale Italiano di Endodonzia, 32(1):17–24.

Martens, L.C., & Rajasekharan, S. (2021). Bioceramic Materials in Pediatric Dentistry. In: Bioceramic Materials in Clinical Endodontics. İsviçre: Springer, p. 87–101.

Mitchell, P.J.C., Ford, T.R.P., Torabinejad, M., & Mcdonald, F. (1999). Osteoblast biocompatibility of mineral trioxide aggregate. Biomaterials, 20:167–73.

Monteiro Bramante, C., Demarchi, A.C.C.O., de Moraes, I.G., Bernadineli, N., Garcia, R.B., & Spångberg, L.S.W. (2008). Presence of arsenic in different types of MTA and white and gray Portland cement. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, 106(6):909–13.

Nam, O.H., Kim, J.H., Choi, S.C., & Kim, Y. (2020). Time-dependent response of human deciduous tooth-derived dental pulp cells treated with theraCal LC: Functional analysis of gene interactions compared to MTA. Journal of Clinical Medicine, 9(2):1–15.

Neelakantan, P., Grotra, D., Sharma, S. (2013). Retreatability of 2 mineral trioxide aggregate-based root canal sealers: A cone-beam computed tomography analysis. Journal of Endodontics, 39(7):893–6.

Nepal, M., Shubham, S., Tripathi, R., Khadka, J., Kunwar, D., Gautam, V. (2020). Spectrophotometric analysis evaluating apical microleakage in retrograde filling using GIC, MTA and biodentine: An in-vitro study. BMC Oral Health, 20(1):1–7.

Ochoa-Rodríguez, V.M., Tanomaru-Filho, M., Rodrigues, E.M., Guerreiro-Tanomaru, J.M., Spin-Neto, R., & Faria, G. (2019). Addition of zirconium oxide to Biodentine increases radiopacity and does not alter its physicochemical and biological properties. Journal of Applied Oral Science, 27:1–10.

Parirokh, M., & Torabinejad, M. (2010). Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties. Journal of Endodontics, 36(1):16–27.

Park, J.W., Hong, S.H., Kim, J.H., Lee, S.J., & Shin, S.J. (2010). X-Ray diffraction analysis of White ProRoot MTA and Diadent BioAggregate. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, 109(1):155–8.

Patil, U., Yeli, M., Tapashetti, S., Naik, B., & Tilakchand, M. (2019). Effect of varying durations of intracanal medicament application used in regenerative endodontic treatment on the push-out bond strength of a novel cement: NeoMTA Plus. Journal of conservative dentistry, 22(1):48.

Pérard, M., le Clerc, J., Meary, F., Pérez, F., Tricot-Doleux, S., & Pellen-Mussi, P. (2013). Spheroid model study comparing the biocompatibility of Biodentine and MTA. Journal of Materials Science: Materials in Medicine, 24(6):1527–34.

Piconi, C., & Maccauro, G. (1999). Zirconia as a ceramic biomaterial. Biomaterials, 20:1–25.

Poggio, C., Ceci, M., Beltrami, R., Dagna, A., Colombo, M., & Chiesa, M. (2014). Biocompatibility of a new pulp capping cement. Ann Stomatol (Roma), 5(2):69–76.

Prati, C., & Gandolfi, M.G. (2015). Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dental Materials, 31(4):351–70.

Qi, Y.P., Li, N., Niu, L.N., Primus, C.M., Ling, J.Q., & Pashley, D.H. (2012). Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate. Acta Biomaterialia, 8(2):836–42.

Quintana, R.M., Jardine, A.P., Grechi, T.R., Grazziotin-Soares, R., Ardenghi, D.M., & Scarparo, R.K. (2019). et al. Bone tissue reaction, setting time, solubility, and pH of root repair materials. Clinical Oral Investigations, 23(3):1359–66.

Qureshi, A., Soujanya, E., Kumar, N., Kumar, P., & Hivarao, S. (2014). Recent advances in pulp capping materials: An overview. Journal of Clinical and Diagnostic Research, 8(1):316–21.

Raina, A., Sawhny, A., Paul, S., & Nandamuri, S. (2020). Comparative evaluation of the bond strength of self-adhering and bulk-fill flowable composites to MTA Plus, Dycal, Biodentine, and TheraCal: an in vitro study . Restorative Dentistry & Endodontics, 45(1):1–8.

Rajasekharan, S., Martens, L.C., Cauwels, R.G.E.C., & Verbeeck, R.M.H. (2014). BiodentineTM material characteristics and clinical applications: A review of the literature. European Archives of Paediatric Dentistry, 15(3):147–58.

Ramachandran Nair, P.N., Sjogren, U., Krey, G., Kahnberg, K.E., & Sundqvist, G. (1990). Intraradicular Bacteria and Fungi in Root-filled, Asymptomatic Human Teeth with Therapy-resistant Periapical Lesions: A Long-term Light and Electron Microscopic Follow-up Study. Journal of Endodontics, 16(12):580–8.

Rodd, H.D., Waterhouse, P.J., Fuks, A.B., Fayle, S.A., & Moffat, M.A. (2006). Pulp therapy for primary molars. International Journal of Paediatric Dentistry, 16(1):15–23.

Rodrigues, E.M., Cornélio, A.L.G., Mestieri, L.B., Fuentes, A.S.C., Salles, L.P., & Rossa-Junior, C. (2017). Human dental pulp cells response to mineral trioxide aggregate (MTA) and MTA Plus: cytotoxicity and gene expression analysis. International Endodontic Journal, 50(8):780–9.

Sarkar, N.K., Caicedo, R., Ritwik, P., Moiseyeva, R., & Kawashima, I. (2005). Physicochemical Basis of the Biologic Properties of Mineral Trioxide Aggregate. Journal of Endodontics, 31(2):97.

Schwartz, R.S., Mauger, M., Clement, D.J., & Walker, W.A. (1999). Mineral trioxide aggregate: A new material for endodontics. Journal of the American Dental Association, 130(7):967–75.

Shahi, S., Rahimi, S., Lotfi, M., Yavari, H.R., & Gaderian, A.R. (2006). A Comparative Study of the Biocompatibility of Three Root-end Filling Materials in Rat Connective Tissue. Journal of Endodontics, 32(8):776–80.

Shayegan, A., Jurysta, C., Atash, R., Petein, M., & Abbeele, A.V. (2012). Biodentine Used as a Pulp-Capping Agent in Primary Pig Teeth. Pediatric Dentistry, 34(7):202–8.

Shen, Y.A., Peng, B., Yang, Y., Ma, J., & Haapasalo, M. (2015). What do different tests tell about the mechanical and biological properties of bioceramic materials? Endodontic Topics, 32(1):47–85.

Siboni, F., Taddei, P., Prati, C., & Gandolfi, M.G. (2017). Properties of NeoMTA plus and MTA plus cements for endodontics. International Endodontic Journal, 50(Special Issue 2): e83–94.

Soheilipour, E., Kheirieh, S., Madani, M., Baghban, A.A., & Asgary, S. (2009). Particle size of a new endodontic cement compared to Root MTA and calcium hydroxide. Iranian Endodontic Journal, 4(3):112–6.

Song, J.S., Mante, F.K., Romanow, W.J., & Kim, S. (2006). Chemical analysis of powder and set forms of Portland cement, gray ProRoot MTA, white ProRoot MTA, and gray MTA-Angelus. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology, 102(6):809–15.

Suh, B., Yin, R., Cannon, M., & Martin, D.E. (2008). Polymerizable dental pulp healing, capping, and lining material and method for use. U.S. Patent Application No. 12/034,093.

Tanalp, J., Karapinar-Kazandağ, M., Dölekoğlu, S., & Kayahan, M.B. (2013). Comparison of the radiopacities of different root-end filling and repair materials. The Scientific World Journal, 2013:1–4.

Tanomaru-Filho, M., Viapiana, R., & Guerreiro-Tanomaru, J.M. (2016). From MTA to new biomaterials based on calcium silicate. Odovtos-International Journal of Dental Sciences, 18(1):18–22.

Tawil, P.Z., Duggan, D.J., & Galicia, J.C. (2015). Mineral trioxide aggregate (MTA): its history, composition, and clinical applications. Compend Contin Educ Dent, 36(4):247–64.

Tenório De França, T.R., da Silva, R., Sedycias De Queiroz, M., & Aguiar, C.M. (2010). Arsenic content in Portland cement: A literature review. Indian Journal of Dental Research, 21(4):591–5.

Tomás-Catalá, C.J., Collado-González, M., García-Bernal, D., Oñate-Sánchez, R.E., Forner, L., & Llena, C. (2018). Biocompatibility of New Pulp-capping Materials NeoMTA Plus, MTA Repair HP, and Biodentine on Human Dental Pulp Stem Cells. Journal of Endodontics, 44(1):126–32.

Torabinejad, M., Hong, C.U., Lee, S.J., Monsef, M., & Pitt Ford, T.R. (1995a). Investigation of Mineral Trioxide Aggregate for Root-End Filling in Dogs. Journal of Endodontics, 21(12):603–8.

Torabinejad, M., Hong, C.U., Mcdonald, F., Pitt Ford, T.R. (1995b). Physical and Chemical Properties of a New Root-End Filling Material. Journal of Endodontics., 21(7):349–53.

Torabinejad, M., & Chivian, N. (1999). Clinical Applications of Mineral Trioxide Aggregate. Journal of Endodontics, 25(3):197–205.

Tuna, E.B., Dinçol, M.E., Gençay, K., & Aktören, O. (2011). Fracture resistance of immature teeth filled with BioAggregate, mineral trioxide aggregate and calcium hydroxide. Dental Traumatology, 27(3):174–8.

Vats, S., & Maheshwari, P. (2019). Comprehensive Estimation and Evaluation of Antimicrobial Efficiency of Different Pulp Capping Materials: An (In-Vitro) Original Study. Journal of Advanced Medical and Dental Sciences Research, 7(5):25–8.

Vidal, K., Martin, G., Lozano, O., Salas, M., Trigueros, J., & Aguilar G. (2016). Apical Closure in Apexification: A Review and Case Report of Apexification Treatment of an Immature Permanent Tooth with Biodentine. Journal of Endodontics, 42(5):730–4.

Voicu, G., Didilescu, A.C., Stoian, A.B., Dumitriu, C., Greabu, M., & Andrei, M. (2019). Mineralogical and microstructural characteristics of two dental pulp capping materials. Materials, 12(11):1–13.

Yoldaş, S.E., Bani, M., Atabek, D., & Bodur, H. (2016). Comparison of the Potential Discoloration Effect of Bioaggregate, Biodentine, and White Mineral Trioxide Aggregate on Bovine Teeth: In Vitro Research. Journal of Endodontics, 42(12):1815–8.

Yousef, M., Abuzeid, S., Taha Hassan Abu Zeid, S., Alothmani, O.S., & Khalil Yousef, M. (2015). Biodentine M. Biodentine and Mineral Trioxide Aggregate: An Analysis of Solubility, pH Changes and Leaching Elements. Life Science Journal, 12(4):1097–8135.

Zarrabi, M.H., Javidi, M., Naderinasab, M., & Gharechahi, M. (2009). Comparative evaluation of antimicrobial activity of threecements: new endodontic cement (NEC), mineral trioxideaggregate (MTA) and Portland. Journal of Oral Science, 51(3):437–42.

Zhang, H., Pappen, F.G., & Haapasalo, M. (2009). Dentin Enhances the Antibacterial Effect of Mineral Trioxide Aggregate and Bioaggregate. Journal of Endodontics, 35(2):221–4.

CHAPTER 8

CLASSIFICATION, APPLICATIONS, PROPERTIES OF GLASS IONOMER-BASED MATERIALS IN PEDIATRIC DENTISTRY FROM PAST TO PRESENT[1]

Hanife Betül KARAASLAN[2]

Emin Caner TÜMEN[3]

1 Bu çalışma, 16.05.2022 tarihinde "Trikalsiyum Silikat Esaslı Biyomateryallerin Farklı İçerikteki Cam İyonomer Esaslı Restoratif Simanlara Bağlanım Dayanımının Değerlendirilmesi" adlı uzmanlık bitirme tezi olarak, Dicle Üniversitesi Çocuk Diş Hekimliği Anabilim Dalı'nda sunulmuştur. Tezi hazırlayan; Dt. Hanife Betül Karaaslan, tezin danışmanı Prof. Dr. Emin Caner Tümen'dir.

2 Arş. Gör. Dicle Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği A.D./ Diyarbakır/Türkiye/dentistbetulokumus@gmail.com (Orcid ID: 0000-0002-9732-177X)

3 Prof. Dr. Dicle Üniversitesi Diş Hekimliği Fakültesi Çocuk Diş Hekimliği A.D./ Diyarbakır/Türkiye /ect1976@gmail.com (Orcid ID: 0000-0002-0905-1096)

General Properties of Glass Ionomer Cements

Glass ionomer cements, which were first used for dental purposes in 1969, were discovered by Wilson and Kent (Prosser, Powis, Brant, & Wilson, 1984). Glass ionomer cements consist of a combination of two different structures: the powder part consisting of calcium, strontium, aluminosilicate glass particles and the liquid consisting of water-soluble polymer acid. When the powder and liquid form are mixed, the hardening reaction takes place (Upadhya, 2005). This hardening reaction takes place chemically as an acid-base reaction. In the first stage, the surface of the glass particles in the powder content is abraded by the acids in the liquid, and ions such as calcium, aluminum and fluorine are released. This phase is called the 'dissolution phase'. After the release of aluminum and calcium ions, the 'gelation phase (freezing phase)' begins. Calcium ions cross-link, forming the viscous structure of the cement. Within 24 hours after mixing the powder liquid, aluminum ions cross-link tightly to the polymeric acid chains, forming the 'maturation phase'. With this final phase, the physical properties of glass ionomer cement are strengthened (Walls, 1986).

Traditional glass ionomer cements have been introduced to the market as a powder-liquid system. The inability to adjust the powder-liquid ratio as determined during the preparation of this type of glass ionomer cement may adversely affect the physicochemical properties of the cement. In order to avoid this problem, glass ionomer cements in unactivated mixture were placed in the capsule form. They are activated by an automatic mixer and become ready for clinical use (Upadhya, 2005).

Glass ionomer cements can be used as a filling material in the restoration of primary and permanent teeth, in bonding stainless steel crowns and orthodontic brackets, as a base material, or in protective treatments for fissure sealing purposes Glass ionomer cements used in pediatric dentistry have advantages such as chemical bonding to teeth, releasing fluoride and being preferred as a restorative material. Glass ionomer cements used in pediatric dentistry have advantages such as chemical bonding to teeth, releasing fluoride and being preferred as a restorative material. When these materials are used for restorative purposes, they can be clinically preferred by preventing caries formation at the tooth-restoration interface thanks to their acceptable level of impermeability, and by ensuring the longevity of the restoration (the ability to prevent material loss that may occur in the tooth in the future) (Berg, 2002).

Classification of Glass Ionomer Cements

Classification of glass ionomer cements according to usage areas

Glass ionomer cements are classified as type I, type II and type III according to their clinical use:

• Type I cements are low film thickness cements used for bonding crowns, bridges and orthodontic brackets.

• Type II cements are used for restorative purposes. It has two subtypes.

Type II-a: It is used in aesthetic restorations in the anterior region. Conventional and resin modified glass ionomer cements are in this group.

Type II-b: It is used for restorative purposes in the posterior region due to its high abrasion resistance.

• Type III cements are used as pit-fissure sealant and base material (Almuhaiza, 2016).

Classification of Glass Ionomer Cements by Content

Today, with glass ionomer cements becoming clinically preferred materials, the need to strengthen the physical and mechanical properties of these cements has arisen. Thanks to the ongoing researches with the current developments in technology, various glass ionomer-based materials that have been accepted for clinical use have been introduced to the market by strengthening their existing properties.

Classification of glass ionomer cements according to their content is as follows:

• Conventional glass ionomer cements

• Resin modified glass ionomer cements

• Polyacid modified composite resins (Comomers)

• High viscosity glass ionomer cements

• Giomers

• Nano-ionomers (Kanık, & Türkün, 2016).

Conventional Glass Ionomer Cements

Conventional glass ionomer cements consist of two different components: a powdered part containing glass particles and a liquid part, which is an aqueous solution of polymer acids (Moshaverinia, Roohpour, Chee, & Schricker, 2011). In the powder composition, there are fluoro-alminosilicate glass particles, and in the liquid part, there are acids such as tartaric, itaconic and polyacrylic acid (Lohbauer, 2010).

When powder and liquid are mixed, the cement hardens through the acid-base reaction and as a result, the translucency and compressive strength of the cement increase (Nicholson, 1998).

Advantageous features of traditional CISs have been reported as being able to bond to the tooth structure without the need for adhesive material,

being biocompatible, preventing caries due to long-term fluoride release, and a similar color to dental tissues (Walls, 1986; Swartz, Phillips, & Clark, 1984).

Conventional glass ionomer cements initially begin to release large amounts of fluorine. In the next period, although there is no high emission as in the beginning, fluorine continues to be released in low amounts for a long time. Fluorine ions activate the remineralization of demineralized enamel and uninfected dentin. Thus, the formation of secondary caries in the tooth can be prevented with long-term fluoride release (Forsten, 1990).

The disadvantages of glass ionomer cements are low wear resistance, brittleness, and relatively low material strength (Cattani-Lorente, Godin, & Meyer, 1994).

Due to the lack of sufficient mechanical properties, the content of conventional glass ionomer cements has been strengthened physicochemically and different types of glass ionomer cements have been put into clinical use (Al-Shaibani et al., 2019).

Resin Modified Glass Ionomer Cements

Resin-based glass ionomer cements were invented in 1989 by Mathis and Ferracane to reduce the setting time and moisture sensitivity of conventional glass ionomer cements, which are affected by moisture sensitivity during the curing reaction (Attin, Buchalla, Kielbassa, & Hellwig, 1995).

Resin modified glass ionomer cements were originally developed as base and liner materials. Later, its content was strengthened and started to be used as a restorative filling material (Forsten, 1995).

RMCIS are composed of a hybrid composition of glass ionomer cement and composite resins. It contains components such as fluoroaluminosilicate glass, photoinitiators, polyacrylic acid, hydroxyethyl methacrylate monomer (HEMA) and water (Berzins et al., 2010).

The curing reaction is completed by the polymerization of the monomers after the acid-base reaction. As with composite resins, resin modified glass ionomer cements exhibit a stronger structure due to the added monomers (Mccabe, 1998). The polymerization of these materials should generally be carried out with light curing devices at a wavelength of 470 nm (Nicholson, & Czarnecka, 2008).

Compared to conventional glass ionomers, the advantageous properties of resin modified glass ionomer cements are that they are resistant to moisture, have high abrasion and fracture resistance, and provide long-term working time for the dentist (Sudha et al., 2017). With the long-term use of RMCIS, surface wear and loss of anatomical form occur. When the material is applied to the tooth, although the color match is similar to the

tooth in the beginning, a darkening in the color of the material has been observed due to surface wear over time (Sidhu, 2010).

In addition, it was stated that these materials, whose biocompatibility was compared with conventional glass ionomer cements, were less biocompatible due to the HEMA added to their monomer content (Nicholson, & Czarnecka, 2008).

Polyacid Modified Composite Resins (Compomers)

The polymerization depth of resin modified glass ionomer cements, which were first produced for restorative purposes, was limited to a maximum of 2 mm. In order to prevent this negative feature, "Polyacid modified resin materials (Compomers)" were developed in 1991 (Andersson-Wenckert, Folkesson, & van Diljken, 1997).

Compomers are materials formed from the combination of the advantageous properties of conventional glass ionomer cements, such as adhesion and fluoride release, and the clinical aesthetic appearance of resin composites (Nicholson, 2007).

Composite resins constitute 70% of the structure of compomers and glass ionomer cements constitute 30%. The curing reaction basically takes place by photopolymerization of the resin in the material (Hes, Leung, & Wei, 1999). The polyacid modified composite resin that was first introduced to the market was the product of Dentsplay company named "Dyract (Dentsply, NewYork, USA)" (Tyas, 2000).

In an in vitro study using Dyract material, compomers were found to be significantly bonded to dentin and enamel. Since compomers are similar to resin composites, primer-adhesive systems should be used before the material is placed into the cavity during clinical application. It has been stated that the bond strength of the compomer material will be higher if etching is applied to the enamel of the tooth to be treated before the use of the primary-adhesive system (Abate, Bertacchini, & Polack, 1997).

Polyacid modified composite resins have become the preferred materials in pediatric dentistry due to their fluorine release, easy application and good aesthetic appearance (Nicholson, 2007).

It has clinical uses such as restorative purposes in class II and class V cavities, as fissure sealant and for bonding orthodontic bands (Qvist, Laurberg, Poulsen, & Teglers, 2004; Chinelatti, Ramos, Chimello, & Palma-Dibb, 2004; Demirci, Ersev, Topçubaşı, & Üçok, 2005).

In randomized clinical studies, although compomer has better physical properties than conventional and resin modified glass ionomer cements, no significant difference was found between them in terms of karyostatic effect (Welbury et al., 2000; Daou, Attin, & Göhring, 2009).

The disadvantages of compomers are limited clinical use, requiring a bonding agent before the use of the material as in composites, micro-breaking and discoloration in the marginal area, more wear than the composite material, and weaker physical properties (Burke, Ray, & McConnell, 2006).

High Viscosity Glass Ionomer Cements

High viscosity glass-ionomer cement (YVCIS) was developed by varying the powder/liquid ratio and powder particle size to improve the poor mechanical properties of conventional CISs. Therefore, YVCIS has been considered as an alternative restorative material for teeth in the posterior region (Friedl, Hiller, & Friedl, 2011). While the powder/liquid ratio is 3:1 or 4:1 in conventional glass ionomer cements, this ratio is 6:1 or 7:1 in high-viscosity glass ionomer cements (Crowley et al., 2006).

In the early 90s, high-viscosity glass ionomer cements were started to be used for "Atraumatic Restorative Treatment (ART)" in developing countries (Yilmaz, Eyuboglu, Kocogullari , & Belduz, 2006).

In the meta-analysis findings of a study, it was reported that, as a result of 3-year follow-up of atraumatic restorative treatments using high-viscosity glass ionomer cement, 71% of the material still exists in the fissures and provides sealing and 97% of the caries preventive effect (van 't Hof, Frencken, van Palenstein Helderman, & Holmgren, 2006).

Since high viscosity glass ionomer cements are not affected by moisture, they do not need a dry surface when applied to the tooth to be treated. This material can be applied even if the tooth surface is moist (Frencken, & Wolke, 2010).

When high viscosity glass ionomer cements are used in fissure sealant treatments, they prevent the development of future carious lesions in healthy dentin (Beiruti, Frencken, van 't Hof MA, & van Palenstein Helderman, 2006).

Frencken et al., in a study they conducted, reported that the high-viscosity glass ionomer cements used as fissure sealant material had a caries-preventing effect even if they were eroded or ruptured over time in the cases they followed up clinically (Frencken, & Wolke, 2010).

In randomized clinical trials, after six years of follow-up, no significant difference in failure was found between single-surface high-viscosity glass ionomer cement and conventional amalgam restorations in permanent teeth (Mickenautsch, & Yengopal, 2012).

High Viscosity Glass Ionomer Cements - CHEMFIL ROCK (YVCIS with Zinc added)

The surface hardness, wear resistance and compressive strength of YVCIS are increased and the curing mechanisms are the same as

conventional glass ionomer cements. Their resolution has been reduced. Chemfil Rock (Dentsplay De Trey, Konstanz, Germany) (Molina et al., 2013). is a glass ionomer cement that has been recently developed by adding zinc particles to the powder content to increase the mechanical properties of high-viscosity glass ionomer cements. In a study, it was stated that Chemfil Rock, a zinc-enhanced glass ionomer cement, exhibits lower micromechanical properties, although it has more macromechanical properties than conventional glass ionomer cements. The reason why the material has low micromechanical properties is thought to be related to the morphology of the glass particles in its structure and the amount of filler it contains. It is also assumed that the zinc polycarboxylate complex formed during the curing reaction of Chemfil Rock provides the material with a high level of fracture resistance (Zoergiebel, & Ilie, 2013).

The bending and tensile strength increased with itaconic acid added as a comonomer to the material content (Moshaverinia, Roohpour, Darr, & Rehman, 2009).

In a study evaluating the amount of microleakage in class V cavities using conventional glass ionomer cement and Chemfil Rock, it was stated that cavities filled with conventional cement showed higher microleakage values (Giray, Peker, Durmus, & Kargül, 2014).

Baba et al. used compomer, Chemfil Rock and Equa Forte as restorative materials for class II cavities in primary teeth. After one year of clinical follow-up, it was reported that compomer exhibited the best clinical performance and that the zinc-enhanced glass ionomer cement, Chemfil Rock, performed better than Equa Forte (Gok Baba, Kirzioglu, & Ceyhan, 2021).

Giomers

Giomers are produced with pre-reacted glass ionomer (PRG) technology and are presented to the market as hybrid materials consisting of a combination of glass ionomer cements and resin composites. The resin matrix of the material contains fluoroaluminosilicate glass particles and polyacrylic acid pre-reacted. In addition, catalysts were added to the matrix to initiate the polymerization. Just like composites, adhesive systems are needed for the bonding of the giomer material to the tooth surface. They are materials that can release fluorine, are easy to apply and light-cure (Kooi et al., 2012).

The giomer material produced by Shofu company in 2000 is Beautifil II. It is basically a nanohybrid composite resin containing S-PRG technology. Pre-reacted glass particles (S-PRG) and fluoro-boroaluminosilicate glass are used as fillers in the resin matrix structure. These impart the fluorine charge feature to the material (Tamilselvam, Divyanand, & Neelakantan, 2013).

The formation of PRG occurs by the acid-base reaction between fluoroaluminosilicate glass (FASG) and polyalkenoic acid formed in the presence of water, and a silica hydrogel layer is formed at the end of the reaction. It contains two types of filling materials with PRG technology. These are the full glass ionomer filler F-PRG and the surface glass ionomer filler S-PRG. S-PRG material consists of 3 layers. The inner part is multifunctional fluoroboro-aluminum silicate glass, the middle part is a pre-reacted glass ionomer layer, and the outermost is a reinforced layer (Ikemura et al., 2003; Akimoto, Ohmori, Hanabusa, & Momoi, 2011; Shiiya et al., 2016).

Bisphenol A glycerate dimethacrylate (Bis-GMA), urethane dimethacrylate (UDMA), 2,2-bis[4-(2-methacryloxy-ethoxyphenyl] propane (Bis-MPEPP) and riethylene glycol dimethacrylate (TEGDMA) components in the organic matrix of the giomers It contains "comphoroquinone" as a photoinitiator.The filling ratio in the resin content is 74.5-87% (Kaya, Bakkal, Durmus, & Durmus, 2018).

With the release of fluoride from the dental material, a stronger fluoropatite compound is formed in the tooth structure instead of hydroxyapatite. Thus, the demineralization process in the presence of caries is slowed down and the remineralization process is accelerated. Fluoride release from the giomer material occurs continuously until the fluoride reservoir is depleted. To recharge the giomer in terms of the fluoride reservoir, it is necessary to use fluoride-containing toothpastes, mouthwashes, or similar products (Markovic et al., 2019; Eldesouky, Hanno, Bakry, & Ahmed, 2016).

In a study evaluating the mechanical and structural properties of the giomer material by polymerization using different light curing devices, it was stated that the amount of filler in the material is a more effective parameter than the power of the beam device. Thanks to the amount of filler in the giomer structure, it becomes more resistant to compression strength (Kaya, Bakkal, Durmus, & Durmus, 2018).

Giomers can be used in all restorations of primary and permanent teeth (classes I, II, III, IV and V), pit and fissure sealants, and treatment of non-carious cervical lesions (Markovic et al., 2019; Rusnac et al., 2019).

In a study evaluating the marginal microleakage of primary teeth using giomer and compomer material, it was observed that the giomer material showed less microleakage values. It has been stated that this material can be preferred for class II restorations in children with high caries risk (Eldesouky, Hanno, Bakry, & Ahmed, 2016).

Giomers are materials that can be preferred for restorative purposes in primary teeth due to their ability to recharge fluorine, prevent secondary

caries, aesthetically compatible color options and acceptable mechanical properties.

Nanoionomers

With the advancement of molecular engineering studies in the field of nanotechnology, it is aimed to improve the existing physicochemical and mechanical properties of materials (Zhang, Lim, Ramakrishna, & Huang, 2005; Beun et al., 2007).

Nano-scale fillers used in materials in dentistry have a particle size of approximately 40 nanometers. However, the innovation made in the material is not directly related to the particle size. It has been reported that the physical and mechanical properties of the material are improved by increasing the amount of nano filler in its content (Beun et al., 2007; Taylor, Kalachandra, Sankarapandian, & Mcgrath, 1998).

Ketac™ N100 is a restorative material developed with nano technology by 3M company in 2007 and introduced to the market. Its clinical uses are stated as temporary filling material in the restoration of primary teeth, small class I, III and V cavities, core material construction and sandwich technique (3M ESPE, Ketac N100, 2007).

In a randomized clinical study using nanoionomer, giomer, and resin-modified glass ionomer for restorative purposes on teeth with non-carious cervical lesions, it was reported that after 1-year follow-up, resin-modified glass ionomer cement and nanoionomer material better preserve the integrity of the structure (Priyadarshini et al., 2017).

Mitra et al., in a study using conventional glass ionomer cement, resin modified glass ionomer cement and nanoionomer, compared the fluorine release and recharge capacity of these materials. It has been reported that nanoionomers release fluoride and recharge similar to conventional and resin-modified glass ionomer cements (Mitra, Oxman, Falsafi, & Ton, 2011).

In a study evaluating the shear bond strength of nanoionomer, conventional glass ionomer cement and resin modified glass ionomer cement to the composite, it was stated that resin modified glass ionomer cement was the best bonding material by showing low polymerization shrinkage values (Babannavar, & Shenoy, 2014).

Glass Carbomers

Glass Carbomer® is a monomer-free restorative cement with carbomized nano-glass based on conventional glass ionomer cement. It contains fluorapatite and hydroxyapatite nanoparticles (Gorseta et al., 2014). It has better physical and mechanical properties than conventional glass ionomers (Moshaverinia et al., 2008).

Thanks to the nano particles in its content, the material gains a strong structure. The nanoparticles that dissolve from the moment they come into contact with the liquid contribute to the formation of the florapatite structure. Thus, the remineralization process is supported (Zainuddin, Karpukhina, Law, & Hill, 2012).

In addition, the beam device used for the curing reaction has a power of 1400 mW/cm^2 (Koenraads, van der Kroon, & Frencken, 2009). The curing reaction is completed by applying heat during the curing reaction of the light curing device used, making the material resistant to compressive strength. The clinical application steps of glass carbomers are similar to conventional glass ionomer cements. After the material is placed in the cavity, GCP Gloss varnish, which is a silicone-based coating, should be applied to the surface in order to maintain the surface smoothness. This coating is used to protect it from moisture and saliva during the initial curing phase and from dehydration during the secondary curing phase (Menne-Happ, & Ilie, 2013).

Çehreli et al., in a study where they measured the marginal integrity and microleakage values of primary molar teeth with glass carbomer material, reported that glass carbomer materials without surface coating showed higher microleakage values by causing microcracks in the teeth (Cehreli, Tirali, Yalcinkaya, & Cehreli, 2013).

The advantage of glass carbomers is that they are easy to use in pediatric patients where humidity control may be difficult due to their moisture tolerance and provide aesthetics close to the natural tooth appearance (Babu, & Subramaniam, 2015).

Multiple factors such as material composition (components), powder-liquid ratio, mixing method, solubility and porosity of the material mass, material surface in contact with the environment, pH of the environment and storage conditions, and the way the material is finished on the surface play a role in the fluoride release from dental materials. Bayrak et al., in their study, evaluated fluorine release using glass carbomer, giomer, high viscosity glass ionomer cement, amalgomer and compomer materials. The glass carbomer group showed the highest fluorine release values. The heat applied during the curing reaction of the glass carbomer prepared in accordance with the manufacturer's instructions increases the mechanical properties of the material. The fluorine release properties of the material change with the application of heat. In the in vitro experimental studies, the acid-base reaction increased with the application of external heat, and the fluorine salts added to the matrix decreased. As a result, fluorine release from glass ionomer-based materials decreases. In the study of Bayrak et al., contrary to in vitro experiments, the amount of fluorine release from glass carbomer prepared by applying external heat showed higher values

than other glass ionomer based materials. Therefore, when evaluating the fluorine release amounts from a material, not only the external heat application factor is effective, but also the composition of the material (Bayrak et al., 2017).

It has been reported that glass carbomers can be used in class I cavities of primary and permanent teeth, in class II cavities where the use of glass ionomer is not recommended, and in class V cavities (Altan, Altan, & Arslanoğlu, 2013).

Bekmezoğlu et al., in a study where they used glass carbomer, giomer, conventional glass ionomer and resin modified glass ionomer cement-containing materials as fissure sealants, stated that the most important disadvantage of resin modified glass ionomer cement is the presence of residual monomers after the curing reaction is completed. For this reason, glass carbomer and glass ionomer cement; reported that it can be used as an alternative to resin modified glass ionomer cements in fissure sealant applications (Bekmezoglu, Güngör, & Karayilmaz, 2019).

In a study evaluating the mechanical properties of glass carbomer and high viscosity glass ionomer cement, it was observed that the microhardness and microshear bond strength of glass carbomers were lower than those of high viscosity glass ionomer cements (Olegário et al., 2015).

Equia Forte HT (Glass hybrid restorative material)

Hybrid materials based on glass ionomer have been modified with glass particles of different sizes. With the developed content, the physical and mechanical properties of the material are significantly affected (Najeeb et al., 2016).

Equia Forte HT (GC, Tokyo, Japan) is a new type of glass hybrid restorative material developed in 2019 based on and modifying glass ionomer cements. Highly reactive glass particles of different sizes are added to the standard fillers. The high reactivity significantly affects the mechanical properties of the material. This feature increases the long-term durability of the material in posterior fillings (Najeeb et al., 2016; Šalinović et al., 2019).

With the glass hybrid technology, the glass's diffraction is reduced and a good match with the matrix is ensured. The material gains a translucent appearance. Therefore, the aesthetic appearance properties of the material have been improved. Equia Forte HT Coat, on the other hand, is a nano-filled coating agent applied to protect the physical properties of the material after the material is placed in the cavity. It provides high wear resistance to the material (GC Corporation, Equia Forte HT, 2019).

In a study, after applying Equia Forte HT, it was left in environments

with different pH. The effects of different pH media on the nanofilled coating agent and wear resistance were compared. As a result, the amount of wear against brushing was found to be less in the materials using the coating agent. In addition, the material dissolves more at low pH (Brkanović et al., 2021).

Future Role of Glass Ionomer Cements in Pediatric Dentistry

Glass ionomer cements are up-to-date materials that have been used for restorative purposes, with the advantages of being anticariogenic due to fluoride release and recharging, biocompatibility with teeth and surrounding tissues, and low microleakage values. With various clinical studies, the safety of the use of glass ionomer cements in pediatric dentistry has been proven (Şirinoğlu-Çapan et al., 2020; Sagmak, Bahsi, Ozcan, & Satici, 2020; Durmus et al., 2021).

Glass ionomer cements are available in two forms for clinical use. The first is the traditional two-bottle powder-liquid system, which is mixed manually and consists of polyalkenoic acid liquid and powder containing glass particles. The second one is in encapsulated form and contains a mixture of glass powder and vacuum-dried polyalkenoic acid in one chamber. In the second compartment is the prepackaged formulation containing a mixture of distilled/deionized water or a solution of tartaric acid and water. The encapsulated form is mixed using mechanical mixing devices. It has been reported that glass ionomer cements, the contents of which are in encapsulated form, are more advantageous than conventional glass ionomer cements that are manually mixed and cured by acid-base reaction, due to the preset powder-liquid ratio, standardized mixing time and technique (Dowling, & Fleming, 2009; Baig, & Fleming, 2015). The compressive strength of glass ionomer cements used in encapsulated form was found to be higher than those prepared manually. Therefore, the use of glass ionomer cements in the form of encapsulation becomes more advantageous than manually prepared cements in areas exposed to greater occlusal pressure forces (Arnold, Warren, Buchanan, & Lombard, 2022).

In addition, with the use of glass ionomer cements in the ART technique, a significant reduction in fissure caries was observed. ART is a treatment option approved by the World Health Organization (WHO) developed to prevent or restore caries in populations with limited access to treatment. The COVID-19 pandemic seen in recent years has had an impact on emergency clinical applications in pediatric dentistry. For this reason, the use of glass ionomer cements in the ART technique ensures successful caries management in both primary and permanent teeth (Al-Halabi et al., 2020).

REFERENCES

Abate, P.F., Bertacchini, M., & Polack, A. (1997). Adhesion of a compomer to dental structures. Restorative Dentistry, 1997;28:509–12.

Akimoto, N., Ohmori, K., Hanabusa, M., & Momoi, Y. (2011). An eighteen-month clinical evaluation of posterior restorations with fluoride releasing adhesive and composite systems. Dental Materials Journal, 30(3):411–8.

Al-Halabi, M., Salami, A., Alnuaimi, E., Kowash, M., & Hussein, I. (2020). Assessment of paediatric dental guidelines and caries management alternatives in the post COVID-19 period. A critical review and clinical recommendations. European Archives of Paediatric Dentistry., 21(5):543–56.

Almuhaiza, M. (2016). Glass-ionomer cements in restorative dentistry: A critical appraisal. Journal of Contemporary Dental Practice, 17(4):331–6.

Al-Shaibani, D., Bamusa, B., Bajafar, S., al Eidan, S., Almuhaidib, D., Alhakeem, F. (2019). Modification of Glass Ionomer Restorative Material: A Review of Literature. EC Dental Science, 18:1001–6.

Altan, H., Altan, A., & Arslanoğlu, Z. (2013). Glass Ionomer Cement, Derivates and Glass Carbomer Cement. ADO Klinik Bilimler Dergisi Journal of Clinical Sciences, 6:1319–22.

Andersson-Wenckert, I.E., Folkesson, U.H., van Diljken, J.W.V. (1997). Durability of a polyacid-modified composite resin (compomer) in primary molars A multicenter study. Acta Odontologica Scandinavica, 55(4):255–60.

Arnold, S., Warren, N., Buchanan, G.D., & Lombard, R. (2022). Comparison of capsule-mixed versus hand-mixed glass ionomer cements Part 1: compressive strength and surface hardness. South African Dental Journal, 77(02):57–64.

Attin, T., Buchalla, W., Kielbassa, A.M., & Hellwig, E. (1995). Curing shrinkage and volumetric changes of resin-modifed glass ionomer restorative materials. Dent Mater, 11:359–62.

Babu, G.K., & Subramaniam, P. (2015). Evaluation of Solubility and Microleakage of Glass Carbomer Sealant. The Journal of Clinical Pediatric Dentistry, 39(5):429–34.

Baig, M.S., & Fleming, G.J.P. (2015). Conventional glass-ionomer materials: A review of the developments in glass powder, polyacid liquid and the strategies of reinforcement. Journal of Dentistry. 2015;43(8):897–912.

Babannavar, R., & Shenoy, A. (2014). Evaluation of shear bond strength of silorane resin to conventional, resin-modified glass ionomers and nano-ionomer cements. J Investig Clin Dent, 5(4):295–300.

Bayrak, G.D., Sandalli, N., Selvi-Kuvvetli, S., Topcuoglu, N., & Kulekci, G. (2017). Effect of two different polishing systems on fluoride release, surface roughness and bacterial adhesion of newly developed restorative materials. Journal of Esthetic and Restorative Dentistry, 29(6):424–34.

Beiruti, N., Frencken, J.E., van 't Hof, M.A., & van Palenstein Helderman, W.H. (2006). Caries-preventive effect of resin-based and glass ionomer sealants-over time: a systematic review. Community Dentistry and Oral Epidemiology, 34(6):403–9.

Bekmezoglu, Z., Güngör, Ö., & Karayilmaz, H. (2019). Comparison of Glass Carbomer, Giomer, Glass Ionomer and Resin Fissure Sealants on Permanent Molar Teeth. Journal of Dentistry Indonesia, 26(1):10–8.

Berg, J.H. (2002). Glass ionomer cements Medical management of dental caries View project. Pediatric Dentistry, 24(5):430–8.

Berzins, D.W., Abey, S., Costache, M.C., Wilkie, C.A., & Roberts, H.W. (2010). Resin-modified glass-ionomer setting reaction competition. Journal of Dental Research, 89(1):82–6.

Beun, S., Glorieux, T., Devaux, J., Vreven, J., & Leloup, G. (2007). Characterization of nanofilled compared to universal and microfilled composites. Dental Materials, 23(1):51–9.

Brkanović, S., Ivanišević, A., Miletić, I., Mezdić, D., & Krmek, S.J. (2021). Effect of nano-filled protective coating and different ph enviroment on wear resistance of new glass hybrid restorative material. Materials, 14(4):755.

Burke, F.M., Ray, N.J., & McConnell, R.J. (2006). Fluoride-containing restorative materials. International Dental Journal, 56(1):33–43.

Cattani-Lorente, M.A., Godin, C., & Meyer, J.M. (1994). Mechanical behavior of glass ionomer cements affected by long-term storage in water. Dent Mater, 10:37–44.

Cehreli, S.B., Tirali, E., Yalcinkaya, Z., & Cehreli, Z.C. (2013). Microleakage of newly developed glass carbomer cement in primary teeth. European Journal of Dentistry, 7(1):15–21.

Chinelatti, M.A., Ramos, R.P., Chimello, D.T., & Palma-Dibb, R.G. (2004). Clinical performance of a resin-modified glass-ionomer and two polyacid-modified resin composites in cervical lesions restorations: 1-year follow-up. Journal of Oral Rehabilitation, 31:251–7.

Crowley, C.M., Doyle, J., Towler, M.R., Hill, R.G., & Hampshire, S. (2006). The influence of capsule geometry and cement formulation on the apparent viscosity of dental cements. Journal of Dentistry, 34(8):566–73.

Daou, M.H., Attin, T., & Göhring, T.N. (2009). Clinical Performance of Compomer and Amalgam restorations in Primary Molars in a Prospective 36 Months follow up. Schweizer Monatsschrift für Zahnmedizin SMfZ, 119(11):1082–8.

Demirci, M., Ersev, H., Topçubaşı, M., Üçok, M. (2005). Clinical evaluation of a polyacid-modified resin composite in class V carious lesions: 3-year results. Dent Mater J, 24(3):321–7.

Dowling, A.H., & Fleming, G.J.P. (2009). Are encapsulated anterior glass-ionomer restoratives better than their hand-mixed equivalents? Journal of Dentistry, 37(2):133–40.

Durmus, B., Sezer, B., Tugcu, N., Caliskan, C., Bekiroglu, N., & Kargul, B. (2021). Two-Year Survival of High-Viscosity Glass Ionomer in Children with Molar Incisor Hypomineralization. Medical Principles and Practice, 30(1):73–9.

Eldesouky, H.I., Hanno, A.G., Bakry, N.S., & Ahmed, D.M. (2016). Marginal leakage evaluation of giomer and compomer in primary teeth (in-vitro study). Alexandria Dental Journal, 41(2):188–93.

Forsten, L. (1990). Short- and long-term fluoride release from glassionomers and other fluoride-containing fillingmaterials in vitro. Scand J Dent Res, 98:179–85.

Forsten, L. (1995). Resin-modified glass ionomer cements: fluoride release and uptake. Acta Odontologica Scandinavica, 53(4):222–5.

Frencken, J.E., Wolke, J. (2010). Clinical and SEM assessment of ART high-viscosity glass-ionomer sealants after 8-13 years in 4 teeth. Journal of Dentistry, Jan;38(1):59–64.

Friedl, K., Hiller, K.A., & Friedl, K.H. (2011). Clinical performance of a new glass ionomer based restoration system: A retrospective cohort study. Dental Materials, 27(10):1031–7.

GC Corporation, Equia Forte HT. (2019). (erişim tarihi: 19.02.2022, https://europe.gc.dental/sites/europe.gc.dental/files/products/downloads/equiaforteht/manual/MAN_Comprehensive_Guide_EQUIA_Forte_HT.pdf)

Giray, F.E., Peker, S., Durmus, B., & Kargül, B. (2014). Microleakage of new glass ionomer restorative materials in permanent teeth. European journal of paediatric dentistry, 15:122.

Gok Baba, M., Kirzioglu, Z., & Ceyhan D. (2021). One-year clinical evaluation of two high-viscosity glass-ionomer cements in class II restorations of primary molars. Australian Dental Journal, 66(1):32–40.

Gorseta, K., Borzabadi-Farahani, A., Gorseta, K., Glavina, D., Borzabadi-Farahani, A., & van Duinen, R. (2014). One-Year Clinical Evaluation of a Glass Carbomer Fissure Sealant, a Preliminary Study The effects of ozone on the NF-kB system pathway View project One-Year Clinical Evaluation of a Glass Carbomer Fissure Sealant, a Preliminary Study-GMA resin sealant. J Prosthodont Rest Dent, 22(2):67–71.

Hes, K.M.Y., Leung, S.K., & Wei, S.H.Y. (1999). Resin-ionomer restorative materials for children: A review. Australian Dental Journal, 44(1):1–11.

Ikemura, K., Tay, F.R., Kouro, Y., Endo, T., Yoshiyama, M., & Miyai, K., (2003). Optimizing filler content in an adhesive system containing pre-reacted glass-ionomer fillers. Dental Materials, 19:137–46.

Kaya, M.S., Bakkal, M., Durmus, A., & Durmus, Z. (2018). Structural and mechanical properties of a giomer-based bulk fill restorative in different curing conditions. Journal of Applied Oral Science, 26:1–10.

Kanık, Ö., & Türkün, L.Ş. (2016). Recent Approaches In Restorative Glass Ionomer Cements. Journal of Ege University School of Dentistry, 37(2):54–65.

Koenraads, H., van der Kroon, G., & Frencken, J.E. (2009). Compressive strength of two newly developed glass-ionomer materials for use with the Atraumatic Restorative Treatment (ART) approach in class II cavities. Dental Materials, 25(4):551–6.

Kooi, T.J.M., Tan, Q.Z., Yap, A.U.J., Guo, W., Tay, K.J., & Soh, M.S. (2012). Effects of food-simulating liquids on surface properties of giomer restoratives. Operative Dentistry, 37(6):665–71.

Lohbauer, U. (2010). Dental glass ionomer cements as permanent filling materials? -Properties, limitations and future trends. Materials, 3(1):76–96.

Markovic, D.L., Petrovic, B.B., Peric, T.O., Trisic, D., Kojic, S., & Kuljic, B.L. (2019). Evaluation of Sealant Penetration in Relation to Fissure Morphology, Enamel Surface Preparation Protocol and Sealing Material. Oral health & preventive dentistry, 17:349–55.

Mccabe, J.F. (1998). Resin-modified glass-ionomers. Biomaterials, 19(6):521–7.

Menne-Happ, U., & Ilie, N. (2013). Effect of gloss and heat on the mechanical behaviour of a glass carbomer cement. Journal of Dentistry, 41(3):223–30.

Mickenautsch, S., & Yengopal, V. (2012). Failure rate of atraumatic restorative treatment using high-viscosity glass-ionomer cement compared to conventional amalgam restorative treatment in primary and permanent teeth: a systematic review update [protocol]. South African Dental Journal, 7(7):329–31.

Mitra, S.B., Oxman, J.D., Falsafi, A., & Ton, T.T. (2011). Fluoride release and recharge behavior of a nano-filled resin-modified glass ionomer compared with that of other fluoride releasing materials. Am J Dent, 24(6):372.

Molina, G.F., Cabral, R.J., Mazzola, I., Brain Lascano, L., & Frencken, J.E. (2013). Mechanical performance of encapsulated restorative glass-ionomer cements for use with Atraumatic Restorative Treatment (ART). Journal of Applied Oral Science, 21(3):243–9.

Moshaverinia, A., Ansari, S., Movasaghi, Z., Billington, R.W., Darr, J.A., & Rehman, I.U. (2008). Modification of conventional glass-ionomer cements with N-vinylpyrrolidone containing polyacids, nano-hydroxy and fluoroapatite to improve mechanical properties. Dental Materials, 24(10):1381–90.

Moshaverinia, A., Roohpour, N., Darr, J.A., & Rehman, I.U. (2009). Synthesis and characterization of a novel N-vinylcaprolactam-containing acrylic

acid terpolymer for applications in glass-ionomer dental cements. Acta Biomaterialia, 5(6):2101–8.

Moshaverinia, A., Roohpour, N., Chee, W.W.L., & Schricker, S.R. (2011). A review of powder modifications in conventional glass-ionomer dental cements. Journal of Materials Chemistry, 21(5):1319–28.

Najeeb, S., Khurshid, Z., Zafar, M.S., Khan, A.S., Zohaib, S., & Martí, J.M.N. (2016). Modifications in glass ionomer cements: Nano-sized fillers and bioactive nanoceramics. International Journal of Molecular Sciences, 17(7):1–14.

Nicholson, J.W. (1998). Chemistry of glass-ionomer cements: a review. Biomaterials, 19:485–94.

Nicholson, J.W. (2007). Polyacid-modified composite resins ("compomers") and their use in clinical dentistry. Dental Materials, 23(5):615–22.

Nicholson, J.W., Czarnecka, B. (2008). The biocompatibility of resin-modified glass-ionomer cements for dentistry. Dental Materials, 24(12):1702–8.

Olegário, I.C., Malagrana, A.P.V.F.P., Kim, S.S.H., Hesse, D., Tedesco, T.K., & Calvo, A.F.B. (2015). Mechanical properties of high-viscosity glass ionomer cement and nanoparticle glass carbomer. Journal of Nanomaterials, 2015:1–4.

Priyadarshini, B.I., Jayaprakash, T., Nagesh, B., Sunil, C.R., Sujana, V., & Deepa, V.L. (2017). One-year comparative evaluation of Ketac Nano with resin-modified glass ionomer cement and Giomer in noncarious cervical lesions: A randomized clinical trial. Journal of conservative dentistry: JCD, 20(3):204–8.

Prosser, H.J., Powis, M.D.R., Brant, P., Wilson, G.A.D. (1984). Characterization of glass-ionomer cements 7. The physical properties of current materials. Journal of Dentistry, 12(3):231–40.

Qvist, V., Laurberg, L., Poulsen, A., & Teglers, P.T. (2004). Class II restorations in primary teeth: 7-year study on three resin-modified glass ionomer cements and a compomer. European Journal of Oral Sciences, 112:188–96.

Rusnac, M.E., Gasparik, C., Irimie, A.I., Grecu, A.G., Mesaroş, A.Ş., & Dudea, D. (2019). Giomers in dentistry - at the boundary between dental composites and glass-ionomers. Medicine and Pharmacy Reports, 92(2):1–6.

Sagmak, S., Bahsi, E., Ozcan, N., & Satici, O. (2020). Comparative Evaluation of Antimicrobial Efficacy and Fluoride Release of Seven Different Glass-Ionomer-Based Restorative Materials. Oral Health Prev Dent, 18(1):521–8.

Šalinović, I., Stunja, M., Schauperl, Z., Verzak, Ž., Malčić, A.I., & Rajić, V.B. (2019). Mechanical properties of high viscosity glass ionomer and glass hybrid restorative materials. Acta Stomatologica Croatica. 2019;53(2):125–31.

Shiiya, T., Tomiyama, K., Iizuka, J., Hasegawa, H., Kuramochi, E., Fujino, F. (2016). Effects of resin-based temporary filling materials against dentin demineralization. Dental Materials Journal, 35(1):70–5.

Sidhu, S.K. (2010). Clinical evaluations of resin-modified glass-ionomer restorations. Dental Materials, 26(1):7–12.

Sudha, K., Kotaiah, T.H., Reddy, O.S., Laxmi, P.S., Rao, C.L., & Pavani, T.L.N. (2017). A Comparative Evaluation of Shear Bond Strength of Resin-modified Glass Ionomer and Zirconomer Incorporated with 1.5% Doxycycline: An In Vitro Study. Journal of Adhesive Dentistry, 9(3):159–63.

Swartz ML, Phillips RW, & Clark HE. Long-term F Release from Glass lonomer Cements. J Dent Res. 1984;63(2):158–60.

Şirinoğlu-Çapan, B., Akyüz, S., Alev, B., Tacal-Aslan, B., Kadir, T., & Yarat, A. (2020). In Vitro Fluoride-Release/Recharge Pattern and Antimicrobial Effects of Current Restorative Materials Used in Pediatric Dentistry. Experimed, 10(1):7–15.

Tamilselvam, S., Divyanand, M.J., & Neelakantan, P. (2013). Biocompatibility of a Conventional Glass Ionomer, Ceramic Reinforced Glass Ionomer, Giomer and Resin Composite to Fibroblasts: In vitro Study. The Journal of Clinical Pediatric Dentistry, 37(4):403–6.

Taylor, D.F., Kalachandra, S., Sankarapandian, M., & Mcgrath, J.E. (1998). Relationship between filler and matrix resin characteristics and the properties of uncured composite pastes. Biomaterials, 19:197–204.

Tyas, M.J. (2000). Three-year clinical evaluation of a polyacid-modified resin composite (Dyract). Oper Dent, 25(3):152–4.

Upadhya, N.P. (2005). Glass Ionomer Cement-The Different Generations. Trends Biomater Artif Organs, 18(2):158–65.

van 't Hof, M.A., Frencken, J.E., van Palenstein Helderman, W.H., Holmgren, C.J. (2006). The Atraumatic Restorative Treatment (ART) approach for managing dental caries: A meta-analysis. International Dental Journal, 56(6):345–51.

Walls, A.W.G. (1986). Glass polyalkenoate (glass-ionomer) cements: a review. JDent, 14:231–46.

Welbury, R.R., Shaw, A.J., Murray, J.J., Gordon, P.H., & McCabe, J.F. (2000). Clinical evaluation of paired compomer and glass ionomer restorations in primary molars: Final results after 42 months. British Dental Journal, 189(2):93–7.

Yilmaz, Y., Eyuboglu, Ö., Kocogullari, M.E., & Belduz, N. (2006). A one-year clinical evaluation of a high-viscosity glass ionomer cement in primary molars. Journal of Contemporary Dental Practice. 2006;7(1):071–8.

Zainuddin, N., Karpukhina, N., Law, R.V., & Hill, R.G. (2012). Characterisation of a remineralising Glass Carbomer ® ionomer cement by MAS-NMR Spectroscopy. Dental Materials, 28(10):1051–8.

Zhang, Y., Lim, C.T., Ramakrishna, S., & Huang, Z.M. (2005). Recent development of polymer nanofibers for biomedical and biotechnological applications. Journal of materials science: materials in medicine, 16:933–46.

Zoergiebel, J., & Ilie, N. (2013). Evaluation of a conventional glass ionomer cement with new zinc formulation: Effect of coating, aging and storage agents. Clinical Oral Investigations, 17(2):619–26.

3M ESPE, Ketac N100. (2007). (erişim tarihi: 19.02.2022, https://multimedia.3m.com/mws/media/443480O/ketac-n100-brochure-ebu.pdf)

CHAPTER 9

USE OF MESENCHYMAL STEM CELLS IN NERVOUS SYSTEM DISEASES

Kemal YÜCE[1]

Ahmet ISMAIL OZKAN[2]

1 Corresponding author, Selcuk University, Medicine Faculty, Department of Basic Medical Sciences, Physiology. Email: yuce2020@hotmail.com, Orcid: 0000-0001-8915-4900

2 Dicle University, Faculty of Science, Department of Biology. Email: aiozkan.2015@gmail.com, Orcid: 0000-0002-4511-2386.

Introduction

Mesenchymal Stem Cells (MSCs) can differentiate into chondrocyte, fibroblast, osteoblast, hepatocyte, neural cell, adipocyte and myocyte, and are multipotent.They are found in almost all tissues, including spleen, cartilage, thymus, milk, muscle, dental pulp, skin, adipose, umbilical cord, bone marrow, endometrium and synovium, and have self-renewable capability (Meirelles, 2009; Patki, Kadam, Chandra, & Bhonde, 2010; Qi, Li, Zhang, & Melino, 2018; J. Wang et al., 2020). MSCs taken from these tissues are not pure in the beginning. The MSCs must be separated, propagated and brought to a certain degree of purity. Before MSCs are used, they must be proven to be MSCs. For this, these cells must be able to adhere to the petri dish, differentiate into osteoblasts, chondrocytes, and adipocytes and they must be positive in terms of markers such as CD105, CD90, CD73. And these cells must also be negative for markers such as CD19, CD11b, CD34, CD14, CD45, and HLA-DR. MSCs also have antifibrotic, proangiogenic, antiapoptotic, antibacterial, neuroprotective, anti-inflammatory, and chemoattractive effects (Song, Scholtemeijer, & Shah, 2020; Viswanathan et al., 2019). Immunomodulation, differentiation and paracrine mechanisms provide that MSCs have the regenerative capacity (Ward, Abadeh, & Connelly, 2018). Secreted molecules by MSCs for paracrine effects are macrophage colony-stimulating factor, interleukins (IL-6,-7,-8,-11,-12,-14,-15), hepatocyte growth factor, transforming growth factor beta, leukemia inhibitory factor, vascular endothelial growth factor, exosomes, angiopoietin and insulin-like growth factor 1 (He, Wang, Chen, Li, & Liu, 2009). Depending on the features stated above, MSCs can be used for treatment of diseases, such as immune system disorders, cardiovascular diseases, bone, skeletal and cartilage damage, Parkinson's, Alzheimer's, multiple sclerosis, ischemia and spinal cord injury (Yuce & Ozkan, 2019).

The number of transferred cells, the injection route, the injection frequency, and the exposure time to the disease are the main factors in determining the effectiveness of cell therapy (Zanganeh, Soudi, Zavaran Hosseini, & Khosrojerdi, 2019). As a result, MSCs promise a future in the treatment of many diseases. Therefore, in this chapter, it has been decided to discuss the MSC applications that may lead to further researches.

Mesenchymal stem cell (msc) applications and experiments

Amyotrophic lateral sclerosis (ALS)

ALS, a destructive neurodegenerative disease, is characterized by the damage of upper and lower motor neurons that causes progressive weakness and death. It has been noted that stem cells can modulate the local microenvironment which might lead motor neurons to cope up with

the disease and keep living (K. S. Chen, Sakowski, & Feldman, 2016). It has been revealed that various growth molecules, which have antiapoptotic and immunomodulatory features, are secreted by MSCs. In such a study of ALS, hMSCs (human mesenchymal stromal cells) have been administered as an intraventricularly single dose or repeated intraspinal implantations in transgenic ALS mouse model. It has been stated that intraventricular implantation of hMSC led to an important decrease in animal's lifespan and an increase in microgliosis of animals. It has been reported that an important healing of motor function and no hMSC-related changes were detected (such as astrogliosis, motor neuron numbers, and microgliosis). Consequently, intraspinal hMSCs implantation may be an encouraging approach to ALS healing. It has also been noted that repeated implantations might bring important therapeutic advantages (Bursch et al., 2019). In another study of ALS, a transgenic animal model was used for the test. The animal model of ALS, symptomatic hSOD1G93A rat, was expressed as a mutated form of human superoxide dismutase (hSOD). MSCs were implanted into the cerebrospinal fluid of symptomatic hSOD1G93A rats. It has been stated that the MSCs leaked into the nervous parenchyma and migrated significantly to the damaged motor neurons in the ventral gray matter (Boucherie, Schäfer, Lavand'homme, Maloteaux, & Hermans, 2009).

Multiple sclerosis (MS)

MS is a central nervous system demyelinating disease caused by immune system disorders. Immune system inflammasomes are among the causes of MS. Inflammasomes are multiprotein complexes of the innate immune response involved in the activation of the proinflammatory cytokines interleukin 1β and IL-18 and the processing of caspase 1, as well as the pyroptosis mechanism regulated by cell death and acquired immunity (Govindarajan, de Rivero Vaccari, & Keane, 2020). It has been noted that MSCs and methylprednisolone (MP) administered mice group had an significantly milder disease and less clinical scores than non-administered (control) mice group. It has been reported that the TNF-α and myeloperoxidase (MPO) were suppressed by MSCs and MP, and the interleukin 10 (IL-10) was increased by MSCs and MP administrations. The brain glutathione (GSH) level of MSCs-administered mice was significantly higher than the brain GSH level of non-administered mice. Consequently, it has been stated that the MSCs might be utilized for cell therapy because they showed a protective effect in the MS animal model (Mahfouz et al., 2017). Experimental autoimmune encephalomyelitis (EAE) is an MS experimental animal model. In a study of EAE, green fluorescent protein-positive MSCs were collected in the CNS inflammation zone after implanted intravenously and intraventricularly. It has also been noted that

glial fibrillary acidic protein, O4 (oligodendrocytic marker), beta-tubulin type III, and galactocerebroside were expressed by the green fluorescent protein-positive MSCs. It has been stated that MSC-implanted animals reduced the clinical aspect of chronic EAE. A serious decrease in CNS inflammation and remarkable recovery of the axons have been reported in MSCs implanted animals (particularly after the intraventricular injection). It has been stated that the intravenously implanted MSCs went to lymph nodes and showed systemic immunomodulatory activity and decreased the lymphocytes reproduction (as a response to myelin antigens and mitogens). As a result, the BM-MSCs have been noted to be an applicable and easy procedure in disorders such as MS for immunomodulation, neuroprotection, neuroregeneration and remyelination (Kassis et al., 2008). In a study of MS animal models, it has been stated that the BM-hMSCs migrated into the damaged CNS, encouraged the functional healing of the chronic and repeated-remitting EAE mouse models, and moderated the improvement of disease and the reaction of the host immune. It has been reported that the dimension of the injury was decreased and the oligodendrocyte lineage cells in the lesion zone were increased by the collection of implanted BM-hMSCs in the CNS. BM-hMSCs have also been reported to affect the host immune responses. It has been stated that the inflammatory T-cells [Th1 cells produces the interferon-gamma (IFN-γ) and the Th17 inflammatory cells produces the IL-17] and their related cytokines were decreased because of the increase in IL-4 produced by Th2 cells and the anti-inflammatory cytokines (Bai et al., 2009). In another study of the EAE mice model, it has been noted that the Interferon-β (IFN-β) gene was transduced into the AD-MSCs. It has also been stated that the functional features of AD-MSCs were increased by transduced IFN-β gene via both ameliorating the MS symptoms in EAE mice models and decreasing the peripheral and central neuro-inflammation indications (Marin-Bañasco et al., 2017).

Spinal cord injury (SCI)

SCI is a neurological condition that seriously affects life unfavorably. Worldwide, two to three million people experience an SCI-related disability each year, and 250,000 to 500,000 people suffer from this disease. The economic cost of this disease is high. Besides, bladder, bowel, and sexual problems are seen in the early stages of life in an individual with spinal cord injury that can cause devastating psychological effects. (Quadri et al., 2018). In an experiment, BM-MSCs have been transfected by the pGL4.51 vector containing luciferase gene to find out the localization feature. It has been observed that the implanted BM-MSCs mostly accumulated at the center of the damaged area. It has been shown by body reflexes and recovery score that Stem cell-administration had seriously better therapeutic potential outcomes than the non-administered group

(Bhat et al., 2018). In a renovation study of chronic SCI, the collagen scaffold (NeuroRegen Scaffold; NRS) and human UC-MSCs (hUC-MSCs) have been administered into the cut place after removing the damaged tissue surgically. Movement in rats was encouraged, and the cortical somatosensory and motor evoked potentials were improved as a result of the administration of NRS and hUC-MSCs. The astrocyte growth was blocked outside the lesion zone by the administration of hUC-MSCs and NRS, whereas the neurofilament, remyelination, and β tubulin III positive neural regeneration were supported by hUC-MSCs and NRS. Consequently, it has been noted that the combined administration of hUC-MSCs with NRS demonstrated important curative effects against chronic SCI via neural regeneration (N. Wang et al., 2017). In a study, SCI has been generated in ICR (CD-1) mice by extradural compression on T6–T7 levels of the spinal cord. It has been stated that the moringin (MOR)-administered gingival MSCs (G-MSCs) showed a few activities such as anti-inflammatory and anti-apoptotic. The MOR-administered G-MSCs have been reported to reduce the COX-2, inflammatory cytokines (IL-1β and IL-6) and GFAP levels in the spinal cord and repair the spinal cord. The apoptotic pathway was affected by MOR-administered G-MSCs. Here, MSCs gave rise to reducing Bax, CASP3 (Caspase3), and caspase CASP9 (CASP9) expressions (Mammana et al., 2019). In a recent study, the hUC-MSCs and laser, have been tested separate or combined, against SCI. As a result, it has been reported that the Basso–Beattie–Bresnahan (BBB) scores of combined administration significantly increased when compared to the separated administrations. It has also been stated that the Nissl bodies of the combined administration group were innumerable and nerve fibers were longer and thicker, and the GFAP was the highest in the combined administration group. The diffusion tensor imaging (DTI) has revealed that the structure and arrangement of neurofilament were improved optimally by the combined administration (H. Chen et al., 2020).

Alzheimer's disease (AD)

AD, which is defined as the worldwide common neurodegenerative disease, brings progressive memory damage and ruining of cognition (Ge, Zhang, Hao, Zhao, & Dong, 2018). Ruining in cognition has been noted to be significantly healed in the $A\beta_{1\text{-}42}$ infused mice administered with placenta-derived MSCs (PD-MSCs) Amyloid precursor protein (APP), the β-site APP cleaving enzyme 1 (BACE1), amyloid β (Aβ) expressions, and the γ-and β- secretase function were decreased by the changes in behavior. The administration of PD-MSCs blocked the activation of glial cells and the generation of both inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Downregulation of inflammatory cytokines, prevention of neuronal cell death, and differentiation of neuronal progenitor

cell into neuronal cell in Aβ1-42-infused mice were accomplished by PD-MSCs. As a result, the neuroprotection (neurogenesis, neuronal death, cytokine expression, and glial cell activation in the hippocampus) has been recorded to be mediated by the PD-MSC (Yun et al., 2013). The amyloid β-peptide (Aβ) was significantly decreased by the BM-MSCs intracerebral implantation in APP/PS1 mice. The decrease in Aβ deposition was related to the renewal of damaged microglial function because the increase in Aβ-degrading factors led to both the reduction of inflammatory responses and the increasing of alternatively activated microglial markers. The BM-MSCs implanted in APP/PS1 mice decreased the tau hyperphosphorylation and progressed the cognitive function. Consequently, it has been stated that the BM-MSCs can regulate either immune or inflammatory responses in AD mice, repair the pathophysiology of them, and develop the cognitive regression related to Aβ deposits (Lee et al., 2009). In a recent study, it has been noted that cytokine-preconditioned MSCs were transplanted intranasally into the triple-transgenic 3xTg mice. As a result, cytokine-preconditioned MSCs originating from extracellular vesicles (EVs) had the potential to stimulate the immunomodulatory and neuroprotective effects in AD. It has been noted that the MSC-EVs in the brain decreased the microglia cells activation and increased the density of dendritic spine. It has been determined that the polarization from primary microglia to an anti-inflammatory appearance was emerged by MSC-EVs. It has been stated that the neuroprotective features of transgenic mice might be a consequence of the affirmative regulation of inflammation state (Losurdo et al., 2020). In another study of the AD mouse model, healing effects of the hUC-MSCs on SAMP8 mice cognition capability have been researched and it has been concluded that the hepatocyte growth factor (HGF) secretion of hUC-MSCs had significantly effective on defective neural cells healing via decreasing the hyperphosphorylation of tau, increasing the spine recovery and supporting the synaptic plasticity (Jia et al., 2020).

Schizophrenia

Schizophrenia is a mental disorder with heterogeneous syndromes. Examples of schizophrenia's syndromes include hallucinations, excessively disorganized thinking, speech and behavior, lack of motivation, lack of energy, failure to perform body cleansing, and cognitive disorders. Biological markers of schizophrenia are stress, mitochondrial dysfunction, neurinflamation, oxidative stress and circadian rhythm disorders (Fisar, 2022). Another feature of schizophrenia patients is that mentalization and the ability to understand other people's intentions and emotions are impaired. (Bradley, Tai, Hankin, & Woolley, 2021). An important part of the information about schizophrenia is based on experimental animal models. In experimental animal models, various chemicals are used to produce

schizophrenia -like symptoms in animals. Phencyclidine (PCP) is one of the substances used to reveal schizophrenia in experimental animals.. PCP reveals the positive, negative and cognitive -like behavior of schizophrenia in rodents. It was shown that the behavioral phenotypes of mice treated with PCP could be alleviated by intracranial transplantation of mesenchymal stem cells (MSC). Extraselular vesicles derived from MSCs concentrate in the lesion areas in the brain. In the injury caused by the PCP, extra cellular vesicles (home) were determined to migrate to the prefrontal cortex (PFC), which is the most affected region of the brain in schizophrenia. The implementation of MSC-EVs was found to improve social interaction and deterioration in the Prepulse inhibition (PPI) observed in mice treated with PCP. MSC-EVs improved schizophrenia-like behaviors and biochemical markers of schizophrenia (Tsivion-Visbord et al., 2020). Behaviors related to schizophrenia also occur when amphetamine is applied to animals.. With TNF-alpha rise, neurinflamation was associated with schizophrenia in amphetamine-sensitive mice and hUC-MSC inhibited neurinflammatory changes related to schizophrenia. (You et al., 2020). In another study using ketamine in forming schizophrenia, behavioral recovery occurred shortly after MSC transplantation. The reason for this recovery was the development of FGF2 gene expression and neurogenesis. (Gobshtis, Tfilin, Fraifeld, & Turgeman, 2021).

References

Bai, L., Lennon, D. P., Eaton, V., Maier, K., Caplan, A. I., Miller, S. D., & Miller, R. H. (2009). Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia, 57*(11), 1192-1203. doi:10.1002/glia.20841

Bhat, I. A., T. B, S., Somal, A., Pandey, S., Bharti, M. K., Panda, B. S. K., . . . Sharma, G. T. (2018). An allogenic therapeutic strategy for canine spinal cord injury using mesenchymal stem cells. *JOURNAL OF CELLULAR PHYSIOLOGY, 234*(3), 2705-2718. doi:10.1002/jcp.27086

Boucherie, C., Schäfer, S., Lavand'homme, P., Maloteaux, J.-M., & Hermans, E. (2009). Chimerization of astroglial population in the lumbar spinal cord after mesenchymal stem cell transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. *Journal of Neuroscience Research, 87*(9), 2034-2046. doi:10.1002/jnr.22038

Bradley, E. R., Tai, M., Hankin, M., & Woolley, J. D. (2021). Preliminary evidence that oxytocin does not improve mentalizing in women with schizophrenia. *Horm Behav, 128*, 104915. doi:10.1016/j.yhbeh.2020.104915

Bursch, F., Rath, K. J., Sarikidi, A., Böselt, S., Kefalakes, E., Osmanovic, A., . . . Petri, S. (2019). Analysis of the therapeutic potential of different administration routes and frequencies of human mesenchymal stromal cells in the SOD1 G93A mouse model of amyotrophic lateral sclerosis. *Journal of Tissue Engineering and Regenerative Medicine, 13*(4), 649-663. doi:10.1002/term.2846

Chen, H., Wang, Y., Tu, W., Wang, H., Yin, H., Sha, H., & Li, Y. (2020). Effects of photobiomodulation combined with MSCs transplantation on the repair of spinal cord injury in rat. *JOURNAL OF CELLULAR PHYSIOLOGY*. doi:10.1002/jcp.29902

Chen, K. S., Sakowski, S. A., & Feldman, E. L. (2016). Intraspinal stem cell transplantation for amyotrophic lateral sclerosis. *Annals of Neurology, 79*(3), 342-353. doi:10.1002/ana.24584

Fisar, Z. (2022). Biological hypotheses, risk factors, and biomarkers of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry, 120*, 110626. doi:10.1016/j.pnpbp.2022.110626

Ge, M., Zhang, Y., Hao, Q., Zhao, Y., & Dong, B. (2018). Effects of mesenchymal stem cells transplantation on cognitive deficits in animal models of Alzheimer's disease: A systematic review and meta-analysis. *Brain and Behavior, 8*(7), e00982. doi:10.1002/brb3.982

Gobshtis, N., Tfilin, M., Fraifeld, V. E., & Turgeman, G. (2021). Transplantation of mesenchymal stem cells causes long-term alleviation of schizophrenia-like behaviour coupled with increased neurogenesis. *Mol Psychiatry, 26*(8), 4448-4463. doi:10.1038/s41380-019-0623-x

Govindarajan, V., de Rivero Vaccari, J. P., & Keane, R. W. (2020). Role of inflammasomes in multiple sclerosis and their potential as therapeutic targets. *Journal of Neuroinflammation, 17*(1). doi:10.1186/s12974-020-01944-9

He, A., Wang, S., Chen, T., Li, J., & Liu, X. (2009). Biological Characteristics of MSCs. In J. Wang & X. Xie (Eds.), *Mesenchymal Stem Cells for the Heart From Bench to Bedside* (pp. 1-11.). Berlin Heidelberg: Zhejiang University Press, Hangzhou and Springer-Verlag GmbH.

Jia, Y., Cao, N., Zhai, J., Zeng, Q., Zheng, P., Su, R., . . . Pei, X. (2020). HGF Mediates Clinical-Grade Human Umbilical Cord-Derived Mesenchymal Stem Cells Improved Functional Recovery in a Senescence-Accelerated Mouse Model of Alzheimer's Disease. *Advanced Science*, 1903809. doi:10.1002/advs.201903809

Kassis, I., Grigoriadis, N., Gowda-Kurkalli, B., Mizrachi-Kol, R., Ben-Hur, T., Slavin, S., . . . Karussis, D. (2008). Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Arch Neurol, 65*(6), 753-761. doi:10.1001/archneur.65.6.753

Lee, J. K., Jin, H. K., Endo, S., Schuchman, E. H., Carter, J. E., & Bae, J.-s. (2009). Intracerebral Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells Reduces Amyloid-Beta Deposition and Rescues Memory Deficits in Alzheimer's Disease Mice by Modulation of Immune Responses. *STEM CELLS*, N/A-N/A. doi:10.1002/stem.277

Losurdo, M., Pedrazzoli, M., D'Agostino, C., Elia, C. A., Massenzio, F., Lonati, E., . . . Coco, S. (2020). Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. *Stem cells translational medicine*. doi:10.1002/sctm.19-0327

Mahfouz, M. M., Abdelsalam, R. M., Masoud, M. A., Mansour, H. A., Ahmed-Farid, O. A., & kenawy, S. A. (2017). The neuroprotective effect of mesenchymal stem cells on an experimentally induced model for multiple sclerosis in mice. *Journal of Biochemical and Molecular Toxicology, 31*(9), e21936. doi:10.1002/jbt.21936

Mammana, S., Gugliandolo, A., Cavalli, E., Diomede, F., Iori, R., Zappacosta, R., . . . Mazzon, E. (2019). Human gingival mesenchymal stem cells pretreated with vesicular moringin nanostructures as a new therapeutic approach in a mouse model of spinal cord injury. *Journal of Tissue Engineering and Regenerative Medicine*. doi:10.1002/term.2857

Marin-Bañasco, C., Benabdellah, K., Melero-Jerez, C., Oliver, B., Pinto-Medel, M. J., Hurtado-Guerrero, I., . . . Suardíaz, M. (2017). Gene therapy with mesenchymal stem cells expressing IFN-ß ameliorates neuroinflammation in experimental models of multiple sclerosis. *British Journal of Pharmacology, 174*(3), 238-253. doi:10.1111/bph.13674

Meirelles, L. d. S. (2009). Methodology, biology and clinical applications of mesenchymal stem cells. *Frontiers in Bioscience, 14*, 4281-4298. doi:10.2741/3528

Patki, S., Kadam, S., Chandra, V., & Bhonde, R. (2010). Human breast milk is a rich source of multipotent mesenchymal stem cells. *Hum Cell, 23*(2), 35-40. doi:10.1111/j.1749-0774.2010.00083.x

Qi, K., Li, N., Zhang, Z., & Melino, G. (2018). Tissue regeneration: The crosstalk between mesenchymal stem cells and immune response. *Cellular Immunology, 326*, 86-93. doi:10.1016/j.cellimm.2017.11.010

Quadri, S. A., Farooqui, M., Ikram, A., Zafar, A., Khan, M. A., Suriya, S. S., . . . Mortazavi, M. M. (2018). Recent update on basic mechanisms of spinal cord injury. *Neurosurgical Review, 43*(2), 425-441. doi:10.1007/s10143-018-1008-3

Song, N., Scholtemeijer, M., & Shah, K. (2020). Mesenchymal Stem Cell Immunomodulation: Mechanisms and Therapeutic Potential. *Trends in Pharmacological Sciences, 41*(9), 653-664. doi:10.1016/j.tips.2020.06.009

Tsivion-Visbord, H., Perets, N., Sofer, T., Bikovski, L., Goldshmit, Y., Ruban, A., & Offen, D. (2020). Mesenchymal stem cells derived extracellular vesicles improve behavioral and biochemical deficits in a phencyclidine model of schizophrenia. *Transl Psychiatry, 10*(1), 305. doi:10.1038/s41398-020-00988-y

Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I., . . . Sensebe, L. (2019). Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy, 21*(10), 1019-1024. doi:10.1016/j.jcyt.2019.08.002

Wang, J., Chen, Z., Sun, M., Xu, H., Gao, Y., Liu, J., & Li, M. (2020). Characterization and therapeutic applications of mesenchymal stem cells for regenerative medicine. *Tissue and Cell, 64*, 101330. doi:10.1016/j.tice.2020.101330

Wang, N., Xiao, Z., Zhao, Y., Wang, B., Li, X., Li, J., & Dai, J. (2017). Collagen scaffold combined with human umbilical cord-derived mesenchymal stem cells promote functional recovery after scar resection in rats with chronic spinal cord injury. *Journal of Tissue Engineering and Regenerative Medicine, 12*(2). doi:10.1002/term.2450

Ward, M. R., Abadeh, A., & Connelly, K. A. (2018). Concise Review: Rational Use of Mesenchymal Stem Cells in the Treatment of Ischemic Heart Disease. *Stem cells translational medicine, 7*(7), 543-550. doi:10.1002/sctm.17-0210

You, M. J., Bang, M., Park, H. S., Yang, B., Jang, K. B., Yoo, J., . . . Kwon, M. S. (2020). Human umbilical cord-derived mesenchymal stem cells alleviate schizophrenia-relevant behaviors in amphetamine-sensitized mice by

inhibiting neuroinflammation. *Transl Psychiatry, 10*(1), 123. doi:10.1038/s41398-020-0802-1

Yuce, K., & Ozkan, A. I. (2019). MESENCHYMAL STEM CELLS (MSCs). In A. TULI, S. POLAT, & O. OGUZ (Eds.), *Basic Medical Sciences* (pp. 23-34.): Akademisyen Kitabevi A.Ş.

Yun, H. M., Kim, H. S., Park, K. R., Shin, J. M., Kang, A. R., il Lee, K., . . . Hong, J. T. (2013). Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1–42-infused mouse model of Alzheimer's disease. *Cell Death & Disease, 4*(12), e958-e958. doi:10.1038/cddis.2013.490

Zanganeh, E., Soudi, S., Zavaran Hosseini, A., & Khosrojerdi, A. (2019). Repeated intravenous injection of adipose tissue derived mesenchymal stem cells enhances Th1 immune responses in Leishmania major-infected BALB/c mice. *Immunology Letters, 216*, 97-105. doi:10.1016/j.imlet.2019.10.008

CHAPTER 10

ANTIMICROBIAL ORGANIC ACIDS

Nurhan GÜMRÜKÇÜOĞLU[1]

1 Prof. Dr. , ORCID ID https:// orcid.org/0000-0002-9669-6318 Karadeniz Technical University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Trabzon, TURKEY ngumrukcuoglu@ktu.edu.tr

Recently, with the development of food technology, the importance of food safety in terms of public health is increasing day by day. Similarly, consumers' demands for safe and high-quality foods are constantly increasing. In developed countries, a large part of the population is exposed to food-borne microbial diseases. The presence of pathogens, which is an undesirable element in food products, is one of the main causes of foodborne diseases worldwide [1]. For this reason, in recent years, alternative methods that can be used instead of preservative methods that cause losses in the nutritional value of foods have gained importance due to the fact that consumers prefer less processed food products [2]. The most important microbial hazards in food products are pathogens such as *Salmonella, Listeria monocytogenes, Escherichia coli, Campylobacter, Staphylococcus aureus, Yersinia enterocolitica* and *Clostridium*. *Escherichia coli, Staphylococcus aureus, Salmonella spp. Yersinia spp.* and *Clostridium spp*. are responsible for many cases associated with stomach and intestinal disorders such as vomiting and diarrhea [3, 4].

Chemical protective additives and artificial antimicrobials are used to inactivate or prevent the growth of pathogenic bacteria [5]. While chemical preservatives and artificial antimicrobials are preferred due to their cheapness, high stability and strong activity, the use of natural antimicrobials is of great importance in controlling microorganisms, since it has been determined that they have toxic activity in living organisms and have carcinogenic effects for humans [6, 7].

There are many substances with antimicrobial effect in the composition of foods. Organic acids, which are found as antimicrobial substances in the food composition, are chemically considered as organic carboxylic acids such as fatty acids and amino acids. The general formulas of organic acids are RCOOH. Organic acids are essential nutrient components for humans. Most of the organic acids are harmless, and these acids contain aroma and flavor compounds in their structures. They can be found in nature purely in plant and animal organisms, and they can also be obtained naturally. After being used in the animal body and metabolized, they are oxidized to carbon dioxide and water. Therefore, they do not leave any residue that may pose a health problem or a risk to the living organism. Some organic acids found in foods show antimicrobial effects by lowering the pH of the environment or intracellularly, or by changing the permeability of the cell membrane and disrupting the substrate transport, or by forming chelates with some metals necessary for the life of microorganisms [8].

The antimicrobial effect of organic acids in foods depends on the type of acid used, its concentration and the method of application. The antimicrobial effect of organic acids also varies depending on temperature, pH, water activity, oxygen, salt and other antimicrobials. These factors

can increase or decrease the antimicrobial effect of acids, such as the numbers and types of microorganisms present, their metabolic activity and microbial interactions.

In general, the effect of organic acids increases at low pH or with antimicrobial factors. It is known that weak acids show stronger antimicrobial activity at low pH than neutral pH. Among these acids, acetic and propionic acid are the strongest inhibitors and are used to inhibit the growth of yeast, mold and bacteria [9]. In addition, low pH reduces cell biomass, growth rate and lag phase,while temperature has a significant effect on the antimicrobial activities of organic acids. Organic acids, which are widely used in the industry for various purposes, can be produced by various microorganisms (Figure 1) [10].

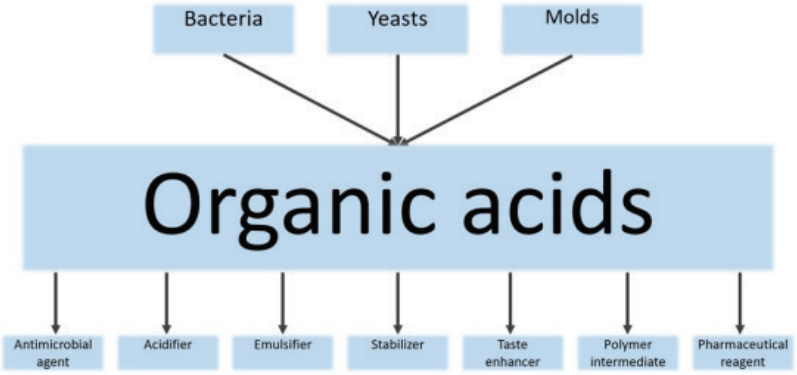


Figure 1: Microbial producers and application areas of organic acids [10]

Not all organic acids have an effect on the microflora. Simple monocarboxylic acids such as short-chain (C1-C7) formic, acetic, propionic and butyric acids, and carboxylic acids, such as lactic, malic, tartaric and citric acids, whose hydroxyl group is usually attached to the α carbon, have specific antimicrobial effects. Salts of organic acids also showed positive effects on microflora. The pH value at which organic acids with antimicrobial activity are semi-dissociated varies between 3-5 [11]. Some organic acids found in foods also show antimicrobial effects by lowering the pH of the environment or intracellularly, by changing the permeability of the cell membrane and disrupting the substrate transport, or by forming chelates with some metals necessary for the life of microorganisms [8]. As a result of their study, it is reported that organic acids produced by lactic acid bacteria have a potential as a biopreservative due to their pH-lowering and antibacterial effects [12]. These organic acids are lactic acid, acetic acid, formic acid, phenyllactic acid, caproic acid and propionic acid [13,

14]. The antimicrobial effects of different organic acids vary depending on the concentration and pH in the environment. At the same time, each acid has a unique spectrum of antimicrobial activity. For example; While lactic acid is more effective on bacteria, sorbic acid has an anti-mold effect. Some acids, such as formic and propionic, have a wider antimicrobial activity and can be effective on fungi, including bacteria and yeasts [11].

Lactic acid, like all other organic acids, is a weak acid that belongs to the group of carboxylic acids. It is found in muscle, blood and various organs of the body. It is used synonymously with lactate. Lactate is the anion of lactic acid. Lactose (milk sugar) is pre-fermented by the starter bacteria used in fermented milk products. As a result, lactic acid is formed, which is easier to absorb [15]. It is a by-product formed as a result of the breakdown of carbohydrates, the main source of which is called glycogen. Lactic acid has been widely used for many years, especially in the food industry as an acidifier, sweetener and antimicrobial agent, as well as in the leather, textile, pharmaceutical and cosmetic industries [16].

Today, acetic acid is one of the most important industrial organic acids used because it is widespread in nature and its usage area is quite wide in industry. Acetic acid has a protective effect on bacteria, molds and yeasts. However, it is more effective on bacteria and yeasts. It has a lethal effect especially on Salmonella and coliform bacteria [17]. Acetic acid used in the food industry is obtained by fermentation, and this constitutes approximately 10% of the world's acetic acid production [18].

It is stated that lactic acid and acetic acid, which are characteristic fermentation products of LAB members, limit or inactivate the growth of spoilage or pathogenic bacteria (Salmonella and Listeria spp.) by reducing the pH. At the same time, it has been observed that both organic acids have a direct antimicrobial effect [19].

It has been reported that the undissolved forms of lactic and acetic acid, which are weak acids, penetrate into the bacteria due to their hydrophobic properties and dissolve in the cell, and as a result, cell death occurs due to the pH decrease in the cytoplasm. Due to its higher solubility stability of acetic acid (pKa 4.75), it has a stronger antimicrobial effect than lactic acid (pKa 3.1) at a certain pH and concentration [20].

Some metabolites produced by lactic acid bacteria show inhibitory effects on some microorganisms [14]. Metabolites, which are the main source of antimicrobial effect, are organic acids, especially lactic acid. Although the main metabolite of lactic acid bacteria is lactic acid, it is stated that the inhibitory effect of acetic acid is more. The stronger effects of acetic acid and propionic acid than lactic acid can be explained by their pKa values (4.87, 4.75 and 3.08, respectively). Considering the effect of the

combined use of lactic acid and acetic acid on *Salmonella typhimurium*, it was seen that the inhibition effect on the development of this acid was more than the individual effect of both acids and it was determined that the acids had synergistic activity. It has been observed that the decrease in pH in the environment also increases the effect of other antimicrobial metabolites. The mechanism of action of organic acids against the target microorganism is explained as the organic acids in undissolved form dissolve the oil in the cell membrane and penetrate into the cell, lowering the normally neutral pH of the cell, leading to inactivation of the cell.

The weak lipolyphic acids lactic acid, acetic acid and propionic acid; It passes through the cell membrane of gram-negative microorganisms and changes the intracellular pH and amino acid metabolism of microorganisms. The microorganism spends a large part of its energy to maintain the decreased intracellular pH balance, and therefore its growth and development slows down. Microorganisms that make up the natural microflora of the digestive system produce organic acids such as lactic acid, acetic acid and propionic acid. With the use of these organic acids as feed additives, the balance of microflora in the digestive tract is turned in favor of beneficial microorganisms and the reproduction of pathogenic microorganisms is prevented. Organic acids such as lactic acid, fumaric acid, propionic acid, citric acid, formic acid, acetic acid have wide usage possibilities in animal nutrition [21]. Classification and some properties of organic acids are given in Table 1, Figure 2.

Table 1. Classification of organic acids and some properties

ACID	MOLAR MASS ($_{g/mol}$)	pKa
Formic	46,03	3,75
Acetic	60,05	4,76
Propionic	74,08	4,88
Butyric	88,12	4,82
Lactic	90,08	3,83
Sorbic	112,14	4,76
Fumaric	116,07	3,02
Hydroxymethylthiobutanoic acid	149,00	3,86
Malik	134,09	3,40
Tartaric	150,09	2,93

Citric	192,14	3,13

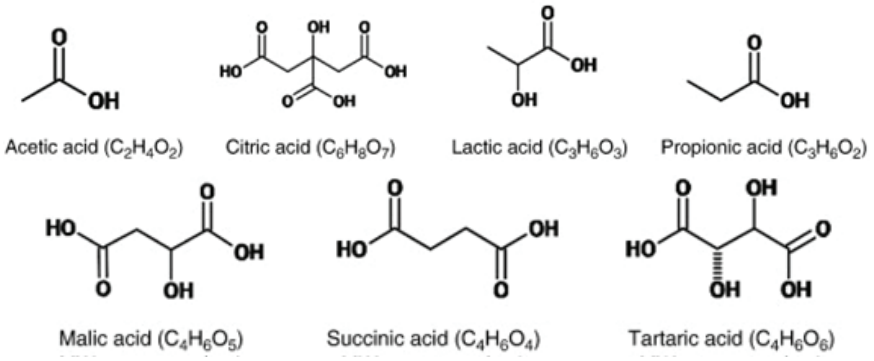


Figure 2. Structures of some organic acids

Formic acid (HCO_2H), also called methanoic acid and the first member of carboxylic acids, is a colorless and corrosive liquid with a pungent odor [22]. Formic acid and its derivatives have a very important place in the industry. Since it contains a hydrogen atom attached to the carbonyl group, it is both an aldehyde and an acid. Formic acid is used as a nutrient for farm animals, as an anti-corrosion and antibacterial agent for food products [23].

Fumaric acid is an organic acid that is widely found in nature and is used as a safe food additive in many processed products to keep the product stable and add tartness. Fumaric acid is also used as an acidifier in the pharmacological industry due to its non-toxic and non-hydroscopic properties [24]. The dosage for use in foods with added sugar is 0.12-0.50%. It is protective against the pathogens of *Listeria monocytogenes*, *Escherichia coli* 0157:H7 and *Salmonella typhimurium* when used at a rate of 1-1.5%.

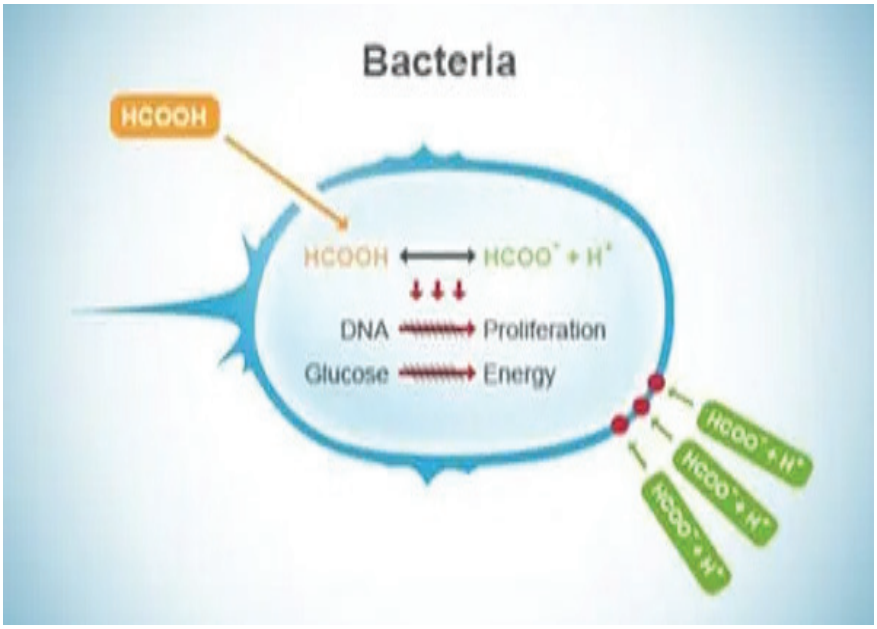
Succinic acid is an odorless organic acid with a slightly bitter and sour taste. It is naturally found in some vegetables. This acid is formed during the Krebs cycle or when microorganisms metabolize fats. Since succinic acid is an acid that cannot be metabolized by bacteria, it is a stable acid [25]. Succinic acid can be obtained by fermentation of tartaric acid or malic acid with special bacteria.

Organic acids used as feed additives create an acidic environment by lowering the pH in the digestive tract. The resulting acid environment prevents the development of pathogenic microorganisms, increases enzyme activity. In addition, the digestibility and usefulness of minerals such as iron, calcium, phosphorus, magnesium, zinc, protein and amino acids increase depending on the acidic environment and the increase in enzyme activity [26, 27]. Organic acids are widely used in meat marinations, and

they contribute to obtaining more reliable and quality meat products by lowering the intracellular pH value. Depending on their use, an increase in water holding capacity occurs, the solubility of meat proteins improves and sensory properties [28]. In addition to its contributions to meat quality, its antimicrobial effect against important pathogens also supports this widespread use [29, 30]. Organic acids are of great importance in terms of human health as well as being effective in many physiological events (taste formation, ripening, etc.) in fruits.

Mode of action

The undissociated form crosses the lipid cell wall of the microbe. The higher cellular PH causes the dissociation of acid releasing its H^+ ions which lower the PH of microbial cell. The lower PH has a deleterious effect on various metabolic processes including DNA replication. Microbial cell uses energy to counteract this PH lowering effect which leads to exhaustion and eventual death of microbe. The remaining anions(-COOH) also have negative effects on cell critical metabolic processes [31].



Acetic acid is mostly used for ripening meat, canned vegetables, sauces, mayonnaise, pickles and ketchup. Lactic acid is mostly used in pickles, vegetable and olive products. Citric acid is naturally found in lemons and is most commonly used in soft drinks. It is also used in canned vegetables, mayonnaise, sauces, fruit products, jams and marmalades. Malic acid is naturally found in fruits and vegetables such as apples, apricots, bananas, cherries, grapes, orange peel, peaches, pears, plums, carrots, peas and potatoes. Since tartaric acid is a fruit-based acid, it is

mostly used in processed food products containing fruit [32]. Since organic acids such as citric acid, lactic acid and acetic acid are synthesized in natural environments, they are recommended because they do not pose a health risk for consumers and they have a high effect on food preservation [33]. They stated that organic acids produced by lactic acid bacteria not only give an acidic flavor to the food, but also contribute to the formation of aroma compounds by their proteolytic and lipolytic activities. It is stated that acetic, propionic and lactic acid show antimicrobial activity against foodborne bacterial pathogens. At pH 4.4 to 5.2, the inhibitory effect of acids on the growth of L. monocytogenes was indicated as acetic > lactic > HCl [34].

It is reported that the effects of acetic acid (vinegar acid) and lactic acid (milk acid) used as preservatives are generally due to the lowering of the pH of the environment [35]. However, it has been determined that acetic acid can penetrate the cell wall and enter the cell and act by denaturing the plasma. Since the antimicrobial effect of acetic acid is realized with its non-dissociated molecules, the effect degree of acetic acid increases as the pH value of the environment decreases. While acetic acid has more antimicrobial effect against bacteria, they are more sensitive to pathogenic bacteria and especially Salmonella acetic acid. On the other hand, the antimicrobial effect of lactic acid is very limited and its effect is mostly against anaerobic bacteria. Many yeasts and molds use lactic acid in their metabolism. However, lactic acid has a great importance in reducing the pH of the environment.

In the production of pickles, lactic acid is formed naturally by microorganisms and the durability of the product is ensured. It is stated that organic acids (formic, acetic, propionic acids, etc.) have bacteriostatic and bactericidal effects on gram (-) bacteria, provided that there are acid molecules that are not sufficiently dissociated in the environment [36].

Also it is reported that acetic acid has a greater inhibitory effect against *L. monocytogenes* than lactic acid and citric acid. Acetic acid was more effective in inhibiting the growth of *Bacillus cereus*. While lactic acid lowers the pH, it causes an increase in the efficiency of acetic acid; acetic acid showed a synergistic effect with lactic acid [37].

It is compared with lactic acid, citric acid, salicylic acid and sorbic acid, the water extract of 26 kinds of plants, *E. coli* O157:H7, *L. monocytogenes*. They investigated in vitro activities against *Y. enterocolitica* and *S. aureus strains*. It was determined that all acid solutions tested inactivated *Y. enterocolitica* and *S. aureus* strains, but sorbic acid numerically reduced the growth of *E. coli* O157:H7 and *L. monocytogenes* [38].

CONCLUSION

Organic acids are acids that contain carbon in their structure and these are formic, acetic, propionic, butyric, fumaric, sorbic, citric, malic acid and their salts. Organic acids are widely used in the food industry as food preservation additives. The most abundant organic acids in foods are acetic acid, malic acid, citric acid, lactic acid and carbonic acid.

Some organic acids found in foods have antimicrobial effects by lowering the pH of the environment or intracellularly, or by changing the permeability of the cell membrane and disrupting the substrate transport, or by forming chelates with some metals necessary for the life of microorganisms.

The antimicrobial effect of organic acids varies depending on variables such as the type and concentration of the acid, conditions of use, pH, temperature and the structure of the target microorganism. Organic acids, which easily pass through the cell membrane, dissociate into their ions, thus lowering the cytoplasmic pH value, since the extracellular environment has a lower pH value than the cytoplasm environment. With the decrease in pH, enzymes and proteins are denatured and cell permeability increases. Thus, the proton driving force of the cell is disrupted, the active transport of nutrients through the membrane is prevented, and as a result, the microorganism loses its viability.

In various studies, it is thought that lactic acid, formic acid and propionic acid have antibacterial effects and these organic acids may have a more important place in the future. For this reason, organic acids have great value both in terms of health and economy. In addition, there is a need for more in vitro or in vivo studies on organic acid applications on pathogenic bacteria that can be found in seafood and pose a risk to humans. Contribution to the literature can be made by examining the effects of organic acids on sensory, chemical and microbiological parameters, especially by conducting studies on the preservation of fishery products.

REFERENCES*

1.Telli, R., Gök, V., Çağlar, A. (2006). Aromatik uçucu yağ bileşenlerinin gıdalardaki antibakteriyel etkileri. Türkiye 9. Gıda Kongresi, 24-26 Mayıs, Bolu, 939.

2. Hızarcı, Ö. (2001). Tulum peynirinden izole edilen laktik asit bakterilerinin tanımlanması ve anti-listerial etkilerinin araştırılması. Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.

3. Friedman, M., Henika, P. R., Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacterjejuni, Escherichia coli, Listeria monocytogenes and Salmonella enterica. Journal of Food Protection, 65, 1545–1560.

4. Demirci, F., Guven, K., Demirci, B., Dadandı, M. Y., Baser, K. H. C. (2008). Antibacterial activity of two Phlomis essential oils against food pathogens. Food Control, 19, 1159–1164.

5. Turner, W. R., Brandon, K. W., Brooks, T. M., Costanza, R., Da, F., Gabw, P. (2007). Global conservation of biodiversity and ecosystem services. BioScience, 57, 868–873.

6. Tajkarımı, M. M., Ibrahim, S. A., Clıver, D. O. (2010). Antimicrobial herb and spice compounds in food. Food Control, 21, 1199–1218.

7. Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J. (2010). Food-borne diseases –the challenges of 20 years ago still persist while new ones continue to emerge. International journal of food microbiology, 139, 3–15.

8. Ova, G. (2001). Koruyucular, Gıda katkı maddeleri. Ege Üniversitesi Mühendislik Fakültesi, 128.

9. Suomalaınen, T. H., Mayra-Makınen, A. M. (1999). Propionic acid bacteria as protective cultures in fermented milks and breads. Lait, 79, 165–174.

10. Coban, H. B. (2020). Organic acids as antimicrobial food agents: applications and microbial productions. Bioprocess and Biosystems Engineering, 43, 569-591.

11. Dibner, J. J., Buttin, P. (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research, 11, 453-463.

12.Gálvez, A., Abriouel, H., Benomar, N., Lucas, R. (2010). Microbial antagonists to food-borne pathogens and biocontrol. Currant Opinion in Biotechnology, 21,142-148.

13. Leroy, F., De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures fort he food fermantation industry. Trends in Food Science & Technology, 15, 67-78.

14. Ouwehand, A. C., Vesterlund, S. (2004). Antimicrobial components from lactic acid bacteria. In Lactic Acid Bacteria Microbiological and Functional Aspects. CRC Press, 11, 375-396.

15. Yeniel, N. (2006). *Lactobacillus delbrueckii* ssp. *bulgaricus* ve *Streptococcus thermophilus* suşlarının melas, peynir altı suyu ve pancar suyunda ekzopolisakkarit (Eps) ve laktik asit üretimlerinin belirlenmesi. Yüksek Lisans Tezi, Gazi Üniversitesi, Fen Bilimleri Enstitüsü, Ankara.

16. Harsa, Ş. (2001). L (+) laktik asidinin saflaştırılması projesi. İzmir Yüksek Teknoloji Enstitüsü, İzmir.

17. Ünlütürk, A., Turantaş, F. (2003). Gıda Mikrobiyolojisi. Meta Basım Matbaacılık, İzmir.

18. Yoneda, N., Kusano, S., Yasui, M., Pujado, P., Wılcher, S. (2001). Recent advances in processes and catalysts for the produstion of acetic acid. Applied Catalysis A: General, 221, 253–265.

19. Ray, B., Daeschel, M. A. (1992). Food biopreservatives of microbial origin. CRC Press, Boca Raton, FL.

20. Holzapfel, W. H., Geisen, R., Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures. Bacteriocins and food-grade enzymes. International journal of food microbiology, 24, 343 – 362.

21. Alp, M. R. (1996). Probiyotiklerin hayvan beslemede kullanılması. İstanbul Üniversitesi, Veterinerlik Fakültesi Dergisi, 22 (1), 1-8.

22. Bruice, P. Y. (2003). Organic chemistry. Prentice Hall, New Jersey, A.B.D.

23. Önder, E. (2006). Sulu organik çözeltilerin elektrokimyasal yöntemle arıtılması. Doktora Tezi, Anadolu Üniversitesi Fen Bilimleri Enstitüsü, Eskişehir.

24. Carta, F. S., Soccol, C. R., Ramos, L. P., Fontana, J. D. (1999). Production of fumaric acid by fermentation of enzymatic hydrolysates derived from cassava bagasse. BioresourceTechnology, 68, 23-28.

25. Coulter, A. D., Godden, P. W., Pretorius, I. S. (2004). Succinic acid. Wine Industry Journal, 19 (6), 16 - 25.

26. Porres, J. M., Etcheverry, P., Miller, D. D., Lei, X. G. (2001). Phytase and citric acid supplementation in whole-wheat bread ımproves phytate-phosphorus release and iron dialyzability. Journal of Food Science, 66 (4), 614-619.

27. Omogbenigun, F. O., Nyachoti, C. M., Slominski, B. A. (2003). The effect of supplementing microbial phytase and organic acids to a corn-soybean diet to earlyweaned pigs. Journal of Animal Science, 81, 1806–1813.

28. Aktaş, N., Kaya, M. (2001). The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. European Food Research Technology, 213, 88-94.

29. Alvarado, C., Mckee, S. (2007). Marination to improve functional properties and safety of poultry meat. Journal of Applied Poultry Research, 16, 113-120.

30. Cadun, A., Kışla, D., Çaklı, Ş. (2008). Marination of deep water pink shrimp with rosemary extract and the determination of its shelf life. Food Chemistry, 109, 81-87.

31. https://en.engormix.com/poultry-industry/articles/organic-acids-review-t40995.htm

32. Çakmakçı, S., Çelik, İ. (1995). Gıda katkı maddeleri. Atatürk Üniversitesi Ziraat Fakültesi Ders Notu:164 s.75, Erzurum.

33. Serdaroğlu, M., Abdraimov, K., Onenç, A. (2007). The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of Turkey breast. Journal of Muscle Foods, 18, 162-172.

34. Sorrells, K. M., Enigl, D. C., Hatfield, J. R. (1989). The effect of pH acidulant. Time and temperature on the growth and survival of *Listeria monocytogenes*. Journal of Food Protection, 52 (8), 571-573.

35. Yemenicioğlu, A., Özkan, M. (2004). Gıdaların başlıca dayandırılma yöntemleri. Meyve ve Sebze İşleme Teknolojisi. 2. Baskı Ankara Üniversitesi Mühendislik Fakültesi, 222.

36. Nir, I., Şenköylü, N. (2000). Kanatlılar için Sindirimi Destekleyen Yem Katkı Maddeleri. Roche, 9, 77-120.

37. Yang, Z. (2000). Antimicrobial compounds and extracellular polysaccharides produced by lactic acid bacteria: structures and properties, Helsinki.

38. Duman Aydın, B. (2008). Bazı tıbbi bitki ve baharatların gıda patojenleri üzerine antibakteriyel etkisinin araştırılması. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 14 (1), 83-87.

CHAPTER 11

TREATMENT PLANNING IN 3-DIMENSIONAL GYNECOLOGICAL BRACHYTHERAPY

Osman Vefa GUL[1]

1 Doctor Lecturer Department of Radiation Oncology, Faculty of Medicine, Selcuk University, Konya, Türkiye ORCID: 0000-0002-6773-3132 e-mail: vefagul@selcuk.edu.tr

Introduction

Today, surgery, chemotherapy and radiotherapy are among the basic treatment methods for cancer cell therapy. Depending on the stage, location of the tumor and the general condition of the patient, radiotherapy can be applied before or after surgery. Radiotherapy is divided into two as external and internal according to the method of application. Brachytherapy, commonly used radiotherapy in cancer treatment is one of the methods. Brachytherapy is a word of Greek origin and means treatment from a short distance. Brachytherapy is a treatment by placing radioactive sources into tissue or body cavities (Gul et al., 2022; Khan, 2010; Lee et al., 2009)

The history of brachytherapy began in Paris in 1896 (Podgorsak, 2005). Just after the discovery of X-rays in 1895, A. Henri Becquerel described natural radioactivity in 1896, when a photographic plate came into contact with uranium crystals (Pospieszny, 2019). After the discovery of radium by Marie Curie and her husband Pierre Curie in 1898, Rutherford's discovery of artificial radioactivity in 1919 was the source of important breakthroughs in medical physics applications (Corradini et al., 2021).

In the early 1900s, these rays began to be used in medicine. The first applications were made by creating surface applicators with radium sources. Its use in gynecological malignancy was first reported in 1903 (Skowronek, 2017). The first "Radium Therapy" book was published in 1909 (Gerbaulet et al., 2002). With the discovery of artificial radionuclides by Irene Curie and Frederick Joliot in 1934, a new era in brachytherapy began (Amaldi, 2015). The first brachytherapy application with Ir-192 radionuclide was carried out by Ulrich Henscheke in 1958 and this application formed the basis of Ir-192 sourced brachytherapy units. The first applications were manually installed. In the following years, remote-controlled afterloading devices have been used, aiming to expose the personnel to less radiation. Today, brachytherapy systems with a large number of channels that allow volume optimization is used (Lee, 2014; Gul et al., 2021). The effectiveness of brachytherapy applications increases with the development of technology and increases the success of treatment in patient groups alone or in addition to external beam radiotherapy (EBRT).

The second most common type of cancer in women is gynecological cancers. Brachytherapy is usually applied together with EBRT in the treatment of gynecological cancer types. Since brachytherapy is applied within the target volume or in the near body cavity, it gives a very high dose to the target tissue and ensures that the surrounding healthy tissues receive a minimum dose. The dose distribution in the tissue is obtained by making simulations in the treatment planning system (TPS) (Famulari et al., 2018).

In parallel with the advances in the field of oncology, new treatment modalities are rapidly developing to prolong the life span of patients

with gynecological tumors. External and internal radiotherapy has a very important place in the treatment of gynecological tumors (Banerjee & Kamrava, 2014). While cervical cancer is more common in sexually active women, endometrial cancer is also common in sexually inactive and postmenopausal women. Brachytherapy can be used as a curative treatment option in patients with locally advanced cervical cancer. Brachytherapy is an indispensable part of definitive radiotherapy in vaginal tumors. In vulvar cancer, definitive/adjuvant radiotherapy is preferred in early-stage patients and chemo-radiotherapy is preferred in locally advanced-stage patients (Yavas & Yavas, 2014).

Radioactive sources used in brachytherapy

Welds used in brachytherapy are usually covered with a non-radioactive capsule such as titanium or stainless steel to prevent contamination and leakage. The capsule provides filtration of low-energy alpha and beta particles (Podgorsak, 2005). Physical and dosimetric parameters that change the effectiveness of the treatment are of great importance in brachytherapy practice. These parameters are the energy, half-life, half-value layer (HVL), dose rate, and radiation type resulting from the decay (Veccia et al., 2015; Yue et al., 2009). The physical properties of some radioactive sources used in brachytherapy are shown in Table 1.

Table 1. Characteristics of radioactive sources used in brachytherapy

Element	Radionuclide	Beam Type	Energy (MeV)	Half-life	Source type	Energy Class
Iridium	Ir-192	Gamma	0.38	73.8 days	Seed-needle	High
Radium	Ra-226	Gamma	0.83	1.626 years	Tube-needle	High
Cesium	Cs-137	Gamma	0.662	30 years	Tube-needle	High
Cesium	Cs-131	Gamma	0.030	9.69 days	Seed	Low
Cobalt	Co-60	Gamma	1.25	5.26 years	Seed-wire	High
Palladium	Pd-103	Gamma	0.02	8.06 days	Seed	Low
Iodine	I-125	Gamma	0.028	59.6 days	Seed	Low

^{192}Ir is obtained by neutron capture of the stable ^{191}Ir isotope in a nuclear reactor. It first decays to ^{192}Pt by beta emission and then to ^{192}Os by electron capture. It reaches the ground state by simultaneous gamma decays. The ^{192}Ir has a complex gamma spectrum. The average gamma energy is 0.38 MeV (Dovbnya et al., 2014).

Brachytherapy applications

Intracavitary brachytherapy is applied by placing the radioactive source in the intra-body cavities adjacent to the tumor with the help of applicators and is the most commonly used technique of brachytherapy (Takafumi et al., 2018).

Interstitial brachytherapy is applied by placing the radioactive source into the relevant tissue. A homogeneous dose distribution can be achieved, especially in asymmetric tumor tissue, with radioactive permanent implants in the size of a rice grain. It can also be applied with radioactive iridium wires placed in temporary tubes placed in the tissue. It is either permanent or temporary (Viswanathan et al., 2011).

Intraluminal brachytherapy is a brachytherapy technique used for areas of the body such as the lung and esophagus. The application is made at a low dose rate (Yi et al., 2012).

Intraoperative brachytherapy is performed by placing specially designed applicators inside the macroscopically visible mass during the surgical operation or on the tumor bed after excision. The application should be done in operating room conditions (Skowronek, 2015).

Superficial (mold) brachytherapy is a brachytherapy technique applied for non-deeply located tumors such as skin and eyes, in the form of radioactive plaques or with specially prepared molds. The aim of this technique is to apply the source as close as possible directly to the tumor surface (Bellis et al., 2021).

Intravascular brachytherapy is applied to prevent restenosis after angioplasty with a small radioactive source placed inside the vein (Andras et al., 2014).

Dose rate classification

In low dose rate (LDR) brachytherapy, the dose rate is 0.4-2 Gy/hour. The dose to be administered can be given more slowly and for a long time. Because they are a very low dose rate, they are generally applied as permanent implants. It is mostly preferred in prostate cancer (Scott et al., 2021).

In medium-dose rate (MDR) brachytherapy, the dose rate is 2-12 Gy/hour and the treatment lasts for an average of 6-8 hours. Medium-dose fast sources have relatively high activity. If a post-loading system is not available, it is not widely used because it causes high dose exposure (Chen et al., 2021).

High dose rate (HDR) brachytherapy has a dose rate >12 Gy/hr. It is administered in fractions of the total dose. Generally, Ir-192 and Co-60 radioactive sources are used. It is widely used for gynecological tumors (Yadav et al., 2019).

Pulse dose rate (PDR) brachytherapy utilizes the computer-controlled afterloading technology of HDR, with a dose rate >12 Gy/hr. It is applied as 24 pulses per day. The most commonly used cancers are gynecological and head and neck tumors (Hudge et al., 2018).

Brachytherapy Applicator Selection

Modern applicators used in brachytherapy can be used with different radioactive sources. These applicators are CT and/or MR-compatible. Applicators have a very important place in brachytherapy applications. The applicators used in brachytherapy vary depending on the type of cancer, anatomical structure, pathological condition, and the technique to be applied. Different types of applicators have advantages and disadvantages over each other (Otter et al., 2019). Applicators provide information on many subjects such as the direction of movement of the source, geometry, and dwell positions in dose calculation. The applicator is a determining factor in the effectiveness of the treatment. Depending on the treatment application, applicators such as ring, tandem, ovoid, and cylinder are preferred. With the help of US and MRI imaging, the correct positioning of the applicator is ensured. After correct positioning, if 3D brachytherapy planning is to be done, CT simulation is provided, and 2D brachytherapy planning is simulated with A-P and lateral films.

Modern applicators use composite fiber etc. made of materials. These applicators are fully compatible with 3D imaging systems and create no artifacts. Tandem ovoid applicators consist of tandem and ovoids of different lengths and sizes. The diameter of the ovoid tubes is 2-3 cm, and the length of the intrauterine tube is between 4-6 cm. A tandem is inserted into the uterine cavity and two ovoids are placed in the lateral fornix. The tandem ovoid applicator is shown in Figure 1. The tandem ring applicator is CT/MR compatible. This applicator is based on the Stockholm technique for gynecological brachytherapy. Tube and ring can be easily inserted into the uterus. Various configurations are available; 30°, 45° and 60° sets are available with the angle determined according to the axis of the applicator (Halabian at al., 2016). The tandem ring applicator is shown in Figure 2. Applicators used in gynecological brachytherapy have advantages over each other. Pathology, staging, treatment volume and treatment dose are important determining factors in applicator selection. Tandem-ring applicator has superiority over tandem-ovoid applicator, especially in cervical cancer brachytherapy.

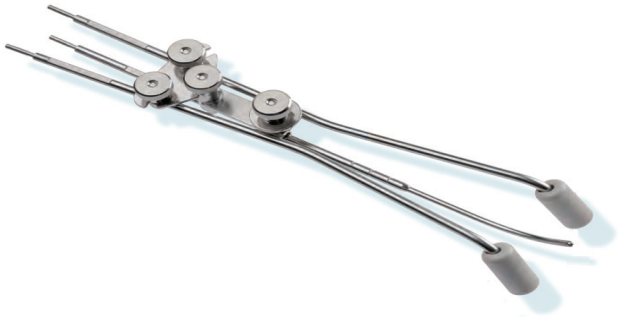


Figure 1. Tandem ovoid applicator

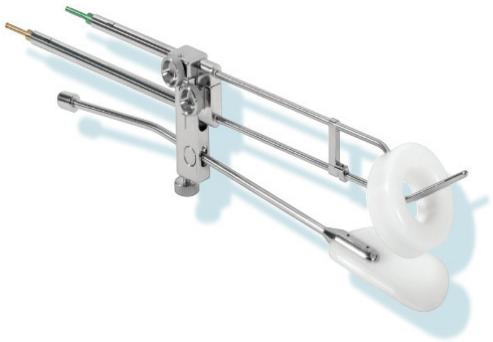


Figure 2. Tandem ring applicator

Single-channel cylinder applicators are widely used in the brachytherapy of patients with early-stage endometrial cancer. These applicators are 20-40 mm in diameter, 2.5-10 cm long and dome-shaped with a central channel. The appropriate applicator size is selected according to the vagina and head (Rangarajan et al., 2022; Malajovich et al., 2020; Kim et al., 2018). The single-channel cylinder applicator is shown in Figure 3.

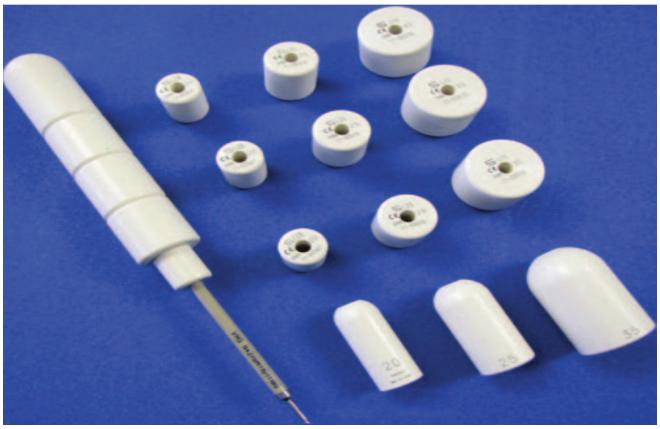


Figure 3. Single-channel cylinder applicators

Contouring in Three-Dimensional Brachytherapy Planning

The Groupe Europe'en Curietherapy-European Society of Therapeutic Radiation Oncology (GEC – ESTRO) has defined a target concept based on MR imaging for the application of volumetric image-based 3D brachytherapy in gynecological cancers, introducing the definitions of gross tumour volume (GTV) , high-risk CTV (HR-CTV) and intermediate-risk CTV (IR-CTV). According to this definition, GTV is defined as a palpable or visible/visible tumor. The clinical target volume (CTV) is the volume of disease-containing tissue to be eliminated, created by margining

the GTV. It is defined as intermediate-risk CTV (IR-CTV) and high-risk CTV (HR-CTV) due to residual macroscopic disease. Cross-sectional imaging methods have an important place in 3D brachytherapy. During the contouring process, T2-weighted MR images and CT images with the applicator attached are used for clinical diagnosis and clinical examination. The rectum, bladder, bowel, and sigmoid are defined as organs at risk (OAR) (Yoganathan et al., 2022).

Dose Description and Reporting in Brachytherapy

In 1985, the International Commission for Radiation Units and Measurements (ICRU) published the report numbered 38 in order to evaluate the treatment reports more clearly due to the application of intracavitary treatment in addition to external radiotherapy in the treatment of brachytherapy patients. In the ICRU 38 report, it was stated that the technique used for each case should be defined, and the total reference air kerma ratio and the absorbed doses at the reference points should be reported. In addition, it was requested to define the doses at the non-anatomical A and B points to define the dose distribution in the target volume. In the ICRU 38, a 60 Gy dose was defined for the CTV as the tumor dose (Haie-Meder et al., 2005; Potter et al., 2006).

According to the report prepared by The Groupe Europe´en Curietherapy–European Society of Therapeutic Radiation Oncology (GEC-ESTRO) gynecological working group consisting of doctors and physicists from different centers in 2000, the dose given to 90% and 100% of the volume depending on the target volume is D90%, and D100%, respectively. In addition, the volume that receives 100% of the prescription dose should be defined as V100%. The bladder, rectum, small intestine, and sigmoid have been defined as OARs. The report recommended 0.1 cc, 1.0 cc and 2.0 cc for bladder, rectum and sigmoid. D2cc doses for bladder, rectum and sigmoid are <80-85 Gy, <70-75 and <75 Gy, respectively (Vinod et al., 2017).

With the recommendations made by the gynecological GEC-ESTRO working group and the use of advanced 3D imaging techniques in the ICRU report no. 89, details for intracavitary brachytherapy have been clarified. According to ICRU report 89, detailed gynecological examination, FIGO staging, the three-dimensional definition of volumes, dose reporting, absorbed dose rate, fraction dose, fraction number, and total EQD2 dose are required for 3D brachytherapy (Schmid et al., 2020).

Brachytherapy Dose Optimization

Before the treatment planning phase, the position of the applicators and the patient's anatomy should be viewed in three dimensions. The target volume and OARs must be defined by the radiation oncologist on the 3D

images obtained. Reference points are defined by the medical physicist and dose distribution is made depending on the source stop times at these points (Laan et al., 2019). The number of welding positions can reach up to 400 cm depending on their TPS. Irradiation times at these positions are different depending on the dose distribution. The position of the source, the distance between the sources, and the irradiation times are adjusted for the desired dose distribution. These adjustment processes are called optimization. The goal of optimization is to best preserve OARs while delivering the desired dose to the target volume (Reniers et al., 2012). In HDR brachytherapy, different models are used for treatment planning that refers to the source posture position (dwell position) and dwell time. In the optimization process, contours defined by the radiation oncologist and reference points defined by the medical physicist are used (Kertzscher et al., 2011; Kertzscher et al., 2014). Optimization models make calculations by considering patient anatomy. Optimization processes are classified as manual optimization, graphic optimization, geometric optimization, point optimization and inverse optimization (Morton et al., 2008; Pekkan et al., 2008).

In the manual optimization technique, optimization is made by manually changing the welding stop times or weights. In this technique, welding dwell times are made by trial-and-error method until the desired dose distribution is obtained. After the pause times have changed, the computer recalculates the dose distribution and the resulting distribution is evaluated. The time to reach the desired dose distribution depends on the experience of the medical physicist (D'Souza et al., 2001; Dinkla et al., 2015).

Graphic optimization is an optimization technique that is made by changing the isodose curves until the desired dose distribution is obtained. Welding dwell times are determined based on isodose curves. Isodose curves can be changed manually until the desired dose distribution is reached (Akimoto et al., 2006; Murali et al., 2010).

Geometric optimization is an optimization technique whose relative dwell times are determined by the geometry of the implant. The dose distribution around the applicator is achieved by normalization to the point within the target. It is a common technique used to improve dose distributions of interstitial implants (Shwetha et al., 2010; Alterovitz et al., 2006).

Spot optimization is an optimization method where welding stop positions are adjusted to calculate the dose of defined spots at a certain distance. This technique does not consider target and OARs. It is necessary to define the target and volume for OARs (Jamema et al., 2010).

Inverse optimization is an optimization technique based on anatomy. In this optimization technique, the target volume and OARs volumes

drawn based on 3D images are very important. Constraints are defined at the planning stage. Dose limitations are made by a medical physicist before starting the optimization process. Accordingly, minimum and maximum dose criteria are defined for the target volume, in addition, volume-dependent dose criteria are defined for OARs. Inverse optimization selects the optimal dwell times in the set of possible source stance positions and determines dwell times that meet the target volume and dose constraints of critical organs (Borot de Battisti at al., 2015).

Dose criteria are revised until the desired dose distribution is achieved. Manual adjustment is not made by the user (Jamema et al., 2011; Trnkova et al., 2009). Inverse optimization selects optimal dwell times for possible welding stop positions. Accordingly, it determines the best source dwell times that meet the target volume and dose limitations for critical organs, without the need for manual adjustments (Gorissen et al., 2013; Badry et al., 2019).

Conclusion

Brachytherapy has a very important place in the treatment of gynecological cancers for a very long time. Many studies have shown that the volume-based therapy recommended by GEC-ESTRO is now implemented in most centers. One of the key advantages of HDR brachytherapy is the optimization of doses to target volume and OARs. There are different optimization techniques used in brachytherapy planning. The optimization technique used varies depending on the location, stage, size of the disease, and desired dose. The applicators used for the optimization to be preferred in the treatment are the determining factors. In addition, the patient density of the clinic and the specific situation of the patient to be planned also affect the optimization preferences. Manual optimization is the most preferred optimization method in many institutions. The development of inverse optimization algorithms is still ongoing. However, manual optimization planning is highly dependent on the user. Inverse optimization is recommended, especially in cases where the desired dose distribution cannot be achieved with manual optimization.

In inverse optimization, if the target volume and dose criteria for OARs are defined correctly, better dose distribution can be obtained compared to manual optimization. In the treatment plans made with inverse optimization, homogeneous dose distribution can be created within the application. In inverse optimization, the dependency on the user is reduced by registering the pre-optimization dose criteria to the system. In addition, a reduction in treatment times can be achieved. 3-dimensional gynecological brachytherapy treatment planning is important for the patient and it is thought that the optimization to be used should be decided on a patient basis.

References

Alterovitz, R., E. Lessard, J. Pouliot, I. C. Hsu, J. F. O'Brien, and K. Goldberg. 2006. 'Optimization of HDR brachytherapy dose distributions using linear programming with penalty costs', *Med Phys*, 33: 4012-9.

Andras A, Hansrani M, Stewart M, Stansby G. Intravascular brachytherapy for peripheral vascular disease. Cochrane Database of Systematic Reviews 2014, Issue 1. Art. No.: CD003504. DOI: 10.1002/14651858.CD003504.pub2.

Akimoto, T., H. Katoh, Y. Kitamoto, K. Shirai, M. Shioya, and T. Nakano. 2006. 'Anatomy-based inverse optimization in high-dose-rate brachytherapy combined with hypofractionated external beam radiotherapy for localized prostate cancer: comparison of incidence of acute genitourinary toxicity between anatomy-based inverse optimization and geometric optimization', *Int J Radiat Oncol Biol Phys*, 64: 1360-6.

Amaldi, U. (2015). The Beginnings of Accelerators in Medicine. In: Particle Accelerators: From Big Bang Physics to Hadron Therapy. Springer, Cham.

Badry, H., Oufni, L., Ouabi, H. *et al.* A new fast algorithm to achieve the dose uniformity around high dose rate brachytherapy stepping source using Tikhonov regularization. *Australas Phys Eng Sci Med* **42**, 757–769 (2019).

Banerjee, R., & Kamrava, M. (2014). Brachytherapy in the treatment of cervical cancer: a review. *International journal of women's health*, *6*, 555–564.

Bellis, R., Rembielak, A., A. Barnes, E., Paudel, M., & Ravi, A. (2021). Additive manufacturing (3D printing) in superficial brachytherapy. Journal of Contemporary Brachytherapy, 13(4), 468-482.

Borot de Battisti, M., M. Maenhout, B. Denis de Senneville, G. Hautvast, D. Binnekamp, J. J. Lagendijk, M. van Vulpen, and M. A. Moerland. 2015. 'An automated optimization tool for high-dose-rate (HDR) prostate brachytherapy with divergent needle pattern', *Phys Med Biol*, 60: 7567-83.

Chen L. (2021). Clinical Applications of Pulsed LowDose-Rate Radiation Therapy. Mathews J Cancer Sci. 6(1):27.

Corradini, S., Marschner, S., Reitz, D. (2021). Historical Development and Current Indications of Image-Guided Brachytherapy. In: Mohnike, K., Ricke, J., Corradini, S. (eds) Manual on Image-Guided Brachytherapy of Inner Organs. Springer, Cham.

Dinkla, A. M., R. van der Laarse, K. Koedooder, H. Petra Kok, N. van Wieringen, B. R. Pieters, and A. Bel. 2015. 'Novel tools for stepping source brachytherapy treatment planning: enhanced geometrical optimization and interactive inverse planning', *Med Phys*, 42: 348-53.

Dovbnya, A.N., Rogov, Y.V., Shevchenko, V.A. et al. A study of ^{192}Ir production conditions at an electron accelerator. Phys. Part. Nuclei Lett. **11**, 691–694 (2014).

D'Souza, W. D., R. R. Meyer, B. R. Thomadsen, and M. C. Ferris. 2001. 'An iterative sequential mixed-integer approach to automated prostate brachytherapy treatment plan optimization', *Phys Med Biol*, 46: 297-322.

Famulari, G., M. A. Renaud, C. M. Poole, M. D. C. Evans, J. Seuntjens, and S. A. Enger. 2018. 'RapidBrachyMCTPS: a Monte Carlo-based treatment planning system for brachytherapy applications', *Phys Med Biol*, 63: 175007.

Gorissen, B. L., D. den Hertog, and A. L. Hoffmann. 2013. 'Mixed integer programming improves comprehensibility and plan quality in inverse optimization of prostate HDR brachytherapy', *Phys Med Biol*, 58: 1041-57.

Gul, O. V., İnan, G. & Başaran, H. (2022). Serviks Kanseri İçin İki Farklı Brakiterapi Tedavi Planlama Tekniğinin Dozimetrik Karşılaştırması . Akdeniz Tıp Dergisi , 8 (1) , 48-54 .

Gul, O. V., G. Inan, and H. Basaran. 2021. 'Impact of different treatment plans on EQD2 for intracavitary brachytherapy of cervical cancer', *J Egypt Natl Canc Inst*, 33: 28.Khan F.M: The Physics of Radiation Therapy 3rd Edition. Lippincott Williams & Wilkins Company, USA, 2010.

Haie-Meder, C., R. Potter, E. Van Limbergen, E. Briot, M. De Brabandere, J. Dimopoulos, I. Dumas, T. P. Hellebust, C. Kirisits, S. Lang, S. Muschitz, J. Nevinson, A. Nulens, P. Petrow, N. Wachter-Gerstner, and G. E. C. Estro Working Group Gynaecological. 2005. 'Recommendations from Gynaecological (GYN) GEC-ESTRO Working Group (I): concepts and terms in 3D image based 3D treatment planning in cervix cancer brachytherapy with emphasis on MRI assessment of GTV and CTV', *Radiotherapy and Oncology*, 74: 235-45.

Halabian, M., Beigzadeh, B., Karimi, A. *et al.* A combination of experimental and finite element analyses of needle–tissue interaction to compute the stresses and deformations during injection at different angles. *J Clin Monit Comput* **30**, 965–975 (2016). https://doi.org/10.1007/s10877-015-9801-9

Hegde, J. V., D. J. Demanes, D. Veruttipong, R. K. Chin, S. J. Park, and M. Kamrava. 2018. 'Head and neck cancer reirradiation with interstitial high-dose-rate brachytherapy', *Head Neck*, 40: 1524-33.

Jamema SV, Kirisits C, Mahantshetty U, Trnkova P, Deshpande DD. Comparison of DVH parameters and loading patterns of standard loading, manual and inverse optimization for intracavitary brachytherapy on a subset of tandem/ovoid cases. Radiother Oncol 2010; 97(3):501-506.

Jamema, S. V., S. Sharma, U. Mahantshetty, R. Engineer, S. K. Shrivastava, and D. D. Deshpande. 2011. 'Comparison of IPSA with dose-point optimization and manual optimization for interstitial template brachytherapy for gynecologic cancers', *Brachytherapy*, 10: 306-12.

Kertzscher, G., C. E. Andersen, F. A. Siebert, S. K. Nielsen, J. C. Lindegaard, and K. Tanderup. 2011. 'Identifying afterloading PDR and HDR brachytherapy errors using real-time fiber-coupled Al(2)O(3):C dosimetry and

a novel statistical error decision criterion', *Radiotherapy and Oncology*, 100: 456-62.

Kertzscher, G., A. Rosenfeld, S. Beddar, K. Tanderup, and J. E. Cygler. 2014. 'In vivo dosimetry: trends and prospects for brachytherapy', *Br J Radiol*, 87: 20140206.

Kim, Y., K. Cabel, and W. Sun. 2018. 'Does the apex optimization line matter for single-channel vaginal cylinder brachytherapy planning?', *J Appl Clin Med Phys*, 19: 307-12.

Laan, R.C., Nout, R.A., Dankelman, J. *et al.* MRI-driven design of customised 3D printed gynaecological brachytherapy applicators with curved needle channels. *3D Print Med* **5**, 8 (2019).

Lee, C. D. 2014. 'Recent developments and best practice in brachytherapy treatment planning', *Br J Radiol*, 87: 20140146.

Lee D.H, Cho J.K, Shin K.H, Shin D, Yoon M, Park S.Y, Lee S.B, Kim J.Y, Cho K.H, Lee J.W, Chung J.B, Choe B.Y, Choi K.S, Suh T.K. Intravaginal Packing Efects of CT-Guided Intracavitary Radiotherapy for Cervical Cancer. Korean Physical Society 2009; 54: 250-254.

Malajovich, I., S. Anamalayil, O. V. Dolney, B. K. Kevin Teo, W. T. Arscott, and N. K. Taunk. 2020. 'Techniques for and uncertainties of MRI-based reconstruction of titanium tandem and ring brachytherapy applicators', *Brachytherapy*, 19: 651-58.

Morton, G. C., R. Sankreacha, P. Halina, and A. Loblaw. 2008. 'A comparison of anatomy-based inverse planning with simulated annealing and graphical optimization for high-dose-rate prostate brachytherapy', *Brachytherapy*, 7: 12-6.

Murali, V., Kurup, P. G., Mahadev, P., & Mahalakshmi, S. (2010). Dosimetric analysis and comparison of IMRT and HDR brachytherapy in treatment of localized prostate cancer. *Journal of medical physics*, *35*(2), 113–119. Otter, S. J., A. J. Stewart, and P. M. Devlin. 2019. 'Modern Brachytherapy', *Hematol Oncol Clin North Am*, 33: 1011-25.

Pekkan, K., Whited, B., Kanter, K. *et al.* Patient-specific surgical planning and hemodynamic computational fluid dynamics optimization through free-form haptic anatomy editing tool (SURGEM). *Med Biol Eng Comput* **46**, 1139–1152 (2008).Podgorsak EB. Radiation oncology physics: a handbook for teachers and students. Vienna: International Atomic Energy Agency; 2005.

Pospieszny, T. (2019). Maria Skłodowska-Curie – the first lady of nuclear physics. Journal of Contemporary Brachytherapy, 11(6), 505-509.

Potter, R., C. Haie-Meder, E. Van Limbergen, I. Barillot, M. De Brabandere, J. Dimopoulos, I. Dumas, B. Erickson, S. Lang, A. Nulens, P. Petrow, J. Rownd, C. Kirisits, and Gec Estro Working Group. 2006. 'Recommendations from gynaecological (GYN) GEC ESTRO working group (II): con-

cepts and terms in 3D image-based treatment planning in cervix cancer brachytherapy-3D dose volume parameters and aspects of 3D image-based anatomy, radiation physics, radiobiology', *Radiotherapy and Oncology*, 78: 67-77.

Rangarajan, R., Saravanan, S. & Kumari Comparison of Vaginal Dosimetry Between Tandem Ovoid (TO) and Tandem Ring (TR) Applicator in CT-Based High Dose Rate Intracavitary Brachytherapy of Cervix. *Indian J Gynecol Oncolog* **20**, 22 (2022).

Reniers, B., G. Landry, R. Eichner, A. Hallil, and F. Verhaegen. 2012. 'In vivo dosimetry for gynaecological brachytherapy using a novel position sensitive radiation detector: feasibility study', *Med Phys*, 39: 1925-35.

Schmid, M. P., L. Fokdal, H. Westerveld, C. Chargari, L. Rohl, P. Morice, N. Nesvacil, R. Mazeron, C. Haie-Meder, R. Potter, R. A. Nout, and Gec-Estro Gyn Working Group. 2020. 'Recommendations from gynaecological (GYN) GEC-ESTRO working group - ACROP: Target concept for image guided adaptive brachytherapy in primary vaginal cancer', *Radiotherapy and Oncology*, 145: 36-44.

Scott, A. A., J. Yarney, V. Vanderpuye, C. Akoto Aidoo, M. Agyeman, S. N. Boateng, E. Sasu, K. Anarfi, and T. Obeng-Mensah. 2021. 'Outcomes of patients with cervical cancer treated with low- or high-dose rate brachytherapy after concurrent chemoradiation', *Int J Gynecol Cancer*, 31: 670-78.

Shwetha, B., M. Ravikumar, A. Katke, S. S. Supe, G. Venkatagiri, N. Ramanand, and T. Pasha. 2010. 'Dosimetric comparison of various optimization techniques for high dose rate brachytherapy of interstitial cervix implants', *J Appl Clin Med Phys*, 11: 3227.

Skowronek, J. (2015). Brachytherapy in the treatment of lung cancer – a valuable solution. Journal of Contemporary Brachytherapy, 7(4), 297-311.

Skowronek, J. (2017). Current status of brachytherapy in cancer treatment – short overview. Journal of Contemporary Brachytherapy, 9(6), 581-589.

Takafumi Toita, Tatsuya Ohno, Hitoshi Ikushima, Tetsuo Nishimura, Takashi Uno, Kazuhiko Ogawa, Hiroshi Onishi, Takushi Dokiya, Jun Itami, The Working Group of the Japanese Group of Brachytherapy/Japan Society for Radiation Oncology (JGB/JASTRO), National survey of intracavitary brachytherapy for intact uterine cervical cancer in Japan, Journal of Radiation Research, Volume 59, Issue 4, July 2018, Pages 469–476.

The GEC ESTRO Handbook of Brachytherapy. Gerbaulet A, Pötter R, Mazeron JJ, Meetens H, Limbergen EV. Printed by ACCO, Leuven, Part 1, 2002. p. 3-20.

Trnkova P, Pötter R, Baltas D, Karabis A. New inverse planning technology for image-guided cervical cancer brachytherapy: Description and evaluation with in a clinical frame. Radiotherapy and Oncology 2009; 93:331-340.

Veccia, A., Caffo, O., Fellin, G. *et al.* Impact of post-implant dosimetric parameters on the quality of life of patients treated with low-dose rate brachytherapy for localised prostate cancer: results of a single-institution study. *Radiat Oncol* **10**, 130 (2015).

Vinod, S. K., K. Lim, L. Bell, J. Veera, L. Ohanessian, E. Juresic, N. Borok, P. Chan, R. Chee, V. Do, G. Govindarajulu, S. Sridharan, C. Johnson, D. Moses, S. Van Dyk, and L. Holloway. 2017. 'High-risk CTV delineation for cervix brachytherapy: Application of GEC-ESTRO guidelines in Australia and New Zealand', *J Med Imaging Radiat Oncol*, 61: 133-40.

Viswanathan, A.N., Erickson, B.E., Rownd, J. (2011). Image-Based Approaches to Interstitial Brachytherapy. In: Viswanathan, A., Kirisits, C., Erickson, B., Pötter, R. (eds) Gynecologic Radiation Therapy. Springer, Berlin, Heidelberg.

Yadav S, Chandel SS, Choudhary S, Yogi V, Singh OP, et al. (2019) Dosimetric Analysis of 3D-CT Image Based High Dose Rate Brachytherapy Treatment Planning of Carcinoma Uterine Cervix: Initial Experiences at Central India Government Institute. J Cancer Sci Ther 11: 244-250.

Yavas G, Yavas C. Current developments in brachytherapy in gynecological tumors. Journal of General Medicine. 2014; 24.3

Yi Chen, Xiao-Lin Wang, Zhi-Ping Yan, Jian-Hua Wang, Jie-Min Cheng, Gao-Quan Gong, and Jian-Jun Luo.The Use of 125I Seed Strands for Intraluminal Brachytherapy of Malignant Obstructive Jaundice.Cancer Biotherapy and Radiopharmaceuticals.Jun 2012.317-323.

Yoganathan, S. A., S. N. Paul, S. Paloor, T. Torfeh, S. H. Chandramouli, R. Hammoud, and N. Al-Hammadi. 2022. 'Automatic segmentation of magnetic resonance images for high-dose-rate cervical cancer brachytherapy using deep learning', *Med Phys*, 49: 1571-84.

Yue, N. J., Z. Chen, R. A. Hearn, J. J. Rodgers, and R. Nath. 2009. 'Time dependence of energy spectra of brachytherapy sources and its impact on the half and the tenth value layers', *Med Phys*, 36: 5175-82.

CHAPTER 12

FATTY ACID ANALYSIS IN FOOD SUPPLEMENTS

Nagihan AKSU[1]

SEDAT SEL[2]

Serap SAGLIK ASLAN[3]

1 İÜ Eczacılık Fakültesi Analitik Kimya AD E-mail: nagihanaksu29@gmail.com GSM No: 05389790229

2 İÜ Eczacılık Fakültesi Analitik Kimya AD E-mail: sedatsel@yahoo.com GSM No: 05065475915

3 İÜ Eczacılık Fakültesi Analitik Kimya AD E-mail: serapsaglik@yahoo.com GSM No: 05425343022

1. INTRODUCTION

1.1. Definition of Food Supplements

Food supplements, in the "Turkish Food Codex Communiqué on Supplementary Foods"; in order to supplement normal nutrition; Concentrates or extracts of nutrients such as vitamins, minerals, proteins, carbohydrates, fiber, fatty acids (FA), amino acids or substances of plant, plant and animal origin, bioactive substances and similar substances that have nutritional or physiological effects, alone or in their mixtures, capsules, tablets, It is defined as the products prepared in liquid or powder forms such as lozenge, disposable powder package, liquid ampoule, dropper bottle and other similar liquid or powder forms and whose daily intake dose is determined [1].

By definition, food supplements are products used for dietary supplements, these products are not included in the scope of drugs. For this reason, they cannot be used for diagnosis and treatment or to prevent, alleviate or cure diseases [2,3].

1.2. Types of Food Supplements

Food supplements are examined in three groups; vitamin-mineral supplements, herbal and animal products.

Vitamin mineral supplements are products in which synthetic or natural vitamins and minerals are prepared individually or together as a tablet, capsule, powder or concentrated solution. While determining the ingredients, it is prepared in accordance with the US Food and Drug Administration (FDA) and World Health Organization (WHO) standards or national standards, taking into account the impurity, bioavailability and daily maximum intake amounts. It is stated that these products are widely used by people who want a balanced diet when they think that their normal diet is insufficient or when they want to support their diet [4].

Herbal based food supplements are obtained from different parts of the plants by various processes and contain different types of elements in their content. It is reported that substances such as green tea, ginseng, grape seed and their extracts obtained by any means are used in production [5,6].

Animal origin food supplements are supplements prepared from marine origin products or bee products. Supplements prepared from marine origin products contain various compounds such as protein, enzyme, and ω-FA. In the preparation of food supplements, crustaceans, sea algae and fish oils are used to a great extent. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are among the polyunsaturated fatty acids (PUFA) group, are found in a large amount of fish oils. The use of

food supplements containing these FAs, called essential fatty acids (EFA), is important for body health [7]. It is reported that food supplements prepared from sea-based products reduce the risk of chronic diseases and have positive effects on health [4].

Propolis, a secondary bee product, is also used as a food supplement due to its antioxidant, antibacterial and antifungal properties. Propolis is registered as a medicine in Romania and Germany [8].

1.3. Use of Food Supplements

According to a study conducted with the participation of 12,000 individuals over the age of 20 on the reasons for the use of food supplements in the USA, women (36%) use calcium supplements to support bone health, while men (18%) use food supplements to support heart health and keep their cholesterol levels within healthy limits reported. It is stated that the purpose of using supplements in individuals over 60 is to protect heart, joint, bone and eye health. In addition, this study reveals that only 23% of the supplements used are recommended by experts. It is reported that the most popular products are multivitamins and minerals, followed by calcium and ω-3 supplements. In another study, it is reported that food supplements are used to supplement the deficiencies in nutrition and to support general body health [9].

The use of food supplements and the drug called Ritalin were compared in a study on children's hyperactivity and attention deficit. Various data were obtained in the study in which food supplements such as vitamins, minerals, probiotics, EFAs, amino acids, phospholipids, plant foods were used. According to these data, the use of food supplements improves attention and improves self-control in children, equivalent to Ritalin [10].

1.4. Chemical Structure of Fats

Fats, consisting of carbon, oxygen and hydrogen, together with proteins and carbohydrates constitute the basic components of living cells. Fats, whose sources can be animal and vegetable, are abundant in nature. Fats, one of the basic nutrients such as protein and carbohydrates, can be taken into the human body with natural or artificial nutrients. Fats synthesized in the body with the help of various enzymes are used for the proper functioning of metabolic activities [11].

Fats, one of the basic nutrients such as carbohydrate and protein, are among the most emphasized research topics in studies related to certain diseases, nutrition and human health. In these studies on fats, the subjects of particular emphasis are whether the oils are oxidatively stable, their degree of saturation or unsaturation, and their cis / trans structure. In addition to these, the EFAs and cholesterol they contain are also examined. Fats

have a great place and importance in human life due to their high energetic nature, providing thermal insulation around the organs and under the skin, their effects on blood lipid values, the importance they have for fat-soluble vitamins, and functional compounds such as EFAs [12].

Almost all oils are produced by esterification of glycerol, a polar trihydroxy alcohol, and FAs with different properties. When looking at all types of oils, it is seen that glycerin is common in their structure. However, oils differ in terms of the FAs they contain. For this reason, the systematic grouping of oils, which are an organic component, according to their physical and chemical properties, is based on the structure and quantity of FAs they contain in figure 1.

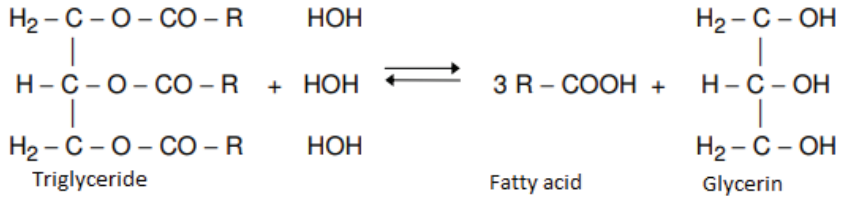


Figure 1: Formulation of the release of fatty acid and glycerin, the building blocks of oils, by oil hydrolysis [13].

1.4.1. Fatty Acids

FAs are carboxylated derivatives of aliphatic hydrocarbons, they contain an alkyl and a carboxyl group in their structure. There are a few FA's structures have been given in figure 2.

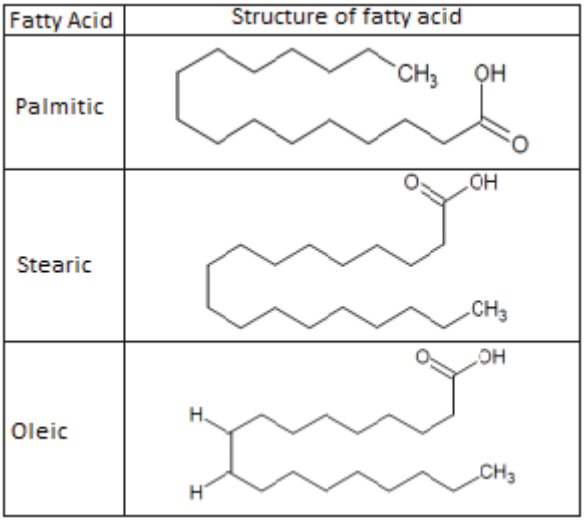


Figure 2: Fatty Acids in the Structure of Some Oils

The number of carbon atoms of a molecule, namely the chain length, the degree of saturation, the number of double bonds in the molecular structure, the position of these double bonds and the bonds of hydrogen atoms with carbon atoms; It determines the nutritional, chemical and physical properties of FAs [14].

Carboxylic fatty acids (CFA) containing 4 carbons (butyric acid) or more are considered as FAs, while FAs that make up natural oils (triglycerides) are considered to contain at least 8 carbons. In many triglyceride types, carbon atoms are even numbered. Because, in their synthesis, acetate containing two carbon atoms in its structure is used [15].

It is known that many vegetable raw materials such as sunflower, olive, coconut, peanut and canola are used in the production of oils. These vegetable oils, which meet almost 97% of FAs, also contain oleic, linoleic, lauric, palmitic, stearic, and myristic fatty acids. Fats in foods can carry FAs specific to that oil as well as non-specific fatty acids.

In order to obtain the oils of animal origin, mostly animals such as fish, cattle, sheep, pigs are used. Among the FAs contained in these animal fats, oleic acid, palmitoleic acid, stearic acid, myristic acid, palmitic acid, EPA, DHA, arachidonic acid and EPA.

FA and their synthesis mechanisms allow a better understanding of metabolic events and biological processes. The relationships of FAs with each other are at least as important as fats. It is reported that FAs in oils found in plants and animals have specific profiles. These FA profiles are not only dependent on plants and animals, but may also differ depending on the environmental conditions and chemical phenomena in which they grow [16].

In the industrial production of FAs, glycerol is separated from the structure by hydrolysis of ester bonds in the fat (triglyceride) chains and FAs are obtained in this way.

1.4.2. According to the Double Bonds in the Structure

When evaluating FAs in terms of their chemical properties, their carbon numbers, double bond numbers, cis / trans isomership of double bonds or the presence of substituents in their structures are examined [17]. FAs are metabolized by specific enzymes called “desaturase”, which are common in plants and animals; In this way, two hydrogen atoms are removed from the structure of FAs and a double bond is formed between carbon atoms [18].

Isomerism can also be seen in the structure of FAs. Isomer structures are concepts related to organic compounds and are defined as the same

closed formulas of compounds and different open formulas. Two of the isomer types seen in unsaturated fatty acids (UFA) are position isomers and geometric isomers. Position isomerism occurs by the different positioning of groups in the molecule or by the placement of double or triple bonds in different places. Its geometric isomerism occurs with different spatial positions of atoms or groups around the double bond. Geometric isomer forms are also called cis and trans isomers [19,20].

Carbon numbers, double bond numbers, cis / trans isomerism of double bonds etc. It gives physical distinguishing properties to FAs. When the cis / trans isomerism is compared, it is stated that the cis form is found in FAs more than the trans form. FAs in cis form have lower melting points than FAs in trans form [17]. While the cis form in UFAs causes breakage in the molecule, the trans form is linear similar to the straight chain of saturated fatty acids. Cis and trans fatty acids configurations shown in fig 3.

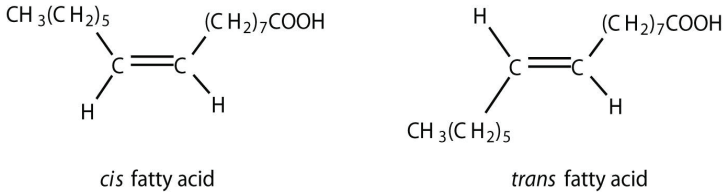


Figure 3: Cis and trans forms of fatty acid

FAs are divided into two basic groups as saturated and UFAs according to the presence of double bonds in their structure, and in the rest of this study, saturation will be considered according to the unsaturation classification.

There are two types of FAs, saturated and unsaturated. UFAs are divided into two. These are called monounsaturated (MUFA) and PUFA [21].

1.4.3. Saturated Fatty Acids

Saturated fatty acids (SFA) do not contain double bonds in their structure. Since all of the carbon atoms in the structure of these FAs bond with hydrogen, they are as stable as possible. They have the lowest reactivity among all FAs since they do not contain a group other than carboxyl as a functional group [22]. SFAs can be synthesized in the human body. They are synthesized by carbohydrates and metabolites of proteins even when no fat is consumed. Generally speaking, oils containing SFAs show that most of them are solid at room temperature. Nutrients mostly contain SFAs called palmitic, myristic and stearic acids in table 1 [20].

Although there is no significant difference between the energy provided by SFAs and the energy provided by other FAs, SFAs cause fat accumulation

in the body. In terms of cardiovascular health, it is reported that restricting the consumption of saturated fat and consuming less than 7% of the total energy will be beneficial. SFAs increase blood lipid values to higher levels than normal. It is reported that these FAS taken into the body increase the level of LDL cholesterol and at the same time make it difficult for the excretion of low-density lipoprotein (LDL) from the body. The increase in the level of LDL in the blood causes the accumulation of cholesterol in the walls of the vessels and causes the formation of atherosclerosis, which is why it is referred to as bad cholesterol among the people. At the same time, it is stated that SFAs, which can cause insulin resistance, should be consumed carefully because they increase the tendency to diabetes [23].

Table 1: A Table Containing the Names and Various Information of Saturated Fatty Acids [20].

Saturated Fatty Acids		
Acetic acid	2 Carbons	$CH_3.COOH$
Propionic acid	3 Carbons	$CH_3CH_2.COOH$
Butyric acid	4 Carbons	$CH_3\ (CH_2)2.COOH$
Caproic Acid	6 Carbons	$CH_3\ (CH_2)4.COOH$
Caprylic acid	8 Carbons	$CH_3\ (CH_2)6.COOH$
Capric Acid	10 Carbons	$CH_3\ (CH_2)8.COOH$
Lauric Acid	12 Carbons	$CH_3(CH_2)10.COOH$
Myristic Acid	14 Carbons	$CH_3(CH_2)12.COOH$
Palmitic acid	16 Carbons	$CH_3(CH_2)14.COOH$
Stearic acid	18 Carbons	$CH_3(CH_2)16.COOH$
Arachidic acid	20 Carbons	$CH_3(CH_2)18.COOH$
Behenic acid	22 Carbons	$CH_3(CH_2)20.COOH$
Lignoceric acid	24 Carbons	$CH_3(CH_2)22.COOH$
Serotic acid	26 Carbons	$CH_3(CH_2)24.COOH$
Montanic acid	28 Carbons	$CH_3(CH_2)26.COOH$

1.4.4. Unsaturated Fatty Acids

UFAs are molecules that contain one or more double bonds in their structure. These FAs contain different numbers and structures and different lengths of bonds in their chain structures. These molecules are named according to the location of the double bond and the number of double bonds [20].The place where the first double bond located closest to the methyl group is ω or “n” is designated as ω or “n”, and the UFAs are divided into 3 groups as n-3, n-6 and n-9 [24].

Those with one double bond in their structure are called MUFAs, and those with more than one double bond are called PUFAs. Oleic acid, one of the MUFAs, and linoleic acid, one of the PUFAs, are among the most common FAs in foods [20].

The reactivity of UFAs is higher than that of SFAs, which is caused by the double bonds in the structure of UFAs. There is an increase in activity according to the number of double bonds in the FA structure. While the human and animal body can synthesize saturated and MUFAs, it cannot synthesize PUFAs and therefore these FAs must be taken from outside, they are essential [7]. It is reported that the vegetable sources of UFAs are sunflower, olive oil, soy, corn, canola, and hazelnut, whereas the animal sources are salmon, mackerel and tuna that are found especially in cold seas and contain abundant SFAs [25]. There is a few UFAs list in table 2.

Table 2: A Table Containing the Names and Various Information of Unsaturated Fatty Acids [20].

Unsaturated Fatty Acids		
Palmitoleic acid	16C	$CH_3(CH_2)5.CH=CH(CH_2)7.COOH$
Oleic acid	18C	$CH_3(CH_2)7.CH=CH(CH_2)7.COOH$
Vaccenic acid	18C	$CH_3(CH_2)5.CH=CH(CH_2)9.COOH$
Linoleic acid	18C	$CH_3(CH_2)4.CH=CH.CH_2.CH=CH.(CH_2)7.COOH$
Linoleic acid	18C	$CH_3CH_2.CH=CH.CH_2.CH=CH.CH_2.CH=CH.(CH_2)7.COOH$
Arachidonic acid	20C	$CH_3CH_2.CH=CH.CH_2.CH=CH.CH_2.CH=CH.CH_2.CH=CH.(CH_2)6.COOH$

1.5. Fatty Acids in Health and Nutrition

The structure of PUFAs and their function in the body were examined and skin rashes were observed in mice consuming lean foods. In subsequent studies, it was determined that in the deficiency of ω -3 and ω -6 PUFAs, serious disorders such as cardiovascular diseases, high blood cholesterol level and blood pressure, asthma and inflammatory diseases, thrombosis, cancer, and febrile diseases [26].

In studies conducted for Eskimos, it has been observed that the rate of getting heart disease is 8 times higher in Eskimos who started living in Denmark compared to those who stayed in Greenland. This is thought to be caused by nutrition. Greenland Eskimos are fed rich in fish, whale and seal oils, which contain a lot of ω-3 FAs [27].

Intensive studies have been carried out on ω-3 PUFAs as they regulate blood pressure, reduce cholesterol levels and prevent the risk of plaque formation after angioplast [28].

It is reported that EPA and DHA taken with foods accelerate the antithrombotic events in the body. Problematic prothrombic situations are

prevented by balanced and regular intake of PUFAs; However, it has been found that people who consume sufficient levels of EPA and DHA have regular heartbeats and heart attacks that cause death are not seen in these individuals [29].

PUFAs play an active role in preventing or slowing down the course of diseases such as eczema and rheumatoid arthritis because they are the precursors of eicosanoids involved in these events [30].

Atopic dermatitis is a skin disease caused by partial or complete absence of the desaturase enzyme that causes GLNA deficiency. Borage flower or evening primrose oil, which contains high levels of GLNA, can reduce the symptoms of this disease. It has even been observed that creams prepared with the addition of this FA are beneficial for diaper rash in babies [31].

It is thought that neurological disorders are related to PUFAs and especially the deficiency of ω-3 PUFAs causes depression. It is stated that not getting enough PUFAs with diet may cause aggressive behavior [32].

It has been stated that symptoms such as non-flexion of the muscle ligaments and severe tissue pain can be reduced in rheumatoid arthritis patients fed with fish oils containing high levels of EPA and DHA [33].

PUFAs also play a critical role in the growth and development of infants and children. EPA and DHA intake is also required for the healthy development of cell membranes in the brain and retina. While DHA required for the unborn baby during pregnancy is transmitted to the brain and retina of the fetus through the mother's placenta, it is negatively affected by the fact that premature babies cannot store this FA at a sufficient level and there are no enzyme systems to synthesize this FA. Therefore, it is stated that enriching baby foods with DHA and other PUFAs will be beneficial for babies. The visual abilities and cognitive states of premature babies can only develop well in this way, like those of those who are breastfed [34].

The recommended amount of ALA for adults is 0.9-1.1 g / day for women and 1.2-1.6 g / day for men. Among the foods that are very rich in ALA are walnuts and flax seeds and their oils. The recommended amount of EPA to be taken with foods is 0.04-0.07 g / day and the amount of DHA is 0.05-0.09 g / day. The best sources of EPA and DHA are shown as fatty fish and fish oils. The amounts of ALA, EPA and DHA in some foods are shown in Table 3 and the recommendations of the American Heart Foundation for intake of ω-3 FAs are shown in Table 4.

		EPA (g)	DHA (g)	ALA (g)
Fish	CatFish	Trace amount	0.2	0.1
	Gadoid Fish	Trace amount	0.1	Trace amount
	Mackerel	0.9	1.4	0.2
	Salmon			
	Farm	0.6	1.3	Trace amount
	Sea	0.3	1.1	0.3
	Canning	0.9	0.8	Trace amount
	Sword	0.1	0.5	0.2
	Canning (in oil)	Trace amount	0.1	Trace amount
Shellfish	Lobster	-	-	-
	Mussel	0.2	0.3	Trace amount
	Shrimp	0.2	0.3	Trace amount
Oily Seeds and Oils	Walnut	-	-	9.1
	Linseed	-	-	18.3
	Canola	-	-	9.3
	Linseed-oil	-	-	53.3

Table 3: ALA, EPA and DHA amounts of some foods (100 g) [35].

		EPA (g)	DHA (g)	ALA (g)
Fish	CatFish	Trace amount	0.2	0.1
	Gadoid Fish	Trace amount	0.1	Trace amount
	Mackerel	0.9	1.4	0.2
	Salmon			
	Farm	0.6	1.3	Trace amount
	Sea	0.3	1.1	0.3
	Canning	0.9	0.8	Trace amount
	Sword	0.1	0.5	0.2
	Canning (in oil)	Trace amount	0.1	Trace amount
Shellfish	Lobster	-	-	-
	Mussel	0.2	0.3	Trace amount
	Shrimp	0.2	0.3	Trace amount
Oily Seeds and Oils	Walnut	-	-	9.1
	Linseed	-	-	18.3
	Canola	-	-	9.3
	Linseed-oil	-	-	53.3

Table 4: Recommendations of the American Heart Foundation on the consumption of ω-3 fatty acids [35,36].

	Suggestions
People without evidence of heart disease	Consuming fish minimum twice meals a week. Especially, oily fishes with high in ALA nutrition should be included on diet.
People with hearth disease	1 g EPA+DHA (3 g fish oil) everyday, preferably consumption of oily fishes, according to doctor suggestion intake of additional fish oil
People with high triglyceride	intake of between 2 and 4 g EPA+DHA under doctor control

The safe use dose of ω-3 FAs in the form of capsules has been announced by the US Food and Drug Administration as 3 g per day [35].

The lack of PUFAs can cause many functional disorders and diseases in the body. In addition, the uniformity of FAs entering into metabolism with foods can disrupt the functioning of other FAs that are deficient in metabolism. This defect prevents the balanced production of prostaglandins, thromboxanes and leukotrienes, which have important roles in preventing diseases [37].

Formation of hormone-like compounds by transformation from PUFAs is summarized in figure 4.

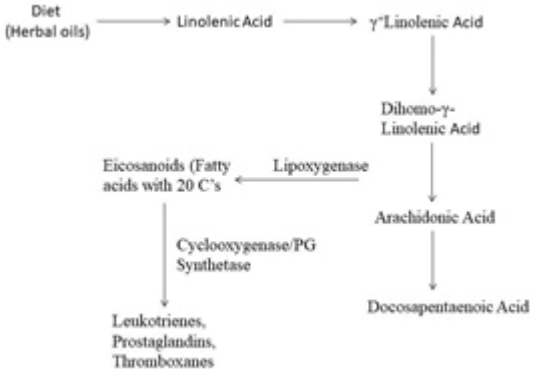


Figure 4: Conversion of dietary polyunsaturated fatty acids to hormone-like compounds [38].

ALA is metabolized to EPA and DHA in the body and factors such as age, gender, health functions and nutritional status can change the effectiveness of this process. High linoleic acid (LA) intake with foods suppresses ALA conversion. ω-3 and ω-6 fatty acids are always in a race in the body due to their duties. ω-3 plays a role in maintaining the fluidity of the blood and ω-6 as an enhancer of coagulation. Excessive intake of ω -6 accelerates blood clotting and the formation of cholesterol plaques,

as well as paving the way for the formation of allergic and inflammatory diseases. ω-3, unlike ω-6, prevents blood clotting, the development of inflammatory diseases and contributes to a healthy level of cholesterol. It is stated that the LA / ALA ratio should not exceed 4: 1 in order for the metabolic functions to progress in a healthy manner and ALA conversion not to be suppressed [39].

1. ANALYTICAL TECHNIQUES FOR DETERMINATION OF FATTY ACIDS IN FOOD SUPPLEMENTS

Maretha Opperman et al. 45 ω-3 fatty acid supplements available from the South African market were analyzed. FID detector was studied in the analysis. The aim of the research is to investigate the FAs, percentages and fish odor levels of these supplements. According to the results of the research, the EPA and DHA contents and rates of the supplements were found. It is stated on the product labels that there is between 90% and 110% EPA and DHA. For this reason, those containing EPA and DHA at 89% or less are considered as sub-standard and those containing more than 110% as above standard. According to the results of the study, it is stated that the supplements analyzed have confirmed the EPA and DHA contents specified on the label [40].

Tobias Tatarczyk et al. conducted a research on preparations prepared to support the western diet which is low in long-chain ω -3 PUFAs. A large number of these preparations are prepared. ω -3 FAs, which have been found to be useful in various studies by national and international societies, are recommended as 1 g / day for atherosclerosis and arrhythmia or 2-4 g / day for lipid-lowering effect. Nine commercially available products were tested for FAs compositions, as preparations prepared and marketed for these purposes differ in their ω-3 PUFA content. Capillary GC was used for analysis. Nine preparations differed greatly in ω-3 fatty acid content up to 63.7 ± 1.58 mol%. Most were unable to achieve a recommended daily dose of one gram, even when administered at the highest dose according to the manufacturer's recommendations. In eight of the preparations, long chain ω-3 PUFAs were detected in amounts equal to or greater than that specified by the manufacturer, while one preparation did not provide any information. The highest EPA and DHA percentages were found in the preparations named Omacor (95.80 ± 0.63%) and Percucor (76.8 ± 7.109%). According to the study, the application of long-chain ω-3 fatty acid preparations can cause great differences in terms of the actual amount taken. Therefore, it is recommended to use the most standard and purified products available [41].

Tao Yi et al. A new GC-MS method has been developed to evaluate the quality of various fish oil capsules, to detect EPA and DHA contents

in fish oil capsules and to analyze them comparatively. Ten batches of fish oil capsules samples obtained from Hong Kong pharmacies were analyzed using this newly developed method. Shimadzu QP2010 GC-MS system (Kyoto-Japan) was used for qualitative and quantitative analysis of fish oil, DB-5 ms high resolution capillary column (thickness: 0.25 µm, length: 30m, diameter: 0.25 mm) was used for sample separation. For temperature programming, after the furnace was kept at 80 ° C for one minute, it was increased by 10 ° C every 10 minutes to reach 250 ° C. Later, it was increased at a rate of 8 ° C per minute, and this speed was reached up to 280 ° C. It was held at this temperature for 5 minutes. Helium was used as carrier gas at a rate of 0.8 ml / min with an injection volume of 1 µl. A comprehensive evaluation of the method developed by the researchers has been made and it has been shown that the method is highly sensitive, reliable, reproducible and accurate. In addition, while the EPA content ranged between 39.52 mg/g to 509.16 mg/g, DHA content ranged from 35.14 mg/g to 645.70 mg/g in all fish oil samples [42].

Alison C. Kleiner et al. 47 commercial fish, krill and algae oil supplements were analyzed for EPA, DHA and other FA contents due to the growing popularity of food supplements. The EPA range for fish and krill-based supplements ranges from 81.8 to 454.6 mg / g oil and DHA ranges from 51.6 to 220.4 mg / g fat, while for algae oil supplements EPA ranges from 7.7 to 151.1 mg / g oil and DHA 237.8 to 423.5 mg / g fat. The percentage of label amount indicated for EPA and DHA ranges from 66 to 184% and from 62 to 184%, respectively. It was observed that only 10 supplements (21% of those tested) had at least 100% of the specified label amount, 12 supplements (25% of those tested) had at least 100% of the specified amount of DHA. It was found that more than 70% of the supplements tested did not contain EPA or DHA in the amounts specified on the label. According to the results of the research, it is stated that the quality of fish oil supplements is not adequately monitored by the producers or government agencies and the tests should be increased to ensure compliance with the legislation [43].

The measurement of long-chain ω -3 polyunsaturated fatty acids (ω-3-PUFA) in gummy dietary supplements was studied by Ziyi Li and Cynthia Srigley. Food supplements containing long-chain PUFA, including EPA and DHA, are frequently used in the USA to support health and reduce the risk of chronic disease. Gummy supplements formulated to contain fish oils are perceived as tastier alternatives to traditional fish oil soft gels or liquids. However, despite the growing popularity of these products, an approved method for analyzing the content of FA has not yet been reported. The aim of this study is to develop and validate a new analytical method for the measurement of long chain ω-3 PUFAs and other lipids in gummy

supplements. This method involves the gentle digestion of cryogenically homogenized gummy samples, followed by liquid-liquid extraction with low toxicity solvents. The extracted lipids are then derivatized to fatty acid methyl esters and analyzed by GC-FID. This method shows high performance in the verification of accuracy, repeatability, linearity, robustness, as well as the absence of interference and pollutants. It is reported that this new method is suitable for measuring long-chain ω -3 PUFA in various gummy supplements currently available in the US market [44].

In the study conducted by Sri Handayani and Cornelia Budimarwanti, ω-3 content in fish oil capsules was determined by alkalimetric titration method. This method is done in three ways. First, FAs were oxidized by KMnO4 using H2SO4 as a catalyst. Second, the propanoic acid is removed by distillation by oxidation. Distilled propanoic acid was further titrated. Analysis of the same sample by GC-MS has already been done. The chromatogram of the analysis result showed the data to be used as comparator. With the alkalimetric titration method, ω-3 contents were found as 3.41% Linoleic acid, 15.48% EPA, 10.18% DHA. It is reported that this method, which was used for the first time by researchers to determine the ω-3 content, is good enough in terms of the precision, accuracy and reproducibility of the study [45].

Benjamin B. Albert et al. The quality and content of fish oil supplements in New Zealand were evaluated in the study conducted by. GC was used to measure the FA content. 1 ml of fish oil was frozen at -80 ° C for storage before analysis. A 50 mg oil sample was weighed into solvent washed methylation tubes and dissolved in 10 ml of toluene. 200 µl (1.0 mg) of this was taken for trans-methylation monitoring by GC analysis. Briefly, 2 ml of methanol: toluene (4: 1 v / v, containing C19: 0, 20 µg / ml as internal standard) was added to the sample. Acetyl chloride (200 microliters) was added during vortexing and the contents were heated at 100 ° C for 1 hour. Tubes were cooled in water (5 minutes) and centrifuged (3000 × g, 5 minutes, 4 ° C) adding 6% K2CO3. The upper toluene phase was collected and stored in a GC bottle at -20 ° C for analysis. The methylated fatty acid samples were analyzed by GC using a stationary carbon-silica column of 30 m x 0.25 mm (DB-225) (J&W Scientific, Folsom, CA, USA). The GC is equipped with a flame ionization detector, autosampler and automatic detector. Injector and detector ports are set to 250 ° C. The furnace temperature was kept constant at 170 ° C for two minutes, increased by 10 ° C / minute to 190 ° C, and then remained constant for one minute. The temperature was then increased by 3 ° C / minute to 220 ° C and this was maintained for a total run time of 30 minutes per sample. A split ratio of 10: 1 and an injection volume of 3 µl were used. A known FA mixture was used to identify peaks by retention

time, and their concentrations were determined using a 6890 Series GC (Hewlett Packard, Palo Alto, CA, USA) with Chemstations Version A 04 (Lepage G AND Roy C). Peroxide values (PV) and anisidin values (AV) were measured and total oxidation values (Totox) were calculated. Only 3 out of 32 fish oil supplements were found to contain EPA and DHA equal to or greater than the labeled ingredients, while most products tested contained less than 67% of these FAs. The vast majority of supplements have been found to exceed recommended oxidation marker levels. 83% of the products included in the study exceed the recommended Perokside Value (PV) levels, 25% exceed the Anisidine Value (AV) threshold values and 50% exceed the recommended Totox levels. Only 8% of them meet the international recommendations not exceeding any of these indices. Almost all fish oil supplements available on the New Zealand market contain concentrations of EPA and DHA that are significantly lower than what is claimed on the labels. Importantly, most of the supplements tested were found to exceed the recommended oxidative marker indices [46].

Tatiane Lima Amorim et al. The quality of dietary supplements containing essential ω-3 fatty acids (ω-3-FA), EPA and DHA in human nutrition were evaluated. The aim of this study was to optimize and validate a rapid capillary electrophoresis (CE) method to determine the EPA and DHA content in marine oil ω-3 supplements. Sample preparation included only one saponification step and analysis took 8 minutes. Verification, detection and quantification were done according to the limits of linearity, accuracy and repeatability. Ten real fish oil supplement samples were analyzed by CE and compared with conventional GC method. There is no significant difference between the two methods at 95% confidence interval [47].

H. Koller et al. In a study conducted by Ş., it was investigated that 22 commercially available fish oil and cod liver oil preparations may be disadvantageous in their accompanying amounts. Cholesterol using GC, heavy metals using atomic absorption, and vitamin A using high resolution liquid chromatography (HPLC) were measured. Both cholesterol and heavy metal content were found in harmless ranges. However, the vitamin A content of cod liver oil capsules was found in amounts that should not exceed the manufacturers' dosage recommendations for pregnant women [48].

In the studies conducted by Nurcan A. Güzelsoy and Belgin İzgi, analyzes were made on the detection of metal contaminants in fish oil supplements. Fish oils used by humans, due to their many health benefits, may contain toxic heavy metals (As, Hg, Cd, Pb). Difficulties are encountered in solubilizing samples, especially when conducting mineral analysis of food supplements with high fat content. To be used in this

study, 33 fish oil, 15 in capsule form and 18 in liquid form, were obtained and inductively coupled plasma mass spectrometry (ICP-MS) was used for the analysis of these samples. The sample preparation process, which is an important step in elemental analysis, has been made more functional. The variables that can affect the extraction efficiency in the microwave-heated solubilization system during the sample preparation process were determined as the sample amount, the amount of HNO_3, HCl and H_2O_2 and optimized by using the Central Composite Design method. Optimum values for microwave system; 0.24 g sample amount is 4.50 ml HNO_3, 1.11 ml HCl and 0.75 ml H_2O_2. Real samples were analyzed with the ICP-MS device after optimim ion of the sample preparation process, which is one of the most important steps of multi-element analysis. The element contents in the fish oils supplied were determined. The maximum limits of Hg, Pb and Cd elements are specified in the Turkish Food Codex Contaminants Regulation, and it has been determined that the analyzed fish oils comply with the legal limits. Arsenic element could not be evaluated due to the lack of a legal limit for this element [49].

Pim Jansson and Bartholomew Kay found that many commercially available ω-3 supplements are unacceptably oxidized and lead to numerous potential health risks, so the prevalence of aldehydes in ω-3 supplements, which have been shown to have cytotoxic, mutagenic and inflammatory properties. It has been researched and evaluated. 12 different ω-3 oils (6 fish, 4 krill, 2 algae) purchased from various retailers were tested using 1 H-NMR (Nuclear Magnetic Resonance) scanning. Both algae samples contained 1.235 (± 0.111) and 1.565 (± 0.618) mMol / L, while 4 krill products contained aldehyde at concentrations between 5.652 (± 0.496) and 6.779 (± 1.817) mMol / L, while two of six fish oils were 1.568 (± 0.291).) and aldehydes at concentrations of 4.319 (± 2.361) mMol / L. There is currently no standard for labeling aldehyde content or ω-3 supplements. Two-thirds (8 out of 12) of the ω-3 supplements tested in this study contained aldehydes. Aldehydes can trigger serious health problems even at very low absolute intake volumes. It is stated that these findings can provide a reason for further studies on this subject [50].

2. CONCLUSION

Although food supplements are not included in the scope of drugs, it is known that individuals are guided by the right people and that they affect health positively by consuming the products correctly. Today, the functional properties of food ingredients have encouraged the use of food supplements for health preservation. Insufficient intake of health-important components due to malnutrition or other reasons increases the tendency towards food supplements. The nutritional supplements industry enables the development of new products in order to meet the needs of individuals.

While food supplements show improvement, they can also cause various problems. In order to prevent these situations from harming health and economy, it becomes very important to make inspections of products at many points from production to licensing, launching and selling.

Fish oils are especially beneficial for many reasons such as containing EFAs (EPA, DHA), being beneficial for serious disorders such as cardiovascular health, cholesterol level, regulation of blood pressure, inflammatory diseases, cancer, meeting the needs of mothers during pregnancy and developing children. It is one of the food supplement types whose use has increased in recent years.

Analyzes are carried out in order to follow the problems caused by label information errors, formation of oxidation products, contamination and auxiliary substances in fish oil supplements. These analyzes have a great effect on preventive health and cost savings. By misguiding the public with incorrect information, detecting contaminated contents such as heavy metals, damages to public health can be prevented in this way.

In the final stage of this review study, the analysis methods developed, optimized or already available by the researchers to determine the amount of FAs in food supplements, oxidation products or various contaminants are presented. Among these analysis methods, methods such as GC, GC-MS, alkalimetric titration method, inductively coupled plasma mass spectrometry, 1 H-NMR scanning, alkalimetric titration method are described. It is thought that the analysis methods developed will provide higher performances in researches and controls in terms of accuracy, repeatability, linearity and durability.

3. REFERENCES

1- Soare, A., Weiss, E.P., Holloszy, J.O., Fontana, L. 2014, Multiple Dietary Supplements Do Not Affect Metabolic and Cardiovascular Health. Aging, 6(2), 149-157.

2- Bailey, RL, Gahche, JJ, Lentino, CV, Dwyer, JT, Engel, JS, Thomas, PR, Betz, JM, Sempos, CT, Picciano, MF. 2011, Dietary Supplement Use İn The United States, 2003–2006. The Journal Of Nutrition,141(2): 261-6.

3- Garcia-Cazarin, M.L, Wambogo, E. A., Regan K. S., Davis C. D. 2014, Dietary Supplement Research Portfolio at the NIH, 2009–2011, *The Journal of Nutrition*, 144(4), 414–418.

4- World Health Organization, (2015), Healthy diet, WHO Fact Sheet, No 394 ,http://www.who.int/mediacentre/factsheets/fs394/en/

5- Ho, C. T., Simon, J. E., Shahidi, F., Shao, Y., 2008, Dietary Supplements: An Overview, Acs Symposium Series; American Chemical Society, Washington, Dc. DOI: 10.1021/bk-2008-0987.ch001

6- Dolan, P., Nortrup, D. A., Bolger, M., Capar, S. G. 2003, Analysis of Dietary Supplements for Arsenic, Cadmium, Mercury and Lead Using Inductively Coupled Plasma Mass Spectrometry, *J. Agric. Food Chem*., 51, 1307-1312.

7- Mol, S. 2008, Consumption of fish oil and its effects on human health, *J. Fisheries Sci. Com*., 2(4): 601-607.

8- Moret, S., Purcaro, G., Conte, L., 2010, Polycyclic Aromatic Hydrocarbons(Pahs) Levels in Propolis and Propolis-Based Dietary Supplements from the Italian Market, *Food Chemistry*, 122, 333-338.

9- Dickinson, A., Blatman, J., El-Dash, N., Franco, J.C., 2014, Consumer Usage and reasons for Using Dietary Supplements: Report of a Series of Surveys, *Journal of the American College of Nutrition*, 33(2), 176-182.

10- Harding, K.L., Judah, R.D., Gant, C.E., 2003, Outcomebased Comparison of Ritalin® Versus Food-Supplement Treated Children with Ad/Hd, Alternative Medicine Review*,* 8(3), 319-330.

11- Lee, R.M.K. 1994, Canadian Journal of Physiology and Pharmacology, 72, 945 - 953.

12- J. Powles, J. Wiseman, D.J. A. 1993, Cole and B. Hardy, Effect of Chemical Structure of Fats upon Their Apparent Digestible Energy Value When Given to Growing/Finishing Pigs, Animal Science, 57, 137-146

13- R.W. Johnson, E. Fritz, 1989, Fatty Acids in Industry**,** Marcel Dekker, New York.

14- Fats and Fatty Oils. 200, Ullmann's Encylopedia of Industrial Chemistry.

15- J. M. Burt, K. D. Massey, and B. N. Minnich, 1991, Uncoupling of Cardiac Cells by Fatty Acids: Structure-Activity Relationships

16- Ahmad, M.U. 2007, Fatty Acids: Chemistry, Synthesis, and Applications: Elsevier Science, Academic Press. 978-0-12-809521-8.

17- Harwood, J.L., Gurr, M.I., Frayn, K.N., Murphy, D.J., Michell, R.H., 2016, Lipids: Biochemistry, Biotechnology and Health, John Wiley & Sons, 6.

18- Lawrence, G.D. 2010, The Fats of Life: Essential Fatty Acids in Health and Disease, Rutgers University Press, 102-106.

19- Larque, E., Zamora, S., Gil, 2001, A. Dietary Trans Fatty Acids in Early Life: A Review, *Early Hum.Dev*., 65, 31-41.

20- Semma, M., 2002, Trans Fatty Acids: Properties, Benefits And Risks, J. Health Sci., 48(1), 7–13.

21- Mensink, R.P., Katan, M.B., 1990, Effect of Dietary Trans Fatty Acids on High-Density and Low-Density Lipoprotein Cholesterol Levels in Healthy Subjects. N. Eng. J. Med., 323:439-445.

22- Wolff, R.L., 1992, Trans Polyunsaturated Fatty Acids in French Edible Rapeseed and Soybean Oils. Journal Of The American Oil Chemists Society, 69(2),106-110.

23- G.R. Thompson. Recommendations for the Use of LDL Apheresis. Atherosclerosis. (2008), 198;2, 247-255.

24- N. M. Zatsick, P. Mayket., 2007 Fish Oil: Getting to the Heart of It. The Journal for Nurse Practitioners, 3;2, 104-109

25- Gogus, U., Smith, 2019, C. n-3 Omega Fatty Acids: Review Of Current Knowledge. Int. J. Food Sci. Technol., 45: 417–436.

26- A. Peter, C. V. Schacky, 2000 n-3 Polyunsaturated Fatty Acids and The Cardiovascular System. Current Opinion in Lipodology, 17, 47-53.

27- F. Kamp, D. Zakim, F. Zhang, N. Noy, and J. A. Hamilton, 1995, Fatty Acid Flip-Flop in Phospholipid Bilayers is Extremely Fast. Biochemistry, 34, 11928—11937.

28- M. J. Calvo, M. S. Martínez, W. Torres, M. Chávez-Castillo, E. Luzardo, N. Villasmil, J. Salazar, M. Velasco, V. Bermúdez, 2017, Omega-3 Polyunsaturated Fatty Acids and Cardiovascular Health: A Molecular View into Structure and Function. Wessel Plus, 1:116-128.

29- Alberto Ascherio, M.D., Eric B. Rimm, Sc.D., Meir J. Stampfer, M.D., Edward L. Giovannucci, M.D., and Walter C. Willett, M.D., 1995, Dietary Intake of Marine n-3 Fatty Acids, Fish Intake, and the Risk of Coronary Disease among Men. New England Journal of Medicine, 332:977 - 982.

30- A P Simopoulos. 1991, Omega-3 Fatty Acids in Health and Disease and in Growth And Development. American Journal of Clinical Nutrition, 54(3):438-63.

31- David T. J., Ferguson A.P., Newton R.W., 1991, Nocturnal Growth Hormone Release in Children With Short Stature and Atopic Dermatitis. Acta Dermato-Venereologica, 71(3):229-231

32- J. R. Hibbeln, N. Salem Jr., 1995, Dietary Polyunsaturated Fatty Acids and Depression: When Cholesterol Does not Satisfy. Am J Clin Nutr. (1995),62(1):1-9.

33- Kremer, J.M. n−3 Fatty Acid Supplements in Rheumatoid Arthritis .American Journal of Clinical Nutrition, 71 (1): 349 – 351

34- Horrocks, L.A. Ve Y.K. Keo., 1995, Health Benefits of Docosahexaenoic Acid (Dha). Pharmacological Research, 40 (3) : 2 1 1 – 225

35- De Filippis, A., Sperling, S.S., 2006. Understanding Omega-3, American Heart Journal, 151(3), 564-570.

36- Larsen, J., Boeckner, L., 2006, Omega-3 Fatty Acids, NebGuide, University of Nebraska,http://www.ianrpubs.unl.edu/epublic/pages/publicationD.j p?publication Id=308.

37- Sprecher, H. Metabolism of Highly Unsaturated n-3 ans n-6 Fatty Acids, Biochimica et Biophysica Açta, (2000), 1486(2-3), 219 - 231

38- B. Lands, 2014, Dietary Omega-3 and Omega-6 Fatty Acids Compete in Producing Tissue Compositions and Tissue Responses. Military Medicine, 179:11, 76-81.

39- Sanders, T.A., 2019 DHA status of vegetarians, Prostaglandins, Leukotrienes and Essential Fatty Acids, 81(2- 3), 137-141.

40- Opperman, M., Marais, D.W., Benade, A.S., 2011, Analysis Of Omega-3 Fatty Acid Content of South African Fish Oil Supplements, Cardiovascular Journal of Africa, 22(6), 324-329.

41- Tatarczyk, T., Engl, J., Ciardi, C., Laimer, M., Kaser, S., Salzmann, K., Lenners, R., Patsch, J.R., Ebenbichler, C.F., 2007, Analysis of Long-Chain Omega-3 Fatty Acid Content in Fish-Oil Supplements, Wiener Klinische Wochenschrift, 119, 417-422.

42- Yi, T., Li, S.M., Fan, J.Y., Fan, L.L., Zhang, Z.F., Luo, P., Zhang, X.J., Wang, J.G., Zhu, L., Zhao, Z.Z., Chen H.B., 2014, Comparative Analysis of EPA and DHA in Fish Oil Nutritional Capsules by GC-MS, Lipids in Health and Disease, 13, 190.

43- Kleiner, A.C., Cladis, D.P., Santerre, C.R., 2014 A Comparison of Actual Versus Stated Label Amounts of Epa and Dha in Commercial Omega-3 Dietary Supplements in The United States, Journal Of The Science Of Food And Agriculture, 95(6), 1260-1267.

44- Li, Z., Srigley, C.T., 2017, A Novel Method for The Quantification of Long Chain Omega-3 Polyunsaturated Fatty Acids (Pufa) in Gummy Dietary Supplements, Journal Of Food Composition And Analysis, 56, 1-10.

45- Handayani, S., Budimarwanti C., 2006, Development of Simple Analytical Method of Omega-3 Fatty Acid by Propanoic Acid Determinetion Using Alkalimetric Titration, Indo. J. Chem., 6(3), 322-324.

46- Albert , B.B., Derraik , J.G.B., Smith , D.C., Hofman , P.L., Tumanov , S., Boas, S.G.V., Garg , M.L., Cutfield, W.S., 2015, Fish Oil Supplements in New Zealand Are Highly Oxidised and Do Not Meet Label Content of n-3 PUFA, Scientific Reports, 5, 7928.

47- Amorim, T.L., Duarte, L.M., Fuente, M.A., Oliveira, M.A.L., Kortes P.G., 2020, Fast Capillary Electrophoresis Method for Determination of Docosahexaenoic and Eicosapentaenoic Acids in Marine Oils Omega-3 Supplements, Journal of Cromatography A., 1613, 460-461.

48- Koller, H., Luley, C., Klein, B., Baum, H., Biesalski, H.K., 1989, Contaminating Substances in 22 Over-the-Counter Fish Oil and Cod Liver Oil Preparations: Cholesterol, Heavy Metals and Vitamin A, Zeitschrift für Ernährungswissenschaft, 28, 76–83.

49- Guzelsoy, N.A., Izgi, B., 2015, Optimization of Analytical Parameters for Determination of (As, Hg, Cd and Pb) in Fish Oil Supplements, Journal of Food and Feed Science – Technology, 15: 19-26.

50- Jansson, P., Kay, B., 2018, Aldehydes Identified in Commercially Available Omega-E Supplements via 1 H NMR spectroscopy, Nutrition, 60, 74-79.

CHAPTER 13

EXPERIENCES OF MOTHERS WITH PHYSICAL DISABILITIES ON THE CARE OF THEIR CHILDREN: A SYSTEMATIC REVIEW

Sümeyra TOPAL[1]

Nursan ÇINAR[2]

1 Corresponding author: MSc. Research Assist. Kahramanmaraş İstiklal University, Faculty of Health Science, Department of Pediatric Nursing, Turkey e-mail: sumeyratopal@ksu.edu.tr Orcid no: https://orcid.org/0000-0001-6316-4043

2 Dr. (Proof) Sakarya University, Faculty of Health Science, Department of Pediatric Nursing, Turkey e-mail: ndede@sakarya.edu.tr Orcid no: https://orcid.org/0000-0003-3151-9975

INTRODUCTION

According to the definition of the World Health Organization (2011), disability has been described as a term covering impairment, activity limitation, and participation restriction. Almost every individual may face with difficulties in performing daily life activities temporarily or permanently in a period of their life. Disability becomes part of life. It is estimated that more than 15% of the world population live together with any disabled individual (WHO, 2014). Therefore, people with disabilities are known as the biggest minority in the world.

In the world, the number of women who want to become parents and raise children although they have physical disabilities has also increased (Malouf, Henderson, & Redshaw, 2017). A woman with a physical disability may face with many problems in maintaining her life as an individual with a physical disability. In particular, the women with a physical disabilities in the reproductive period further need care services in important health issues such as adolescence, sexuality, reproductive health, family planning, pregnancy, delivery, postpartum period and baby care (Shpigelman, 2014). However, many women with a physical disabilities are unable to access the health care services. They ignore their requirements due to various barriers such as, environmental and physical limitations, distinctive attitudes and behaviors, service inadequacies related to medical care and rehabilitation, education and economic restrictions (Shpigelman, 2014; Tarasoff, 2014). However, it is every woman's right to have a motherhood experience and to have a baby healthily (Malouf, Henderson, & Redshaw, 2017).

The women with a physical disability may have more fears and uncertainties about baby care compared to other pregnant women or women who want to become pregnant. A mother with physical disability may face with many impeding factors in breastfeeding, carrying, making her baby sleep, giving her baby a bath and meeting daily routine care needs (Redshaw, Malouf, Gao & Gray, 2013; Shpigelman, 2014; Tarasoff, 2014). However, the difficulties experienced by mothers with physical disabilities during their motherhood process provides mostly them to develop different coping strategies by improving their some aspects (Redshaw et al. 2013; Shpigelman, 2014).

Until recently, society viewed women with a physical disability as incapable of assuming the role of mother (WHO, 2011; Malouf, Gao & Gray, 2013; Redshaw et al. 2013; Shpigelman, 2014; WHO, 2014). Health professionals should provide guidance, support, and facilitation to women with disabilities during the preparation for and transition to motherhood. It can be overcame the challenges that she can face by providing the necessary support to the physically disabled mother who wants to be a mother by health professionnals.

The aim of this study was to reveal the current scientific knowledge about the experiences of mothers with physical disabilities in the care of their children and the strategies they use.

METHODS

This systematic review was carried out using the steps of the phrase Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) to report systematic reviews (Moher et al., 2009a). Working protocol which enables systematic compilation and recording of meta-analysis "PROSPERO" is registered in the database (ID= CRD42020142885) https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020142885.

Search strategy

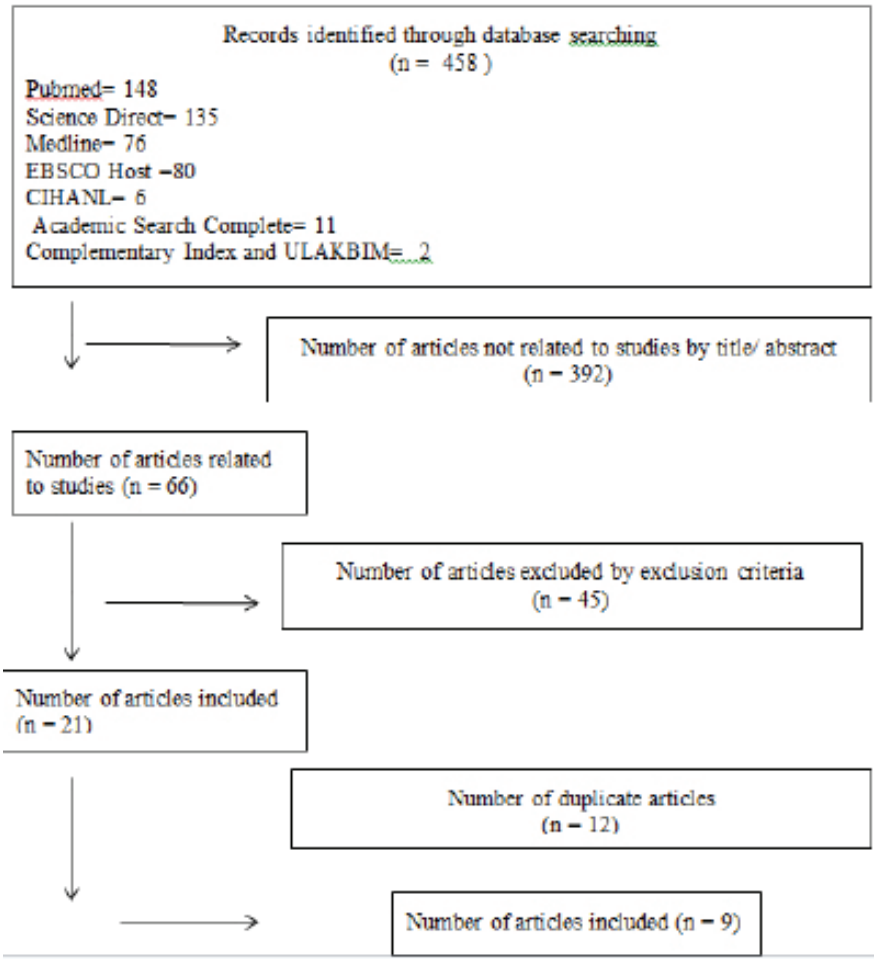
A systematic literature search was conducted on the 13th of November 2019 without limit features in the following databases: PubMed, Embase and CINAHL. Reference lists of included studies were searched by hand, and articles citing those studies were searched in Scopus to identify additional relevant studies. A final search was performed October, 18, 2022. To ensure a comprehensive search, there were no restrictions on the country of research or publishing year. In this section, the design of the study, inclusion / exclusion criteria, screening strategy, evaluation and reporting stages are explained.

Selection criteria

This systematic review focused on the domain 'mothers with physical disabilities', determinant 'children care' and outcome 'experience'. Since the underlying causes are investigated, qualitative studies and qualitative data extracted from mixed methods studies that addressed the experiences of mothers with physical disabilities regarding children care were considered relevant. For practical reasons, only articles in English was included. Studies on the child care experiences of physically disabled mothers, which do not aim to research children care, were excluded. Additionally, studies involving other individuals, such as healthcare providers and family members, were excluded. Duplicate resolution, all titles and abstracts were screened by both investigators (ST and NÇ) for compliance with inclusion and exclusion criteria. Later, the full text was scanned by both investigators (ST and NÇ). Studies that were uncertain whether they met the inclusion or exclusion criteria were independently screened by the second investigator (NÇ) and discussed until consensus was reached. Other members of the research group were consulted if any doubt remained. Studies that deal with the childcare experiences of mothers with physical disabilities and which are presented as full text have been examined in detail.

The PRISMA diagram shows the process of the selecting studies that were included in this review (Figure 1).

Figure 1. Flow- chart of study selection



RESULTS

Table 1 lists nine studies investigating the problems experienced by mothers with physical disabilities in the care of children. When the results of nine studies were examined by focusing on the titles related to child's nutrition, hygienic care (care of diaper area, bath, daily hygienic care etc.), child's safety and adaptation to home environment, transport of a child from one place to another, making the environment accessible to them and the communication and cooperation between caregivers have been seen.

Powell et al. carried out a qualitative research on 25 samples to investigate the factors that facilitate and prevent breastfeeding while mothers with physical disabilities were feeding their babies. Four themes

that facilitated breastfeeding were obtained as a result of the research. These themes were indicated as adaptation and equipment, the use of breast pump, receiving physical help from other people, and husband's support. On the other hand, five themes inhibiting breastfeeding were found in the study. These themes were determined as the lack of support during breastfeeding, disability-related health problems, the lack of knowledge, difficulties related to milk production, and attachment difficulties. In line with the data obtained from the study, it was concluded that information that facilitated breastfeeding by health personnel was inadequate for women with physical disabilities who wanted to breastfeed and that more breastfeeding support should be given (Powell et al. 2018). In the study, it was mentioned that support should be provided to promote breastfeeding in women with disabilities as well as in women without disability. Disabled women with activity limitation especially in the upper extremity should be initially given counseling about appropriate breastfeeding position and that different strategies (such as pillow support) should be developed to breastfeed and hold the baby easily. In a secondary analysis study carried out in the UK in 2013, it was aimed to describe the maternal health services received by women with disabilities during pregnancy, delivery and postpartum period, to understand the perception of care they had, and to determine how different this perception of care was from the women without disability. It was determined that the women with different types of disabilities also differed in terms of experiences. It was determined that the women with physical disabilities further used the prenatal and postnatal care services but they had less options for delivery services. Although the results of the study showed that women mostly had positive thoughts about care services, breastfeeding rates were found to be lower. Mothers stated that they needed another person during their breastfeeding and they had difficulty in this process on their own (Wint, Smith & Iezzoni, 2016).

In the study carried out by Wint et al. (2016), the experiences of mothers with physical disabilities on newborn care, and how they were adapted to the home environment and care responsibilities were examined. It has been determined that baby care in the night, giving baby a bath, and the transport of the child were among the mothers' biggest challenges. In the study, mothers had stated that they had great difficulties in taking care of their babies in the night, had a difficulty in taking their babies from the cradle and putting them on the care table and that it has not easy to give care on their own. In the same study, the mothers had stated that they did not dare to give their babies a bath on their own and needed to get help with the fear of dropping their babies. The majority of mothers stated that they received support from their own mothers, husbands or healthcare personnel while giving their babies a bath. One of the biggest difficulties and the causes of anxiety reported by mothers in the study was the transport

of babies. Mothers use various alternative products to transport babies in order to adapt to these difficulties. To perform that task, many women found that using a wrapping or something similar (scarf, sweatshirt, binding to belly or carrier) to secure the infant are easiest and preferable method. In addition to these, furniture adaptations and support provided by caregivers for mothers using wheelchairs were determined as the basic adaptation actions. The independence of the mother with disability in baby care can be ensured by means of the equipment, such as side opening cradles, low cupboards, and baby carrier which can be added next to the wheelchair, by arranging the physical environment. As a result of the study, it has been mentioned that it was possible for women with physical disabilities to perform the care of a baby completely and to adapt to their environment. New studies are required for it, and that the creation of environments where mothers' experiences can be share with campaign with the experts through Occupational Therapy Intervention would give positive results.

In a qualitative study carried out by Tarasoff (2017) to determine the perinatal care experiences of women with physical disabilities and the obstacles they faced while giving care, five themes that posed an obstacle in baby care were determined. These themes are (1) Inaccessible care environment, (2) Negative attitudes, (3) Lack of knowledge and experience, (4) Lack of communication and cooperation among caregivers and (5) Misunderstanding of disability and disability-related needs. The results obtained from the study revealed that the perinatal care system has not been established in accordance with the women with physical disabilities. Healthcare personnel has negative attitudes, support has not been provided sufficiently regarding the care of the newborn, the staff have deficiencies to communicate with each other, and a mother with disability could not meet the need for health care sufficiently. In the study, it has been emphasized that disability in perinatal care may result in bad consequences. Similar results have been also mentioned in a similar study on the subject (Morrison et al. 2014).

In the results of another study carried out to investigate the access and quality of maternal health service for women with disabilities. [3] Some shortcomings regarding interpersonal communication in the care of women with disabilities were found. These areas of shortcoming are listed as communication, feeling of being listened and supported, being involved in the decision making process, and having a reliable and respectable relationship with clinical staff. All of the women included in the sampling stated that they needed more help in terms of postnatal care and child's nutrition. The mothers stated that they had difficulty while breastfeeding their babies and had difficulty in providing the appropriate breastfeeding position (Malouf, Henderson, & Redshaw, 2017).

In the study carried out by Shpigelman (2014) with the mothers with physical disabilities, the physical difficulties faced by women during motherhood experiences and their strengths were investigated. The main difficulties of mothers are associated with their care roles. For instance, giving baby a bath, changing of clothes, transport of babies from one place to another, playing games, the experiences while participating in social activities etc. A mother who participated in the study "The kids sometimes take from advantage of the disability and it is hard for me setting a limit. I remember in once, when my daughter discovered that I cannot run, she run away, because she knew I will not able to catch her" stated. She stated that they felt inadequate in that case. Despite these negativities, while the women included in the study were evaluating motherhood in the context of disability, they stated that being a mother with disability helped them to feel themselves strong by developing a positive disability identity for them. They developed different coping strategies for the care difficulties faced by them. A mother participated in the study stated that she used lots of baby wipes to clean her baby since she had difficulty in washing her baby and that it was highly important for her although it seemed less important. Another mother also said "I had to adapt to the new situation [raising a kid], coming up with new ideas how to physically deal with the situation. I have discovered new abilities as I can do things that I would not think I could do. For example, it is difficult for me sitting on the floor with the kids so I put them on the table". The mothers stated that they talked all kinds of problems by means of the online sites they had created with other mothers in the same situation with respect to indirect coping strategy, and that they found solutions to the problems by each other's recommendations. The mothers stated that they can feel themselves stronger by means of face-to-face and online meetings from which they would get social and emotional support in the future, and they recommended to come together with mothers who are visually or hearing impaired. In the study, it was also mentioned that the children of mothers with physical disabilities were also affected by their mothers, which enabled them to learn how to deal with the difficulties faced at home, at school and around friends and helped them to get autonomy. In fact, it was stated that the mother feeling strong against her physical disabilities would enable her to be a good role model for her child. The mothers stated that the environment should be made more accessible for them, physical and emotional support should be provided, they should be provided with information on parental guidance and the rights they have, and financial support should be provided to meet the needs of their children.

In a study carried out in Norway in 2002, a woman with physical disability stated that she felt as if she was asexual individual before she became a mother and that she felt like a woman by the birth of her son.

She said that the pregnancy process she experienced and the fact that she became a mother made her more conscious as an individual despite her physical disabilities. Many women included in the study also used similar expressions, and they stated that the basic problem was that the society fit them in a mold and have prejudices for baby care instead of the obstacles restricting their activity levels. Another mother with physical disability stated that she has to change the diaper of her baby when there are other people, and that she does it behind closed doors because of the fear of thinking that she cannot hold her baby properly or think that she is doing something wrong. The mothers with physical disabilities who participated in the study said that their most powerful weapon against obstacles was their children. [10] The aim of the study of Powell et al. to describe the adaptive strategies used by mothers with physical disabilities during early motherhood. This qualitative study included semi-structured telephone interviews between January and March 2014 with US mothers with a range of physical disabilities who had a baby within the past 10 years. As a result of the research, four themes were obtained. Analysis revealed five broad themes indicating important adaptive parenting strategies for mothers with physical disabilities caring for infants and toddlers: They are as follows: (a) acquiring or modifying baby-care equipment, (b) adapting the home environment, (c) accessing information and supports, (d) developing communication strategies to facilitate safety, and (e) receiving assistance from others. The findings from the study are that in order for support and greater availability of equipment for physically disabled mothers, disabled mothers need information on these issues, and in addition, medical staff and social workers need training for disabled parents (Powell et al. 2019).

DISCUSSION

To become a mother is a very special feeling in order to experience by every woman. It is known that women with disabilities like every mother want to become mothers and that they fulfill their duties in society when they become mothers, but in the society, there is a perception that women with disabilities cannot give birth to children and cannot care for them (Karataş & Çifci, 2010). The issues on which women with children receive support for baby care from their social environment and healthcare personnel are the child's nutrition/breastfeeding, making baby sleep/soothing, giving a baby a bath, household chores, spiritual support, giving information, guidance and financial aid. However, it has been stressed that medical staff do not have the knowledge and experience to facilitate parental adaptation for mothers with disabilities and should receive training on this issue (Powell et al. 2019).

The most difficult issues experienced by mothers with physical disabilities, who were examined within the scope of the study, while meeting

the care needs of their babies are breastfeeding, transporting, make sleep of baby, giving baby a bath, and daily care routines (Shpigelman, 2014; Wint, Smith & Iezzoni, 2016). To support a mother with physical disability while breastfeeding her baby and to teach breastfeeding techniques correctly are important for the baby to get enough breast milk and for the continuation of the breast milk (Powell et al. 2018).

In the studies, it was also seen that mothers with physical disabilities had different experiences on how they adapt to care responsibilities and childrearing despite the similar difficulties and physical disabilities in the care of their children. When the studies were examined, it was stated that there were coping strategies developed by mothers in a similar situation and that they were attractive and guiding (Redshaw et al. 2013; Shpigelman, 2014; Tarasoff, 2017). In the studies examined, it was stated that there were problems in participating in social activities with their children. It was indicated that this situation can cause emotional problems and social isolation in mothers with physical disabilities but can turn into gains in mothers with disabilities who develop healthy coping methods (Shpigelman, 2014; Wint, Smith & Iezzoni, 2016).

CONCLUSIONS

The results presented in this review reveal that mothers with physical disabilities have problems in the care of their children.

It has been also observed that physical disability not only has disadvantages but also makes positive contributions to the life of both mother and child. In the studies, it is stated that healthcare professionals are not adequately equipped against individuals with physical disabilities and that mothers with disabilities have to cope with the negative perception that women with disabilities in the society cannot fulfill their maternity and caregiving roles, and that they cannot adequately prevent stigmatizing approach. The fact that researchers Although it is seen that mothers have developed strategies for the care of their children despite their disabilities, the relevant experts and healthcare professionals should have more supportive roles in the further development of these strategies. Carry out more studies in this regard in different cultures will also be guiding for healthcare professionals. It is also important that mothers share their experiences and coping strategies with the individuals in similar situations. It is thought that it is necessary to develop tools and materials to be used by mothers with physical disabilities in the care of their babies and children and to primarily study on the strategies related to the protection of children from accidents and their safety.

In the studies that examined, some of the mothers said that they received support from their husbands, their children and social environment with

respect to caring their babies and reported how important it was for them. It is suggested that the social support for mothers with physical disabilities especially by health professionals should be provided.

Health care providers with ethical and professional responsibilities should be involved in the care of all patients, the needs of the mother in the care of the patient with physical disability should be evaluated, and individualized care should be applied. The healthcare personnel should determine the needs of the mother and her family at home and should provide support and counseling so that the necessary arrangements can be made. It is necessary to collaborate with the institutions where mother and her family can receive social support, and the mother and her family should be supported without prejudice and questioning. It is necessary to take measures to eliminate the negative perceptions of the society about mothers with disabilities, and it should not be forgotten that they have expectations from life and right to become a parent like other women.

LIMITATIONS

The fact that it can not be find too many studies in this regard during the literature review covering the last 16 years, the fact that studies are from different cultures, and the different age ranges of the children of mothers with physical disabilities are limitations of the study.

DISCLOSURE

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

S. T and N.C., designed the study, collected, analyzed and interpreted the data, and approved the final manuscript. The assessment of the studies was made by two Authors (NC and ST) and evaluating the quality of the research was realized at the same time with its coding.

REFERENCES

Grue, L., & Laerum, K. T. (2002). 'Doing Motherhood': some experiences of mothers with physical disabilities. *Disability & Society*, *17*(6), 671-683. https://doi.org/10.1080/0968759022000010443

Karataş, K., & Çifci, E. G. (2010). Türkiye'de engelli kadın olmak: deneyimler ve çözüm önerileri. *Uluslararası Sosyal Araştırmalar Dergisi*, *3*(13), 147-153. Retrieved from: https://www.stgm.org.tr/sites/default/files/2020-09/turkiyede-engelli-kadin-olmak-deneyimler-ve-cozum-onerileri.pdf

Malouf, R., Henderson, J., & Redshaw, M. (2017). Access and quality of maternity care for disabled women during pregnancy, birth and the postnatal period in England: data from a national survey. *BMJ open*, *7*(7), e016757. https://doi.org/10.1136/bmjopen-2017-016757

Morrison, J., Basnet, M., Budhathoki, B., Adhikari, D., Tumbahangphe, K., Manandhar, D., ... & Groce, N. (2014). Disabled women′ s maternal and newborn health care in rural Nepal: A qualitative study. *Midwifery*, *30*(11), 1132-1139. https://doi.org/10.1016/j.midw.2014.03.012

Powell, R. M., Mitra, M., Smeltzer, S. C., Long-Bellil, L. M., Smith, L. D., Rosenthal, E., & Iezzoni, L. I. (2018). Breastfeeding among women with physical disabilities in the United States. *Journal of Human Lactation*, *34*(2), 253-261. https://doi.org/10.1177/0890334417739836

Powell, R. M., Mitra, M., Smeltzer, S. C., Long-Bellil, L. M., Smith, L. D., Rosenthal, E., & Iezzoni, L. I. (2019). Adaptive parenting strategies used by mothers with physical disabilities caring for infants and toddlers. *Health & social care in the community*, *27*(4), 889-898. https://doi.org/10.1111/hsc.12706

Redshaw, M., Malouf, R., Gao, H., & Gray, R. (2013). Women with disability: the experience of maternity care during pregnancy, labour and birth and the postnatal period. *BMC pregnancy and childbirth*, *13*(1), 1-14. https://doi.org/10.1186/1471-2393-13-174

Shpigelman, C. N. (2015). How to support the needs of mothers with physical disabilities?. *Disability and rehabilitation*, *37*(11), 928-935. https://doi.org/10.3109/09638288.2014.948133

Tarasoff, L. A. (2017). "We don't know. We've never had anybody like you before": Barriers to perinatal care for women with physical disabilities. *Disability and Health Journal*, *10*(3), 426-433. https://doi.org/10.1016/j.dhjo.2017.03.017

Wint, A. J., Smith, D. L., & Iezzoni, L. I. (2016). Mothers with physical disability: Child care adaptations at home. *American Journal of Occupational Therapy*, *70*(6), 7006220060p1-7006220060p7. https://doi.org/10.5014/ajot.2016.021477

World Health Organization (2011). World report on disability. Retrieved from: https://www.who.int/disabilities/world_report/2011/report.pdf

World Health Organization (2014). Disability - Draft WHO globaldisabilityaction plan 2014–2021: Better health for all people with disability - Report by the Secretariat. Sixty-Seventh World Health Assembly, A67/16; 2014. Retrieved from: https://apps.who.int/iris/bitstream/handle/10665/199544/9789241509619_eng.pdf;jsessionid=D51A156F91BD36D3479AE78C94A5D1C8?sequence=1

Table 1. Characteristics of the evaluated studies of experiences of mothers with physical disability

Table 1. Characteristics of the evaluated studies of experiences of mothers with physical disability

Author (s),year of publication, country, region	Name of the study	Methods[1]		Aim of the study	Main Results / Reached themes	Strengths, limitations
		Research design	The sample size, inclusion and exclusion criteria			
Powell et al.,2018,United States	Breastfeeding Among Women With Physical Disabilities in the United States	This study is a part of a case study with a larger scale and mixed-method examining the health needs and disabilities of the women with physical disabilities in the USA before, during and after pregnancy. The qualitative component analysis was performed to answer the research questions of the current study.	n=25 The inclusion criteria in the study include the presence of a physical disability that prevents walking or the use of hands and arms during pregnancy, having given birth in the last 10 years, being between 18 and 55 years old and being able to speak English.	In this study, it was aimed to investigate the factors that facilitate and prevent breastfeeding of mothers with physical disabilities.	As a result of the analyses performed, four themes that facilitated breastfeeding were reached (a) adaptation and equipment, (b) the use of breast pump (c) receiving physical help from the environment and (d) husband's support. Furthermore, five themes revealing obstacles to breastfeeding were found: (a) the lack of support (b) disability-related health problems (c) the lack of knowledge (d) difficulties related to milk production and (e) attachment difficulties.	The first one of limitations of the study is that having given birth in the last 10 years, the fact that more than half of the sample has children of 5 years or younger, the fact that only three of the children are 10 years old should be among the inclusion criteria. The second limitation is the facts that the data of the study are based on women's own reports, these reports have not been verified by health care providers, and the likelihood that participant acted biasedly to be socially desirable. Thirdly, there is the possibility of the bias of the participants in the selection of participants. Fourthly, research participants consisted of non-hispanic white, well trained and English speaking women. The negative situation in terms of this diversity does not reflect the heterogeneity of women with physical disabilities but is insufficient to represent the experiences of all women with physical disabilities. Fifthly, the difference of disability-specific experiences was not taken into account in the study due to the small number of disability types since the study sample included 25 women with nine different types of physical disabilities.

Redshaw, Malouf, Gao, & Gray, 2013, United States	Women with disability: the experience of maternity care during pregnancy, labour and birth and the postnatal period	144 foundations in the UK participated in the mail survey. Along with prenatal, delivery and postnatal care sections, the 12-page structured questionnaire covers access, information, communication and selection. The groups with and without disability were compared in descriptive and corrected analyses. Comparisons were performed separately for five disability groups: women with physical disability, sensory impairment, mental disability, learning disability and multiple disabilities	n=24155 Those who were under 16 years of age and those whose babies died were not included in the study.	To define the maternal health service for women with disabilities during pregnancy, delivery and postpartum period	The women with physical disabilities further used the prenatal and postnatal care services but they had fewer options for delivery services. Individuals with disabilities often have positive opinions about the care they receive and have stated that there is adequate access and participation, but breastfeeding rates are lower.	52% survey response rate, and no collection of data on socioeconomic condition or education level are among the limitations of these descriptive studies based on self-report. The number of samples is significantly greater in terms of reflecting individuals who define themselves as disabled despite the fact that women with disabilities are less likely to participate. This made it possible to compare the experiences of mothers with and without disabilities in the study. The differences in the care and perceptions of women with different types of disabilities were investigated
Wint, Smith &Iezzoni, 2016, United States	Mothers With Physical Disability: Child Care Adaptations at Home	Although there are two components of the large study, they constitute the analysis of national survey data and the qualitative descriptive analysis of in-depth individual interviews.	n=22 The women with physical disabilities at the age of 21 and over who have given birth in the last 10 years were included as participants in the study. The people interviewed are required to receiving support for moving (e.g., wheelchair, crutch, walking stick) or	This study describes the experiences of mothers with physical disabilities on newborn care, and how they were adapted to the home environment and care responsibilities.	Night care, giving a bath and the transport of baby were determined as the biggest difficulties. Adaptation actions include a dressing for transporting the baby, furniture adaptations for the mothers using wheelchairs,	The limitations of the study include especially relatively small and non-generalizing sample. When the limited racial and ethnic diversity of the sample are taken into account, it is not possible to argue about the obstacles that could disproportionately affect

			have significant difficulties while using the upper extremities.		and the help provided by caregivers.	minorities or special cultural practices for child care. Furthermore, women's discourses about their physical disabilities or home environments were not controlled. Nevertheless, women's consistent and authentic expressions during 2-hour interviews indicate that they can convey their experiences properly.
Tarasoff, L. A, 2017, Kanada	We don't know. We've never had anybody like you before": Barriers to perinatal care for women with physical disabilities	In-depth interviews were conducted with 13 women with disabilities who gave birth in the last 5 years. The constructivist-based theoretical approach was used in the analysis of data.	n=13 The inclusion criteria in the study consisted of defining yourself as a woman with physical disability or activity limitation, living in Ontario, being 18 years and over, having given birth in the last 5 years, and being able to speak English.	This qualitative study attempts to find out the perinatal care experiences of women with physical disabilities in a state in Canada and its consequences by highlighting the determination of care barriers.	Five themes that posed an obstacle to care were determined based on the analysis of interviews conducted with the participants: (1) Inaccessible care environment, (2) Negative attitudes, (3) Lack of knowledge and experience, (4) Lack of communication and cooperation between caregivers and (5) Misunderstanding of disability and disability-related needs.	The results of the study are limited since they do not reflect the experiences of all physically disabled women in Ontario or Canada. Within the scope of the results, as in other qualitative studies, most of the sample consisted of married, white, heterosexual, well-educated women with physical disabilities residing in large city centers. Therefore, the experiences of the participants do not represent all women with physical disabilities, especially women with low income or different races living in rural and remote areas.
Morrison et al., 2014, Nepal	Disabled women's maternal and newborn health care in rural Nepal: A qualitative study	A qualitative methodology was used in semi-structured interviews.	n=27 mother n=5 midwife The participation of married women who had given birth in different parts of the region in the last 10 years and who had different disabilities was aimed. Moreover, caregivers working in maternal health services were also interviewed	There is little evidence on the access of women with disabilities to maternal and neonatal health services in low-income countries, and few studies refer to women with disabilities to understand their experiences in the care and seeking care. The study investigates the maternal and neonatal care experiences of women with disabilities in rural Nepal.	Married and disabled women consider pregnancy and births as normal and prefer birth at home. The shortcomings of quality, cost and family support are still present in the same way for women with disabilities as in their peers without disabilities. As a result of interviews conducted with health care professionals, was determined that women with disabilities felt they did not have sufficient preparation to meet maternal	The qualitative sample in the study was limited to women participating in previous studies, and therefore, only women who gave a birth between 2001 and 2008 and had disabilities were included. Most of the women gave birth at home, and therefore, the institutional experiences of women with disabilities could not

					health needs.	be identified. Moreover, the access to care of unmarried women with disabilities could not be explained.
Malouf, Henderson, & Redshaw, 2017,United States	Access and quality of maternity care for disabled women during pregnancy, birth and the postnatal period in England: data from a national survey	Secondary analysis was performed on data obtained from the 2015 national survey on women's maternal health services care experiences. Descriptive and adjusted analyses were performed for five disability groups: comparisons were made with physical disability, sensory impairment, mental disability, learning disability and multiple disability, and the answers of women without disabilities.	The questionnaires completed by a total of 20.094 women had an available response rate of 41.2%. Women with disabilities represent 9.5% of the total sample (1958). There are the groups defining themselves with different types of disability among the women who gave birth three months ago. The exclusion criteria from the study consisted of only women whose babies died and the women who were under the age of 16 during the last birth	More women with disabilities become mother day by day, however, the care of these women is rarely the focus of quantitative research. This study aims to investigate the access and quality of maternal health service for women with different disabilities.	The results obtained reveal some shortcomings related to interpersonal aspects of women in care: these are listed as communication, feeling of being listened and supported, being involved in the decision making process, and having a reliable and respectable relationship with clinical staff. The women from all disability groups demand more help in terms of postnatal contacts and child's nutrition.	The participation of all institutions providing maternal health services in the UK, and the answers of a significant number of women with different types of disabilities are among the strengths of the study. Moreover, women's perspectives on their care are also mentioned. All data in the questionnaire were reported by individuals within 3 months after the birth and retrospectively collected. This situation may question the validity of the answers given as far as remembered from pregnancy.
Shpigelman, 2015, İsrail	How to support the needs of mothers with physical disabilities?	The semi-structured in-depth interview method was used.	n=17 Three criteria were determined for the women who were included in the sample: (a) Being a Jewish woman (b) Being 18 years and over (c) having a physical (action) disability.	The objectives of this study are as the following: (1) to understand and explain the difficulties faced by women with physical disabilities during maternity processes; (2) to understand and define their strengths and (3) to produce a list of the support that health professionals and policy makers need to apply to meet the needs of these mothers	The results of the study have revealed the physical and mental difficulties women face during their motherhood experiences. Although these difficulties exist, while the women were evaluating motherhood in the context of disability, they stated that being a mother with disability helped them to develop their own positive disabled identity, to create a framework for mutual dependence and to be resistant. The disabilities of mothers also affected the children and enabled them to learn how to deal with the difficulties. The mothers recommend that the environment should be made more accessible for them,	The results of the study are based only on mothers' perspectives. Children's and fathers' perspectives should also be included in the studies to be carried out in the future.

					physical and emotional support should be provided, they should be provided with information on parental guidance and the rights they have, and financial support should be provided to meet the needs of their children.	
Grue and Lærum, 2002, Norway	'Doing Motherhood': Some experiences of mothers with physical disabilities	The study was conducted through semi-structured interviews.	n=30 All participants gave birth after 1976. Some of the participants are married and some of them live alone with their children. Half of the families of the selected participants have one child and the other half have two or three children.	The main aim of the study is to clarify the experiences on raising children as an individual with disability in today's society. The study basically has two major objectives although more comprehensive. The first of these is to provide information and to get ideas about the lives of women with physical disabilities. The second objective is to present a general discussion on disability and social integration.	To explain briefly, the women in the study are generally accepted as those who take care (disability discourse) instead of giving care (motherhood discourse) by professionals. However, although the stories told by 30 women who willingly shared their experiences impose restrictions on the activity levels of disability effects, the main problem is other people's disbelief in them (disability) and the phenomenon of "fitting in a mold" which is socially and financially present for motherhood in today's Norwegian society.	Limitations are not specified.
Powell RM et al., 2019, United States	Adaptive parenting strategies used by mothers with physical disabilities caring for infants and toddlers	This qualitative study included semi-structured telephone interviews	n=25 Between January and March 2014 with US mothers with a range of physical disabilities who had a baby within the past 10 years	The purpose of this study is to describe the adaptive strategies used by mothers with physical disabilities during early motherhood.	As a result of the analyses performed, (a) acquiring or modifying baby-care equipment, (b) adapting the home environment,(c) accessing information and supports, (d) developing	Limitations are not specified.

communication strategies to facilitate safety, and (e) receiving assistance from others.				