ISSN: 2149-7893 E-ISSN: 2536-507X

CYPRUS JOURNAL OF MEDICAL SCIENCES

Indexed in the Web of Science

Volume: 10 Issue: 1 February 2025

REVIEWS

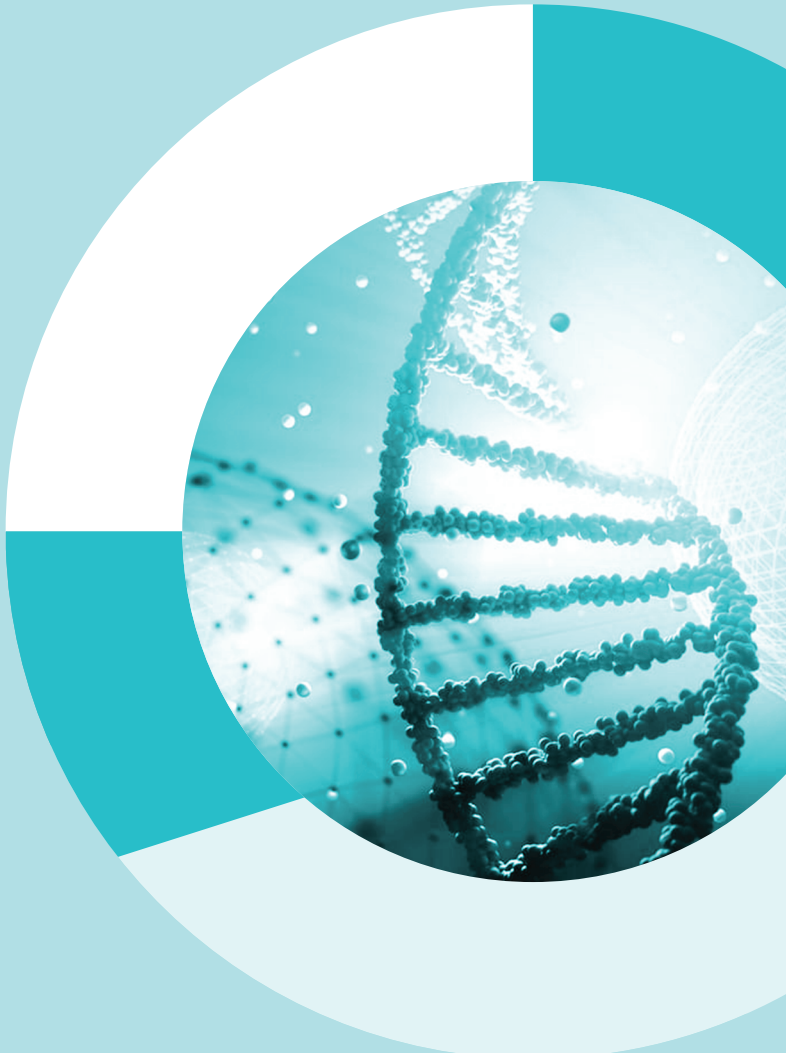
- **Avocado (*Persea americana*) and Potential Anticancer Effects**
  Dayı et al.; Nicosia, Kyrenia, North Cyprus
- **Galectins: An Amazing Marker and a Potential Therapeutic Target**
  Tınazlı and Bakhtiyarova; Nicosia, North Cyprus
- **Implant Scans Accuracy Factors**
  Çise Özal; Morphou, North Cyprus

ORIGINAL ARTICLES

- **The Therapeutic Effect of Extracts of *J. Drupacea* Fruit Against COPD in Rats**
  Akbulut et al.; Konya, İzmir, Türkiye
- **The Impact of Grand Multiparity on Perinatal and Neonatal Outcomes**
  Atlıhan et al.; İzmir, Türkiye
- **Gender Authorship Trends of Ophthalmology Review Articles**
  Delil Özcan; İstanbul, Türkiye
- **Comparison of Different Fixation Methods for Sagittal Split Ramus Osteotomy**
  Özlü et al.; Ankara, Türkiye
- **The Association of Handgrip Strength and Fatigability with Cognitive Function**
  Gürsoy Karaman and Koç; Nicosia, North Cyprus
- **The Role of Contact Alergens on Seborrheic Dermatitis**
  Ünsal et al.; Ankara, Türkiye; Famagusta, Nicosia, North Cyprus
- **Awareness About Dental Implants**
  Sultanoğlu et al.; İstanbul, Türkiye; Nicosia, North Cyprus
- **Effects of Melatonin on Intestinal Tissue Injury in Sepsis**
  Ateş et al.; İstanbul, Türkiye

CASE REPORTS

- **A Scenario of Mature Florid Cemento-Osseous Dysplasia in the Mandible Accompanied by Secondary Chronic Osteomyelitis**
  Efeoğlu et al.; Ankara, Türkiye
- **Iatrogenic Botulism: A Case Report**
  Özlem Önder; Nicosia, North Cyprus



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E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr **Publisher Certificate Number:** 14521

Publication Date: March 2025
E-ISSN: 2536-507X
ISSN: 2149-7893
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The journal is published electronically.
Owner: Ahmet Özant on behalf of Cyprus Turkish Medical Association
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CYPRUS
JOURNAL OF MEDICAL SCIENCES
Indexed in Web of Science
Volume: 10 | Issue: 1 | February 2025

CONTENTS

REVIEWS

1 **Avocado (*Persea americana*) and Potential Anticancer Effects: Do the Effects Suppress Carcinogenesis?**
Taygun Dayı, Serpil Özsoy, Aysel Yaren Bozkurt; Nicosia, Kyrenia, North Cyprus

7 **Galectins: An Amazing Marker and a Potential Therapeutic Target**
Mehtap Tınazlı, Gaukhar Bakhtiyarova; Nicosia, North Cyprus

13 **Guide to Optimizing the Accuracy of Intraoral Implant Scans: A Review Article**
Çise Özal; Morphou, North Cyprus

ORIGINAL ARTICLES

22 **The Therapeutic Effect of Flavan-3-Ols from Organic Extracts of *Juniperus drupacea* Fruit Against Elastase-Induced Chronic Obstructive Pulmonary Disease in Rats**
Hatice Feyza Akbulut, Hüsamettin Vatansev, Bayram Çolak, Hülya Özdemir, Zeliha Esin Çelik, Mehmet Akbulut; Konya, İzmir, Türkiye

33 **The Impact of Grand Multiparity on Perinatal and Neonatal Results in Females Over 35 Years of Age**
Ufuk Atlıhan, Onur Yavuz, Hüseyin Aytuğ Avşar, Can Ata, Selçuk Erkılınç, Tevfik Berk Bildacı; İzmir, Türkiye

38 **Gender Authorship Trends of Review Articles in the Ophthalmology Literature from 2000 to 2022**
Delil Özcan; İstanbul, Türkiye

44 **Comparison of the Effects of Different Fixation Methods on Fragments and Temporomandibular Joint in Sagittal Split Ramus Osteotomy Applied to Patients with Mandibular Asymmetry Using Three-Dimensional Finite Element Analysis**
Mert Özlü, Serpil Altundoğan, Seray Öztürk Kavuncu; Ankara, Türkiye

51 **Association Between Handgrip Strength and Fatigability and Cognitive Performance in Adults Aged 65 and Older**
Nazemin Gürsoy Karaman, Emine Koç; Nicosia, North Cyprus

58 **The Role of Contact Allergens on Facial Seborrheic Dermatitis**
Gülfem Ünsal, İçim Kömürcügil, Nermin Karaosmanoğlu; Ankara, Türkiye; Famagusta, Nicosia, North Cyprus

62 **Questioning The Awareness of Partially Edentulous Patients About Dental Implants and Implant Supported Dental Prostheses**
Elifnur Güzelce Sultanoğlu, Bedirhan Dökülmez, Meryem Hürbağ; İstanbul, Türkiye; Nicosia, North Cyprus

70 **Relationship Between Antioxidant Enzyme Chains and Trace Elements and Electrolytes Levels and Potency of Melatonin on Sepsis-Induced Small Intestine Injury**
Gülten Ateş, Hatice Yorulmaz, Elif Özkök, Şule Tamer, İbrahim Ertuğrul Yalçın, Vakur Olgaç; İstanbul, Türkiye

CASE REPORTS

79 **Secondary Chronic Osteomyelitis of the Mandible and Concomitant Mature Florid Cemento-Osseous Dysplasia: A Case Report**
Serhat Efeoğlu, Eray Aktay, Elif Polat Balkan; Ankara, Türkiye

83 **Latrogenic Botulism Following Botulinum Toxin Injection in Palmar Hyperhidrosis: A Case Report**
Özlem Önder; Nicosia, North Cyprus

REVIEW

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-34
Cyprus J Med Sci 2025;10(1):1-6

Avocado (*Persea americana*) and Potential Anticancer Effects: Do the Effects Suppress Carcinogenesis?

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Abstract

Avocados have become one of the most popular fruits today and contain many anticancer nutrients, such as vitamins A-C-E, polyphenols, carotenoids, glutathione, monounsaturated and polyunsaturated fatty acids, and dietary fiber. Inhibiting cancer cell proliferation, preventing metastasis, and eliminating cancer cells are important goals for cancer treatment. Because of their content, avocados can potentially stimulate apoptosis, inhibit cell differentiation, proliferation, and carcinogen absorption, and reduce reactive oxygen species and inflammatory cytokines. Limited human studies, *in vivo* studies, and *in vitro* studies that examined the effects of each type and part of avocado are included in this narrative review. Some *in vitro* studies have demonstrated the anticancer effects of avocado pulp, peel, and seeds because of their functional nutrient contents that affect apoptotic protein expression (caspase-3,-6,-7,-9), Bax, Bcl-2, and Bcl-xL trigger cell cycle arrest (cellular senescence), superoxide dismutase enzyme activity (antioxidant), and suppress proinflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1 beta and 6. Consuming one-half avocado/day [~68 g (edible part)] supports beneficial nutrient intake. Although some potential anticancer effects of avocados have been demonstrated in *in vitro* studies, more *in vivo* studies are needed to increase the level of evidence.

Keywords: Avocado, *Persea americana*, cancer, anticancer effects, inflammation, proliferation

INTRODUCTION

The avocado (*Persea americana*) is a tropical fruit that grows almost everywhere in the world but originated in Mexico and South America. It is from the *Lauraceae* family and is known as the "butter fruit", "alligator pear", "ahuacate", and also "avocato". There are 50 different types of avocado, and the most well-known and sold types of avocado are the "Fuerte" and "Hass". While the "Fuerte" type is bright, green, and rough, the "Hass" is purple.[1] Due to its beneficial properties and popularity, avocado has been used in both food and cosmetic industries.[2] Avocados contain many beneficial nutrients and substances, so every age group can consume this fruit. Avocado is a source of potassium, magnesium, phosphorus, non-heme iron, vitamins A, B, C, E, and K, dietary fiber (DF), and phytochemicals. Furthermore, the carbohydrate (CHO) content of this fruit is lower than that of many other fruits.[3,4] Some important studies in the current literature have demonstrated the beneficial effects of avocado on cognitive function, diabetes mellitus, cardiovascular health, and cancer.[5-8] As a potential antioxidant agent, these bioactive substances can reduce oxidative stress and inflammation.[1,4] Moreover, avocado phytochemicals have the potential to inhibit cell proliferation and stimulate apoptosis; thus, they have potential benefits against cancer development.[9] With this in mind, this study aimed to review the effects of avocados on carcinogenesis. Thus, the methodologies and results of limited human, *in vivo,* and *in vitro* studies that searched for the proapoptotic, antioxidant, anti-inflammatory, and antiproliferative effects of each type and part of avocado are included in this narrative review article.

To cite this article: Dayı T, Özsoy S, Bozkurt AY. Avocado (*Persea americana*) and potential anticancer effects: do the effects suppress carcinogenesis?. Cyprus J Med Sci. 2025;10(1):1-6

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Received: 30.04.2024
Accepted: 23.07.2024
Publication Date: 14.03.2025

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Nutritional Content of Avocados

The macronutrient and micronutrient contents of avocados are related to factors such as type, growing model, consumed portion, and maturation time. Although there has been an increase in the production and sale of the "Hass" avocado in recent years, Tremocoldi et al.[10] reported that total bioactive compounds are better in the peel and seeds of the "Fuerte" type than the "Hass". The pulp of avocado has a higher water, fat, and ash content than the peel and seed. In contrast, the protein content of the seeds was better.[11] The content of the edible part of the "Hass" is 72% water, 10% fat, 7% CHOs, 2% protein, and 5% DF. Both soluble and insoluble types of DF form a significant part of the CHO content of avocados. The fat content is higher than that of the other fruits, and 71% of this content is monounsaturated fatty acids (MUFA), 13% is polyunsaturated fatty acids (PUFA), and 16% is saturated fatty acids. Although the official serving size of an avocado is 50 g, some micronutrients and nutritional substance content are present in 68 g. This fruit is a good source of fat-soluble vitamins [A (43 µg/68 g); E (1.3 mg/68 g); K (14 µg/68 g)], as well as folate (60 mg/68 g), vitamin C (6 mg/68 g), potassium (345 mg/68 g), and magnesium (19.5 mg/68 g). In addition, although avocado is not as rich in phytochemicals as red fruits and citrus, it contains some antioxidant substances. The antioxidant capacity of avocado is primarily related to its lutein and zeaxanthin (185 µg/68 g), and β (beta) carotene (43 µg/68 g) contents.[2,9,12] The nutritional content of avocados is presented as a graphic abstract in Figure 1.

Cancer

Cancer is one of the most important causes of mortality and morbidity worldwide. The World Health Organization (WHO) reported 19.3 million cancer cases and 10 million deaths worldwide in 2020. The WHO defines cancer as uncontrolled cell growth and progression of mutated cells. The ability of mutated cells to metastasize and migrate to different tissues and organs increases the burden of cancer on patients and the healthcare system. Cancer is the second leading cause of mortality after cardiovascular diseases.[13-15] Conventional cancer therapies are based on suppressing uncontrolled cell growth, preventing metastasis, and eliminating cancerous cells. Accordingly, chemotherapy, radiotherapy, and surgical intervention are the main types of cancer treatment. Furthermore, programmed cell death is an important intracellular defense mechanism for maintaining homeostasis and suppressing carcinogenesis.[16]

Many risk factors may cause cancer development, which can be classified as modifiable or unmodifiable (gender, age, genetic factors, etc.). Factors related to lifestyle behaviors are modifiable, such as nutritional, physical, and sexual habits, alcohol consumption, and smoking.[17,18] Poor diet diversity, low food quality, and hygiene, high refined grains, alcohol consumption, fast food consumption, and low fruit, vegetable, and legume consumption are some of the nutrition-related cancer risk factors.[19] Fruits and vegetables in particular are high in DF and antioxidants, such as A-C-E vitamins, as well as phytochemicals with anticancer properties.[20] Because of the nutritional elements and bioactive nutritional components such as vitamin C, phenolic compounds, and carotenoids, the anticancer effects of avocados have been the subject of research in recent years.[9]

Potential Anticarcinogenic Effects of Avocado

Although there are limited human studies on the effects of avocado consumption on cancer pathogenesis, some *in vitro* studies have been conducted on different cancer cell lines (Table 1).

Potential Pro-Apoptotic Effects

While total fat, saturated fat, trans-fatty acids, and omega-6 fatty acids are associated with increased cancer risk, omega-3 and plant-based MUFAs are related to lower risk.[21] The highest fat content is found in avocados. Most of the fat content was MUFA (71%). In a case-control study involving 209 men with newly diagnosed prostate cancer and 226 healthy men, dietary MUFA intake was associated with a lower prostate cancer risk. The main dietary MUFA source was avocado.[22] Ericsson et al.[8] conducted a cohort study to evaluate the effects of avocado consumption on cancer development in the United States population.

The consumption of a weekly serving of ≥1 avocado reduced overall colorectal, lung, and bladder cancer risks in men.[8] In a randomized controlled trial, daily avocado consumption (175 g for men, 140 g for women) resulted in lower fecal bile acid concentrations, a better abundance of bacteria capable of fiber fermentation, and better fecal short-chain fatty acids, which are known as pro-apoptotic and anti-inflammatory agents in the human body.[23] Apoptosis is a cell death mechanism in the human body. This process occurs regularly to provide homeostasis between cell formation and death. Because apoptosis is a process of sequential cell death, it is also known as programmed cell death.[24] Guzmán-Rodríguez et al.[25] reported that the application of *Persea americana var drymifolia* (PaDef) extract induced apoptosis in the MCF-7 breast cancer cell line. It has been noted that the application of PaDef for 48 h increased the expression of cytochrome c, APAF 1, caspase-7, and -9, decreased the mitochondrial membrane potential, and induced apoptosis by increasing mitogen-activated protein kinase p38 phosphorylation.

Potential Antiproliferative Effects

Cell proliferation is necessary for healthy growth and development and also for being healthy in living. However, sustaining cell proliferation signaling is a hallmark of cancer, which is known as uncontrolled cell growth.[26] In addition to apoptosis, another attractive intracellular pathway that plays an anticarcinogenic and antiproliferative role is cellular senescence. Cellular senescence, a natural process of embryogenesis, is triggered by DNA damage, exposure to oncogenic stimuli, stress, and trauma, and causes the cell cycle to arrest irreversibly in the G1 and G2 phases. The irreversible cell cycle arrest of differentiated

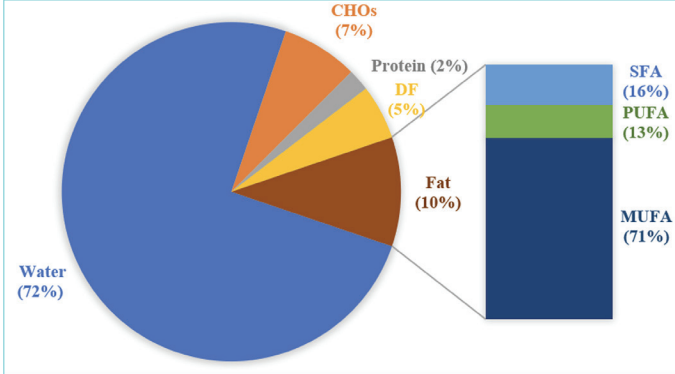


Figure 1. Macronutrient content of "Hass" type of avocado (prepared by the authors, based on the references in the "Nutritional content of avocado" section).

CHOs: Carbohydrates, SFA: Saturated fatty acids, DF: Dietary fiber, PUFA: Polyunsaturated fatty acids, MUFA: Monounsaturated fatty acids.

Table 1. Literature on avocado and its anticancer effects

Human studies

Aim of the study	Material and Methods	Results	References
The effects of all fatty acids on prostate cancer development in Jamaica.	This was a case-control study. A total of 209 prostate cancer patients and 226 healthy men (40-80 years old) were included. Dietary fatty acid intake was determined using a FFQ.	Consuming avocado was found to be the main MUFA source in men from Jamaica. Other fatty acids were not associated with prostate cancer, whereas higher dietary MUFA intake was associated with a lower prostate cancer risk.	22
The association between avocado consumption and cancer development in the United States.	45,289 men in the HPFS (1986-2016) and 67,039 women in the NHS (1986-2014) participated. Consumption of avocado was determined by the FFQ every four years.	While ≥1 serving/week of avocado consumption was found associated with lower risk in overall cancer types in men, this relationship was not significant in women.	8

Animal studies

Aim of the study	Material and Methods	Results	References
Chemopreventive mechanisms of hydroethanolic extracts from avocado pulp and seeds in rats with liver cancer.	Oral intake of 50 mg/kg hydroethanolic extract daily for 20 weeks.	The increase in lipid peroxidation was prevented. Low glutathione peroxidase, glutathione-S-transferase, and superoxide dismutase activities were improved in liver tissue (*antioxidant*). COX-2 and NF-κB expression levels decreased (*anti-inflammatory*). The levels of the suppressor proteins p53 and Bax increased (*proapoptotic*).	39
Evaluation of the effects of avocado oil on inflammation in male C57BL/6J mice.	Oral intake of 4 mL/kg of avocado oil was provided for 90 days.	TNF-α and IL-1β levels were decreased by avocado oil supplementation.	37

***In vitro* studies**

Cancer cell types	Materials	Results	References
Esophageal squamous cell carcinoma and colon adenocarcinoma.	Ethanol, ethyl acetate, petroleum, and chloroform extracts of avocado fruit pulp.	Twenty µg/mL ethanol extract exerted a significant inhibitory effect on cancer cell growth in related cancer cell lines.	40
Liver, breast, and colorectal cancer cells.	Acetogenin-rich extract of the avocado fruit pulp.	8.1 µg/mL of the acetogenin-rich extract exerted anticancer effects on liver carcinoma, 52.1 µg/mL on breast cancer, and 11.3 µg/mL on colorectal cancer cells.	41
Melanoma cell line.	Nanoemulsion containing a procyanidin-rich extract of avocado peels.	The nanoemulsion increased preferential cytotoxicity and decreased migration (antimetastatic effect) in the melanoma cell line.	42
Colon and liver cancer cell lines.	The edible parts of the avocado and seed extracts.	The avocado seed extract was found to be more effective than the edible extract. Furthermore, the sterol compound was even more effective. In a dose-dependent manner, both extracts exert anti-inflammatory and anticancer effects.	4
Determination of the antioxidant effect of avocado.	Avocado seed, peel, and pulp extracts (from 60 different ready-to-eat Hass varieties of avocados).	The peel extract had more phenols (gallic acid equivalents) and flavonoids (quercetin equivalents) components than the seed extracts. The highest antioxidant activity was observed in the peel extract. The maximum antioxidant capacity was observed at a 1 mg/mL concentration for the combination of avocado peel extract (61%) and nisin (39%).	43
Colon cancer cell line.	The lipid-rich extract of Mexican avocado.	Lipid-rich extracts at 100 and 150 µg/mL lipid-rich extract decreased cell growth. Twenty eight µg/mL stimulated apoptosis via activation of caspases -8 and -9.	44

Table 1. Continued

Breast and liver cancer cell lines.	Triterpenoid of avocado seeds (isolated from ethanol extract).	The extracts and the isolated triterpenoid compound exhibited significant cytotoxic activity. 62 µg/mL triterpenoid isolate inhibited cell proliferation in the breast cancer cell line, and 12 µg/mL exerted the same effect in the liver cancer cell line.	[29]

FFQ: Food frequency questionnaire, MUFA: Monounsaturated fatty acids, HPFS: Health professionals follow-up study, NHS: Nurses' health study, COX-2: Cyclooxygenase-2, NF-κB: Nuclear factor kappa-B, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1 beta.

cancer cells constitutes the potential tumor-suppressive property of cellular senescence.[27] Dabas et al.[28] reported that in the LNCap prostate cancer cell line, *Persea americana* seed extract suppressed the expression of cyclin-dependent kinases 1 and E_2, which are responsible for the regulation of the cell cycle, and showed antiproliferative effects by stopping the cell cycle in G0/G1 phase. Moreover, Abubakar et al.[29] showed that the application of 62 µg/mL and 12 µg/mL of triterpenoid extracted from *Persea americana* seed on MCF-7 (breast cancer) and HepG2 (liver cancer) cancer cell lines, respectively, had cytotoxic effects; thus, triterpenoid can suppress the proliferation of cancer cells and exert anti-cancer effects.

Potential Anti-Oxidant Effects

It is known that reactive oxygen species (ROS) such as; superoxide radicals, hydroxyl radicals, and hydrogen peroxide, contribute to carcinogenesis by causing DNA breaks, mutations, and the proliferation of mutated cells. The intracellular antioxidant defense system plays a crucial role in preventing the damage that can be caused by ROS species. The best-known intracellular antioxidant defense mechanism is superoxide dismutase (SOD) activity. SOD catalyzes the conversion of superoxide radicals into less reactive substances such as oxygen and hydrogen peroxide. In addition to its proapoptotic and antiproliferative effects, some *in vitro* studies have reported that avocado seed extract increases SOD activity, thereby affecting the intracellular antioxidant defense system. Kupnik et al.[30] examined the antimicrobial and antioxidant effects of avocado seeds and reported that avocado seed ethanol extract has a higher antioxidant capacity, especially due to its polyphenolic content. Athaydes et al.[31] examined the protective effects of *Persea americana mill* seed extract against gastric ulcers. According to the experimental results of the study, the *Persea americana mill* seed extract has a potential protective effect against gastric mucosal injuries by increasing SOD enzyme activity as well as mucus synthesis.

Additionally, a study performed by Alkhalaf et al.[4] examined the antioxidant properties of *Persea americana* fruit and seed extract on HT 116 (colon cancer) and HepG2 (liver cancer) cancer cell lines and found that *Persea americana* had an antioxidant effect in both extracts. Moreover, although there is limited evidence in the current literature, avocation B, the odd-numbered carbon lipid of avocado, has potential anti-cancer effects as a cytotoxic agent and fatty acid oxidation inhibitor.[32]

Potential Anti-Inflammatory Effects

The bidirectional relationship between inflammation and cancer is known. Although inflammation, especially chronic inflammation, can cause cancer, cancer cells can increase the levels of proinflammatory cytokines in the human body. Inflammation can stimulate nearly all cancer pathways.[33] There are some human studies on the effects of avocado consumption on inflammatory biomarkers. Zhang et al.[34] conducted a study with overweight and obese adults and assessed their high sensitivity to C-reactive protein (hs-CRP), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) after consumption of the Hass-type avocado for 12 weeks (one avocado/day, ~168 g pulp). Although they did not observe significant changes in IL-6, MCP-1, and ICAM-1 expression, significant differences in hs-CRP and VCAM-1 expression were observed. A pilot study added a Hass type of avocado to hamburgers and compared the effects of hamburger consumption with and without avocado. This study showed that 68 g of avocado consumption preserved the nuclear factor kappa B (NF-κB) light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα), thereby reducing the activation of the NF-κB pathway.[35] Another study highlighted the anti-inflammatory effects of *Persea americana* seed extracts. Lipopolysaccharide-stimulated RAW264.7 murine macrophages were treated with avocado seed extracts for 24 hours and reduction of IL-6, IL-1β, and tumor necrosis factor-alpha (TNF-α) were observed. In addition, prostaglandin E_2 production was inhibited by the avocado extract.[36] Additionally, avocado oil supplementation (4 mL/kg, 90 days) was found to be effective for reducing proinflammatory cytokine levels (TNF-α, and IL-1β) in male C57BL/6J mice.[37]

The human, *in vivo*, and *in vitro* studies conducted to determine the anticancer effects of avocados are summarized in Table 1. In addition, all these potential effects are illustrated in Figure 2. In addition to the pulp and peel parts of avocado, the seed is another study area. Although the literature suggests that avocado seeds may help prevent cancer and exert potent anticancer effects, the "California Avocado Commission" claims that there is insufficient data on the safety of seed consumption for human health.[38]

CONCLUSION

An avocado, one of the most popular fruits in recent years, contains many health benefits. As the present review shows, due to the

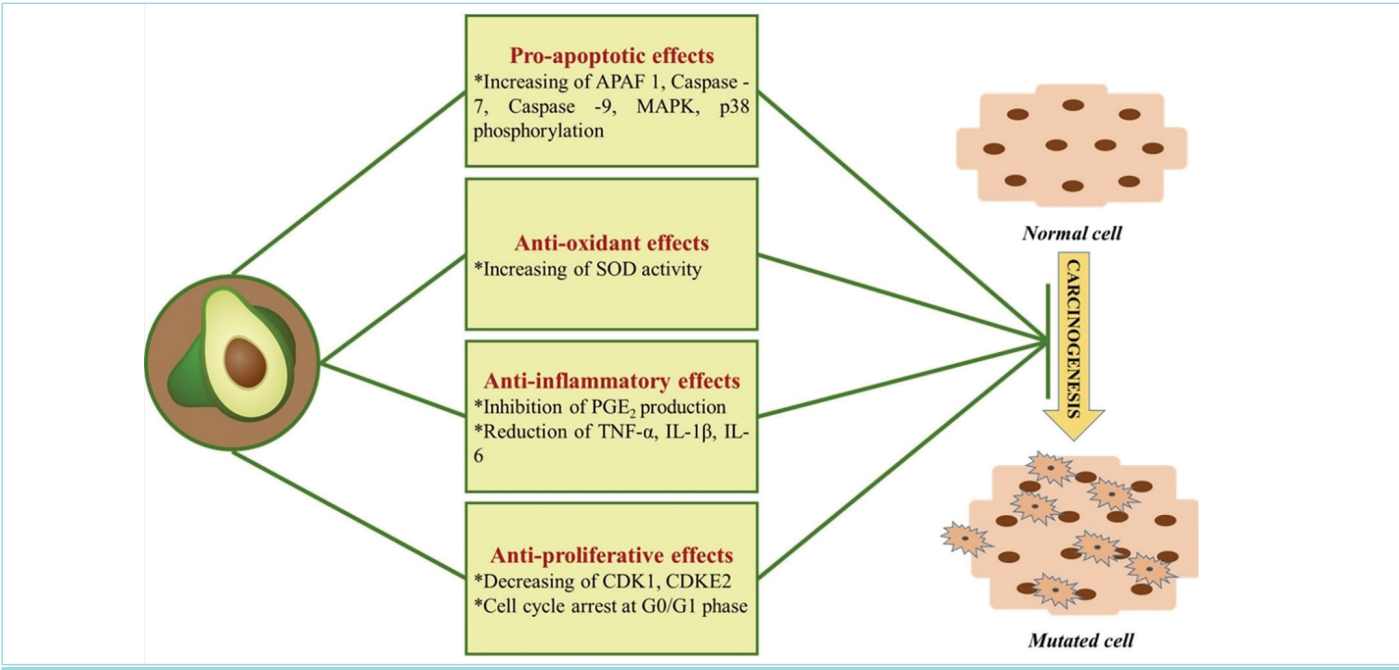


Figure 2. Potential cancerogenesis suppressive effects of avocado (prepared by the authors, based on the references in the "Potential anticarcinogenic effects of avocado" section and also Table 1).

APAF: Apoptotic protease activating factor, MAPK: Mitogen-activated protein kinase, SOD: Superoxide dismutase, PGE_2: Prostaglandin E_2, TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta, CDK: Cyclin-dependent kinases.

beneficial nutritional content of the avocado, its extracts have shown proapoptotic, antioxidant, anti-inflammatory, and antiproliferative effects in many current *in vitro* studies. The limited number of human studies showing that avocado consumption between once/day to once/a week showed anticancer effects. Thus, avocados can potentially suppress carcinogenesis. Nearly half of a portion of avocado (~68 g) can support daily DF, antioxidant vitamins, phytochemicals, MUFA, and PUFA intake. Although some important *in vitro* studies have shown that avocados have anticancer effects, the evidence remains minimal, and the number of *in vivo* studies is still limited.

Consequently, numerous *in vitro* studies on different cancer cell lines and *in vivo* studies are necessary. In particular, there is a need for studies designed to examine the pulp side (the most edible part) of avocado consumption and cancer development in humans.

MAIN POINTS

- Avocado, which has become popular in recent years, has the potential to be effective in carcinogenesis because of its high nutritional composition and high polyphenolic content.
- *In vitro* studies have indicated that due to its rich polyphenolic content and nutritional matrix, the avocado extract may exert a proapoptotic effect by affecting caspases involved in apoptosis.
- Avocado extracts exhibited anti-inflammatory, antioxidant, and antiproliferative effects in some *in vitro* studies.
- Some human studies have shown the potential anticancer and antiinflammatory effects of avocado consumption.

FOOTNOTES

Acknowledgments: The authors thank Mr. Simon Thompson (an English language proofreader) for proofreading this review article.

Authorship Contributions

Concept: T.D., S.Ö., A.Y.B., Design: T.D., S.Ö., Analysis and/or Interpretation: T.D., S.Ö., Literature Search: T.D., S.Ö., A.Y.B., Writing: T.D., S.Ö., A.Y.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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REVIEW

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-30
Cyprus J Med Sci 2025;10(1):7-12

Galectins: An Amazing Marker and a Potential Therapeutic Target

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Abstract

Galectins (Gal) are a wide group of proteins expressed in various cells, and they are particularly known for their ability to recognize and bind carbohydrates. We present current evidence showing that Gal play roles in both acute and chronic inflammation pathways. They participate in extracellular events such as cell proliferation, cell adhesion, bacterial colonization, apoptosis, oncogenesis, chemotaxis, embryogenesis, oncogenesis, and differentiation. To date, 15 Gal have been identified. The most studied enzyme is galectin-3 (Gal-3). This lectin group is defined as a protein that activates oxidative stress and inflammation. Gal-3 is a multifunctional protein that participates in various biological processes, including proliferation, differentiation, angiogenesis, cancer progression, and metastasis. Additionally, Gal play an important role in fibrogenesis. Fibrotic diseases are seen in the lungs, heart, kidneys, and liver, and they cause permanent organ damage and loss of function. For these reasons, Gal are considered potential inflammatory markers. As the structure and functions of Gal become clearer, new molecules are expected to facilitate the diagnosis and treatment of vascular complications associated with inflammation, autoimmune diseases, tumor spread, cancers, allergic events, diabetes, and hypertension. Here, we aimed to review the most recent literature to draw attention to the importance of Gal and to remind patients of their potential benefits in diagnosis and treatment.

Keywords: Galectins, inflammation, fibrosis, biomarker, Gal-3

INTRODUCTION

Galectin-3 (Gal-3), a member of the galectin (Gal) protein family, has garnered significant attention in recent years owing to its diverse roles in various physiological and pathological processes. This multifunctional protein interacts with beta-galactoside-containing glycoconjugates, influencing cell-cell and cell-matrix interactions, as well as signaling pathways. Additionally, recent research has been conducted to understand the complex roles of fibrosis, cardiovascular disease, and cancer progression.[1]

A study by Danguy[2], highlighted the crucial role of Gal-3 in cancer biology and demonstrated its impact on tumor growth, invasion, and metastasis. The study elucidated the mechanisms through which Gal-3 promotes tumor progression, suggesting its potential as a therapeutic target in cancer. Additionally, a review that[3] emphasized the significance of Gal-3 in fibrotic diseases, such as liver and pulmonary fibrosis. The authors discussed the molecular pathways regulated by Gal-3 in fibrosis and proposed Gal-3 inhibitors as promising therapeutic agents for these conditions. Moreover, emerging evidence suggests a link between Gal-3 and cardiovascular diseases, as explored in a study by Henderson[4]. The researchers identified Gal-3 as a biomarker of cardiovascular risk and heart failure (HF), highlighting its potential utility in predicting and managing cardiac disorders.[4] In cardiovascular diseases, Gal-3 is a key point in the pathogenesis of HF, fibrosis, and atherosclerosis. Many studies have demonstrated that elevated Gal-3 levels are associated with inflammation, adverse cardiac remodeling, and myocardial fibrosis, leading to impaired cardiac function.[5]

Moreover, Gal-3 has been implicated in atherosclerotic plaque formation and instability through its effects on vascular inflammation and smooth muscle cell proliferation.[6] In inflammatory conditions, Gal-3 has been shown to modulate immune responses by regulating macrophage

To cite this article: Tınazlı M, Bakhtiyarova G. Galectins: an amazing marker and a potential therapeutic target. Cyprus J Med Sci. 2025;10(1):7-12

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Received: 19.04.2024
Accepted: 06.09.2024
Publication Date: 14.03.2025

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activation, cytokine production, and T-cell function. Gal-3 plays a dual role in inflammation, acting as both a proinflammatory mediator and a regulator of immune tolerance.[1] The dysregulation of Gal-3 expression has been linked to autoimmune diseases, such as rheumatoid arthritis (RA) and inflammatory bowel disease, highlighting the importance of Gal-3 in immune homeostasis and disease pathogenesis. The pathophysiology of Gal-3 encompasses many biological processes, such as immune regulation, cancer progression, and cardiac dysfunction.

Gal-3 is expressed in renal cell types such as tubular epithelial, mesangial, and immune cells within the kidney. Its interactions with cell surface receptors and extracellular matrix components modulate cell adhesion, migration, and survival, thus influencing the cellular responses to injury and repair processes in the kidney.[7]

Generally, Gal-3 seems to play a certain role in inflammation, fibrosis, cancer, and other organ diseases. As an internal medicine specialist, we wondered about the role of Gal-3, which has been accepted as a biomarker in cardiovascular, kidney, gastrointestinal, autoimmune, infection, cancer, and thyroid diseases, and we also conducted a general literature review and found out the role of this biomarker in these diseases and prepared this article by wondering how predictive it is and how much it affects prognosis and mortality.

Gal-3 and Cardiovascular Diseases

Gal-3 mainly induces pathological remodeling and fibrosis; therefore, in HF pathology, it has been called a "culprit" biomarker, such as C-reactive protein or N-terminus pro B-type natriuretic peptide in HF.[8] Zaborska et al.[9] in their review published in 2023, they found the following evidence: HF is a clinical syndrome with increasing frequency and high morbidity and mortality. The search for a biomarker that can be useful in diagnosis and risk classification has continued in recent literature.[9]

Făgărăşan et al.[10] examined the effect of Gal-3 in heart disease (congenital) in the same year and stated the following in their review: According to what we know so far, Gal-3 has the potential to predict cardiac dysfunction in adults and children. It is a novel biomarker. Here, we describe how Gal-3 may be potentially useful in the early detection of failure symptoms and predicting postoperative complications in patients with congenital heart disease. Further studies are needed to determine how Gal-3 may relate to complications and long-term outcomes in both adult and pediatric patients. The reliability of the predictive feature depends on clinical concordance and positive correlation with data obtained from advanced cardiac imaging, such as echocardiography and cardiac magnetic resonance.[10]

Coronary Heart Disease, Myocardial Infarction, Atrial Fibrillation

Elevated serum Gal-3 levels have been linked to a heightened incidence of cardiovascular events in individuals with chronic coronary heart disease. Conversely, another large-scale patient follow-up study spanning a 13-year enrollment period of stable chronic heart patients indicated that Gal-3 could not autonomously forecast recurrence after accounting for biomarkers of inflammation, hemodynamic stress, myocardial dysfunction, and renal dysfunction.[11] Gal-3 serum levels displayed a notably positive correlation with the severity of disease in individuals diagnosed with coronary artery disease, and higher Gal-3 levels were accompanied by an elevated degree of myocardial fibrosis.[12] Moreover, elevated Gal-3 levels expression was correlated with diuretic therapy administration and a higher incidence of acute atrial fibrillation (AF). Pranata et al.[13], according to the results of their study, serum Gal-3 levels after ablation are associated with an increased risk of AF recurrence. However, detailed research is needed to confirm these findings and the use of Gal-3 as a therapeutic biomarker.[13] Mohtasham Kia et al.[14] in 2023, a review titled "Insights into the Role of Gal-3 as a Diagnostic and Prognostic Biomarker of AF" in the Journal Disease Markers also investigated the potential role of Gal-3 as a biomarker of AF.

In conclusion, Gal-3 is a biomarker, prognostic, and mortality determinant of many cardiovascular diseases.

Gal-3 and Kidney Diseases

Gal-3 is the precursor of the kidney, Gal-3 can be found in the apical areas of the branches of the ureteric bud during the metanephros. It is also expressed in the fetal papillary and medullary collecting ducts of both the plasma and cytoplasmic membranes. It becomes evident later in fetal kidney maturation in the basal areas of the medullary collecting ducts. Gal-3 expression has been reported at later stages of nephrogenesis, but it is limited to the primary cilium and collecting tubules in normal adult kidneys.[15]

Numerous studies have reported increased Gal-3 levels in patients. Gal-3 has the potential to be a biomarker for diagnosis and prognosis of diabetic nephropathy (DN). However, the results from individual studies are inconsistent, and the relationship between Gal-3 and DN needs to be investigated in more comprehensive meta-analyses. As a result of this meta-analysis, although some studies have supported the association between Gal-3 expression and DN risk, the actual effect of Gal-3 on DN risk is still controversial because the results of individual studies differ.[16]

Furthermore, Gal-3 plays crucial roles in fibrogenesis across various organs, including the lungs, kidneys, liver, and myocardium, as highlighted in recent research. In a study involving kidney biopsies from 249 patients with chronic kidney disease (CKD), plasma Gal-3 concentrations were assessed. Results showed a direct correlation with tubular atrophy and interstitial fibrosis and an inverse correlation with estimated glomerular filtration rate.[17]

In another study involving 280 patients who underwent kidney biopsy, higher urinary Gal-3 levels were associated with severe interstitial fibrosis.[18]

A new study in 2024 by Kim et al.[19] in their scientific report published in the Journal Nature Portfolio, the authors provided evidence that Gal-3 increases vascular calcification and high inflammatory status, suggesting a potential causal relationship between serum Gal-3 and increased mortality in hemodialysis patients.

The current evidence indicates that Gal-3 is a promising biomarker and therapeutic target in acute kidney injury and CKD. However, further research is warranted to fully elucidate its diagnostic and therapeutic potential in these conditions.

Galectins and Gastrointestinal Diseases

According to the literature, it is associated with chronic liver disease, liver fibrosis, cirrhosis, cholestatic liver diseases, and those with chronic gastritis and *Helicobacter pylori (H. pylori)* positive. Rayane Bernardes Estevametal investigated the modulation of Gal-3 and Gal-9 in the gastric mucosa of patients with confirmed *H. pylori* infection and

chronic gastritis. The authors evaluated mast cell density and the *in situ* expression of Gal-1, -3, and -9 using immunohistochemistry in 44 gastric antrum biopsy samples obtained from patients with chronic gastritis, those with active gastritis, and a control group. Their findings revealed a significant positive association between *H. pylori* infection and chronic gastritis.[20] Additionally, the potential effect of *H. pylori*-associated Gal-3 on neurodegeneration was also reported by Boziki et al.[21] It was researched in 2018. They focused on the role of Gal-3 in shaping the immune system's responses to microbial agents, specifically *H. pylori*, thereby enhancing the effect of the microbe in areas distant from the normal site of colonization, such as the central nervous system (CNS) in this review.[21]

The Relationship Between Liver Fibrosis and Galectin-3

The role of Gal-3 in liver disease is unclear. In this regard, in 2021, An et al.[22] the major scientific research was conducted by. In this study, the researchers conducted searches in PubMed, Embase, and the Cochrane Library databases and identified 43 cohorts and 33 studies involving a total of 4,168 patients with hepatocellular carcinoma (HCC) liver disease. Among patients with HCC, high tissue expression of Gal-1 and Gal-3 was significantly correlated with poor overall survival and positive vascular invasion. Conversely, high tissue expression levels of Gal-4 and Gal-9 were significantly associated with better overall survival and a low risk of vascular invasion.

Furthermore, serum levels of Gal-3 were notably elevated in various liver conditions, including HCC, chronic active hepatitis B, liver failure, and cirrhosis. Additionally, serum Gal-9 levels were significantly higher in HCC and autoimmune hepatitis. The study concluded that low expression of Gal-4 and Gal-9 and high expression of Gal-1 and Gal-3 are indicative of poor prognosis in patients with HCC, as well as serum levels of Gal-3 and Gal-9 in chronic liver diseases, showing a positive association with risk.[22]

Wang et al.[23] published a review in International Immunopharmacology in 2023. In this review, the authors attempted to explain the identity and mechanism of macrophages in liver fibrosis. The etiologies-underlying liver fibrosis are the various; alcohol consumption, viral infections, and drug toxicity. Cirrhosis is a common clinical liver syndrome that causes approximately two million deaths annually worldwide. Although fibrosis is generally considered difficult to reverse, due to the liver's extensive regenerative capacity, liver fibrosis is possible. Additionally, the imbalance in fibrosis formation can be regulated by specific interventions. This treatment may help prevent or even reverse fibrosis before it becomes irreversible. However, the chronic inflammatory environment of fibrotic liver can remodel macrophages and stimulate them toward different phenotypes.[23]

In 2021, Del Turco et al.[24] published an article. In this study, YKL-40 and Gal-3 levels were compared in the evaluation of liver fibrosis in patients with cirrhosis. A total of 46 participants consisted of 24 patients, 10 with HCC, 14 without HCC, and 22 healthy individuals. Gal-3 and YKL-40 were measured in the serum of all patients and candidates in the healthy group. Consequently, YKL-40 and Gal-3 levels were notably elevated in kidney transplant recipients compared with the healthy group. It was determined that 1 day after transplantation, the Gal-3 and YKL-40 levels started to decrease compared with the baseline levels and remained unchanged for 1 month. In this study, Gal-3, but not YKL-40, was shown to be associated with the severity of liver fibrosis; therefore, using this non-invasive, simple, and rapid method, estimating the amount of circulating Gal-3 was associated with both liver damage and fibrosis. Gal-3 may be useful as an indicator of inflammatory processes. However, Gal-3 cannot be used as a general biomarker for HCC because blood levels do not differ between patients with and without HCC.[24]

Galectin and Its Relationship with Other Diseases

Cancer Types and Galectin

Gal-3 levels are elevated in many other diseases, including various types of cancer and chronic obstructive pulmonary disease. Although its role in cancer development is not fully known, it has been reported that it may be associated with neoplastic formation and metastasis. Many studies in the literature have shown that immunohistochemical dyes such as Gal-3 have low specificity for cancer detection.[25] It is known that Gal-3 expression increases especially in the gastrointestinal system, pancreas, breast, head and neck, CNS thyroid, uterus, bladder, and tongue cancers. There are no markers that can definitively differentiate benign from malignant thyroid nodules before surgery. Serum Gal-3 levels were found to be statistically higher in some studies in patients with malignant thyroid nodules. It has been reported that Gal-3 expression increases, especially in papillary carcinomas, and can be used in the differential diagnosis of benign lesions.[26]

The importance of biomarkers such as Gal-3 in the prognosis of PTCs is unclear. In a study where Gal-3 expression was found to be higher in papillary thyroid cancer patients than in anaplastic thyroid cancers, survival was found to be higher in anaplastic thyroid cancers, and Gal-3 was found to not correlate with the advanced stage of the tumor or the presence of lymph node metastasis.[27]

There are no sufficient studies on the relationship between serum Gal levels and lung cancer. However, in clinical use, measuring Gal levels in serum is easier and more practical than measuring Gal expression in tissue. In a study conducted in 2018, serum levels of Gal-1 and -3 values were found to be significantly lower in patients with lung cancer than in the control group. Gal-1 and -3 levels; no relationship was found with survival, tumor diameter, or tumor stage. In the presence of metastasis, both Gal levels were significantly lower. Many preclinical studies on lung cancer have found a relationship between Gal-1 and immune escape and tumor progression. Gal-1 also has prognostic significance in human lung cancer. Tumor cell variants with high potential for lung colonization have high Gal-3 expression levels on the cell surface. Similarly, increased Gal-3 expression is correlated with the metastatic potential of some tumorigenic cells, such as cell motility and invasion into the extracellular matrix. However, the relationship between these findings and the epithelial origin of human tumors is not fully understood. Although it has been explained by various mechanisms that high Gal values in the tissue are related to stage, metastasis, and survival in some types of cancer, it is not clear whether the measurement of Gal in serum is correlated with the level in tissue. There is a need for studies investigating the correlation between high Gal levels in tissue and serum levels. Gal-1 and -3 levels measured in serum are not associated with survival in lung cancer patients.[28]

Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a lung disease of unknown cause, with poor prognosis, advanced fibrosis, chronic and progressive disease, and is ultimately fatal. Factors causing recurrent alveolar damage

include viral infections, gastroesophageal reflux, and environmental and occupational exposure. Additionally, genetic predisposition is an important factor in damage development.[29]

It is known that biomarkers are important for calculating the risk of developing IPF, early diagnosis of the disease, prognosis, and monitoring the response to treatment. The ideal biomarker should be valid and reliable and should be easily obtained using non-invasive methods. Research on more than one biomarker in IPF is ongoing, but a biomarker that can be used primarily in clinical practice has not yet been identified. Gal-3 is defined as a marker that activates oxidative stress, and the inflammatory response is considered a potential inflammatory marker. It has been shown to regulate immune response, cell receptor interactions, inflammation, cancer cell behavior, and scar formation. Much evidence has shown that Gal-3' activates various profibrotic factors, stimulates fibroblast proliferation, and accompanies collagen production.[30] Because safe and effective treatments for IPF are urgently needed, many new treatment options, including TD139 (the Gal-3 inhibitor), are currently being investigated. In recent studies, the effect of inhaled Gal-3 inhibitor combined with nintedanib in patients with IPF is in the phase 2 study phase.[31] There are many studies showing the biochemical and immunological relationship between Gal-3 and lung fibrosis, but in most of them, although Gal-3 was slightly higher than in controls, the difference was not statistically significant.[32,33]

It appears that Gal-3 is a multifunctional protein that triggers inflammation and affects its progression.[34]

Relationship with Rheumatic Diseases

In literature reviews, Gal-1, -3 and -9 are associated with many autoimmune diseases, such as uveitis, Behçet's disease (BD), and RA.[35]

Gals can participate in inflammatory and immune responses as autoantigen. In the studies conducted, the presence of autoantibodies against different Gal was detected in normal healthy individuals and selected patient individuals. In a study conducted by Lee et al.[36] in 2007, Gal-3 levels were found to be higher in patients with BD and RA than in the control group.

Yilmaz et al.[37] reported that Gal-3 levels in serum increased in Familial Mediterranean fever patients. The reason for the increase in Gal-9 expression in inflammatory diseases is that they-3, the ligand of Gal-9 in Th1 cells, triggers Gal-9 and uses this situation to suppress Th1 immunity. In the meantime, Gal-9 is spontaneously activated by interferon gamma (IFN-γ) expression is upregulated. It is thought that the elevation of Gal-3 expression may be due to its role as a positive regulator of inflammation and for increasing the activation of cells in the myeloid series. As observed in all the data, different Gal can create inhibitory or enhancing signals to control the immune system. In addition, many cytokines such as IFN-γ, growth factors [transforming growth factor-alpha (TGF-α), TGF-β], the monomer/dimer balance of Gal, their stability in tissues, and their amount, decrease, or increase due to many effects such as reduction and oxidation may harm the organism. Studies have shown that cytokines increase the expression of some Gal in inflammatory diseases. Therefore, it appears that the body creates a more effective and severe response by increasing the production of endogenous anti-inflammatory Gal to regulate the homeostasis of immune cells. Before the development of Gal-based therapeutic agents, a more comprehensive investigation and comprehension of the mechanisms and pathways involved in the different "immunoregulatory" roles of the less-explored Gal is required. It is not yet known what effect functional deficiency or excess of the Gal family has. We do not know why the same Gal exhibit different functions in different environments. New research shows that Gal play an important role in the development of acute inflammation, infectious diseases, allergies, autoimmune diseases, atherosclerosis, and cancer-related chronic inflammation. Thus, recombinant proteins or specific Gal inhibitors can be used as therapeutic agents for inflammatory diseases.[38]

Several studies have shown that serum Gal-3 binding protein (Gal-3BP), a novel marker of obesity and Metabolic syndrome (MetS),[39] is positively associated with MetS. In a study involving 570 Chinese adults, serum Gal-3BP was found to be a useful biomarker for the risk of developing MetS.[40] There are studies supporting the idea that high serum Gal-3 levels may be a good marker for myocardial fibrosis in adolescents with obesity and MetS and in patients with MetS and AF.[41]

Immunoglobulin-G4-related disease (IgG4-RD) is a rare systemic fibroinflammatory disease. The pathogenesis of this condition is not fully known and appears to be multifactorial. Accepted environmental factors such as blue-collar work are also important risk factors. It was first described in 2003 as an autoimmune disease that causes multi-organ involvement, especially in the chest, head and neck, pelvic, and abdominal organs. Many autoantigen and autoantibodies are used for diagnosis. Most of these antibodies are not specific to the disease, and antibodies against IgG4-related cholangitis (IRC), laminin 511-E8, annexin A11, prohibitin 1, and Gal-3 have been confirmed. Gal-3 has been specifically associated with manifestations of type 1 autoimmune pancreatitis, IRC, and salivary and lacrimal glands. Gal-3 has been indirectly associated with disease activity but has been reported to remain elevated during glucocorticosteroid treatment. Additionally, Gal-3 inhibits the differentiation of B cells into immunoglobulin-secreting plasma cells, and Gal-3 has a profibrotic role that has been attributed to various fibrotic diseases.[42] One study showed an increase in Gal-3 expression in cells involved in immune response activity, including dendritic cells, macrophages, epithelial cells, and myofibroblasts in patients with IgG4-RD, whereas Gal-3 signaling was also found in stromal deposition.

Gal-3 is also required for the effective phagocytosis of macrophages to remove apoptotic cells and thus prevent autoimmune reactions. In addition, endogenous Gal-3 has been found to induce Th2. Gal-3 is highly expressed in affected tissues. In addition to IgG4-RD, Gal-3 expression has been shown to increase in various autoimmune diseases, such as RA, polymyositis-dermatomyositis, systemic lupus erythematosus, systemic sclerosis, BD, and Crohn's disease.[43] In another study, in patients diagnosed with IgG4-RD, Gal-3 levels were found to be high in the tissues of the pancreas, lung, salivary glands, kidney, aorta, retroperitoneum, and bile ducts, especially at higher levels in the lymph nodes, and IgG4 anti-Gal-3 autoantibodies were detected, especially in the lymph nodes was found to be associated.[44]

Autoimmune Endocrine Diseases and Galectins

Graves is an autoimmune disease of the thyroid gland. In Graves' disease, the immunotolerance of thyroid structures is impaired by several factors (endogenous and environmental factors). This results in the induction of TRAb produced by B-cells. Gal-3 is crucial in numerous cell functions, including transformation, pre-mRNA splicing, growth, and apoptosis, as

well as other biological activities, such as inflammation, host defense, angiogenesis, and fibrosis.[45,46]

In light of this information, some recent studies have found that blood Gal-3 levels are increased in patients with Graves' disease. However, there was no difference in Gal-3 levels between Graves' patients with thyroid-related ophthalmopathy and those without compared with healthy controls.[47]

CONCLUSION

Gal-3 is a multifunctional protein involved in a variety of functions types of diseases. Its prognostic value in predicting the outcomes of HF and its diagnostic value in thyroid carcinoma diagnosis have been extensively investigated. It has also been found useful in predicting metastases in laryngeal carcinoma, breast, colorectal cancers, prostate, pancreatic and gastric cancers. Functions of Gal-3 in fibrosis and immunity has also been researched, which suggests it is possible therapeutic benefit in certain types of fibrotic diseases and infection. Gal-may represent a therapeutic approach to delay progression from these diseases. As a result, in our literature review, we see that Gal-3 appears as a diagnostic, prognostic, and mortality biomarker in all diseases that we frequently see in internal medicine practice, in all areas covering the branch of internal medicine. And we see that this Gal-3 biomarker plays a major role in the further follow-up and treatment of these diseases.

MAIN POINTS

- Galectins may be useful biomarkers for the early diagnosis of inflammatory diseases and malignancies.
- New detailed studies should be conducted to increase the specificity and sensitivity.
- This approach may open new horizons for intelligent design of treatment targets after diagnosis.

FOOTNOTES

Authorship Contributions

Concept: M.T., G.B.; Design: M.T., G.B.; Data Collection and/or Processing: M.T., G.B.; Analysis and/or Interpretation: M.T., G.B.; Literature Search: M.T., G.B.; Writing: M.T., G.B.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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REVIEW

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-31
Cyprus J Med Sci 2025;10(1):13-21

Guide to Optimizing the Accuracy of Intraoral Implant Scans: A Review Article

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Abstract

As intraoral scanners (IOS) develop daily, their trueness and precision are increasingly being questioned and evaluated. Accuracy is affected by both patient and operator factors. These factors cause image distortion and impression inaccuracies. To maximize the accuracy, it is necessary to master the scanning process. The purpose of this review is to summarize the knowledge of the factors and highlight the points that should be considered to ensure maximum accuracy. Studies conducted with the IOSs technologies used today have revealed that operator experience, scanning distance, scanning head size, distance between implants, scanned area (full/half arch), implant depth, and the presence of saliva or blood in the area to be scanned are factors that significantly affect accuracy. When choosing and using a scanner, obtaining maximum performance from the scanner and knowing what factors affect the accuracy of the impression taken will enable us to use the scanner more accurately, have a higher accuracy of the impression taken, and therefore produce more successful and long-lasting restorations. Although an optimum condition that increases accuracy cannot be determined due to differences in the evaluation of studies, the lack of a sufficient number of studies for each factor, and the conditions changing from case to case, preliminary conclusions that should be paid particular attention to in increasing accuracy have been determined. In addition, the manufacturer's instructions should also be considered in improving the performance of the IOS.

Keywords: Digital implant scans, accuracy, implant scanning factors, intraoral scanners

To cite this article: Özal Ç. Guide to optimizing the accuracy of intraoral implant scans: a review article. Cyprus J Med Sci. 2025;10(1):13-21

INTRODUCTION

Recent developments in digital technology have provided advantages for both clinicians and patients. These systems, which provide advantages to clinicians from diagnosis to production, also increase patient comfort, reduce treatment time, and prevent human errors. As digital systems have developed, material diversity has also increased.[1] Intraoral scanners (IOS), which have become widely used, have made it possible to produce implant-supported restorations with a digital workflow. Using the scan body, the scanned data are sent to CAD software, and after the prosthetic restorations are designed, the data are sent directly to the milling machine for production.[2] On the other hand, the accuracy of this process directly affects the success of the treatment. However, it is essential to understand the variables that affect the scanning process and results to maximize the effectiveness and accuracy of IOSs.

Digital impression accuracy is critical for the production of accurate and properly fitting implant restorations. Accuracy is given by two measuring techniques in ISO 5725: trueness and precision.[3] Trueness is the degree to which the true or accepted reference value and the arithmetic mean of a large number of test findings match. The degree of agreement between the test results is known as the precision.

Accuracy is affected by various factors. Factors like the file type used in special format or STL format during the design phase, depth of implant, whether the scanned area is wet or dry, lighting of the environment, use of different optical systems, tongue and cheek movements,

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Received: 21.04.2024
Accepted: 02.12.2024
Publication Date: 14.03.2025

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edentulous ridge length, quantity and form of keratinized gingiva, angle, location, and quantity of implants, and features of scan bodies. Factors that reduce intraoral scan accuracy result in the development of accumulated scan distortion.[1,2,4,5]

Consequently, by being aware of and cognizant of these influencing factors, dental treatments carried out through the use of digital workflows can become more predictable and reliable.[5] The aim of this article is to evaluate operator- and patient-related factors that negatively affect the accuracy of intraoral implant scans.

Operator-Related Factors

Intraoral Scanner and Software

IOS has different working principles and imaging techniques. IOS is manufactured by various companies; they operate in various protocols, including laser and video, confocal microscopy, triangulation, structural illumination, interferometry, and wave sampling. These different operating principles affect the image clarity of the scanners. Differences in scanning technologies and systems used in the production of implant-supported restorations have been reported in the literature (Table 1).[6,7]

Operator Experience

In the use of IOS, the scanning time and image volume are taken into account in the formation of image clarity and distortion. With the experience of the operator, it can reduce the extra time and number of images that may occur during recording. Scanners with longer scan times have been reported to be less accurate when associated with less experienced users (Table 1).[8-10] However, some studies reported that operator experience had no significant effect.[11,12]

File Format

The file format used affects how scanned data are stored, processed, and transmitted across software programs and systems. In addition, the file format used when exporting or importing digital impressions across different software programs or systems is crucial. File format incompatibility may result in data loss, conversion problems, or inaccuracies during the transfer process. For storing scanned data, several file formats provide various levels of accuracy and resolution. Higher precision and resolution enable the capture of more fine aspects of the implant surface, resulting in improved accuracy. Choosing a file format that offers higher accuracy and resolution ensures that the digital impression is more accurate. In addition, to minimize file size, certain file formats use compression techniques; however, this compression may result in data loss or degradation. It is critical to select a file format that works with both the scanner used to record the digital impression and the software required to process and design the final restoration.[2]

Scanning Head Size

Various IOSs are available on the market, each with a different scan head size. The literature has reported that when larger scan head sizes are used, higher trueness and precision values and fewer scanning images are required.[13] More studies are required to evaluate the impact of scan head size on precision and trueness in various IOSs.

Scanning Distance

The scanning distance is the distance between the IOS tip and the target surface. Studies have reported that scanning accuracy changes as the scanning distance changes.[14,15] The ideal scanning distance is specified by the IOS manufacturer for each IOS version. These are the recommended distances recommended by the company, and following them maximizes the performance of the IOS accuracy. Miyoshi et al.[15] scanned six implants placed in the edentulous maxilla in their *in vitro* study five times for each scanner, with four different IOSs: True Definition, CEREC Omnicam, Trios Scanner 2, and CS 3600. As a result of the study, the precision declined as the impression ranges expanded.

Table 1. Studies evaluating the impact of intraoral scanner-software and operator experience on intraoral implant scanning accuracy

Study	Variable	Study type	Sample size	Arch type	Number of implant	IOS	Results
Ciocca et al.[11]	Operator experience	*In vitro*	5	Mandible	6	- True Definition, 3M ESPE	The operator experience had no significant effect.
Schmidt et al.[6]	Intraoral scanner and software	*In vitro*	10	Maxilla and mandible	3	- Trios 3 Pod; 3Shape A/S shape)	Accuracy depends on the software and hardware used by intraoral scanners; however, new software and systems do not warrant increased accuracy.
Revell et al.[10]	Operator experience	*In vitro*	8	Maxilla	5	- iTero Element 2, Cadent -Medit i500 -Primescan, CEREC -Trios 3, 3Shape A/S -Trios 4, 3Shape A/S	The deviation in the implant platform was greater in the scans performed by the less experienced operator than in the scans performed by the experienced operator.
Marques et al.[9]	Operator experience	*In vitro*	10	Maxilla	1	- Trios 3, 3Shape A/S	The operator experience had little impact on the accuracy of full and partial arch scans.
Verniani et al.[7]	Intraoral scanner and software	*In vitro*	10	Maxilla	Separately 3	- Trios 3; 3Shape A/S - I700, Medit	When the full arch model was analyzed, Trios 3 performed significantly better than Medit I700 in acquiring the scanbody position.

IOS: Intraoral scanner.

Scanned Area

When performing IOS, whether the area to be scanned is full of half arc affects the accuracy. In full-arch implant-supported restorations, the digital data recorded increase with the increase in scan length and rotation of the opposing arch.[16,17] The increase in the number of images recorded in IOS causes overlapping of images and distortions. Manufacturers recommend a limited number of images to avoid this distortion and folds. It has been stated that impression accuracy is affected because this number of images is exceeded in full-arch intraoral scans, and the scanner cannot combine the images (Table 2).[11,18,19]

Scan Protocol

The term "scan protocol" describes the precise guidelines and procedures followed during the scanning process to record the digital impression. The sequence of scans, number of scan bodies, and order of scanning implant fixtures or abutments are all important. The aspects of the scan protocol that affect the accuracy of the digital impression. A clear and consistent scan protocol helps minimize errors during the scanning process and ensures reproducibility. Since the imaging methods and software used by IOS in digital impression systems differ from each other, each company recommends a scanning protocol that is specific to its own system.[8,17,20] Previous research has shown that changing the scanning pattern can affect the accuracy of intraoral digital scans (Table 3).[12,21,22] Additionally, regardless of the proposed scanning protocol, it is known that the impression accuracy of the scanners is much better in the region where scanning is started than in the region where scanning ends.[8,17]

Scan Body Design

The transfer of the implants' three-dimensional location to the virtual model mayn occasionally deviate due to the use of scan bodies. The material of the scan body, geometric design, clinical height, implant position, connection, and angle in the dental arch are among the other factors that affect the accuracy of the digital implant size.[23,24] There are various implant-scan body designs on the market. Based on their retention system or the material they are composed of, the scan bodies can be categorized. A few manufacturers also offer identical scan bodies at different heights. Scanning parts can be produced from polyetheretherketone (PEEK), titanium alloy, or various resins. Today, PEEK is generally preferred for the production of scanning pieces. This material has a matte appearance and optical properties that do not absorb or reflect light.[25]

Overall, 1-piece PEEK scan body designs showed higher displacements than the metallic-scan bodies.[26,27] Moreover, sterilization processes may also affect the positioning and accuracy of the scan body.[28] As a result, based on the limited data that is currently available, a metallic-scan body design may be selected to minimize displacement caused by PEEK material distortion from tightening or sterilization.[26,27] These results further support the idea that 1-piece PEEK scan bodies should only be used once.[28] Recurrent utilization of the scan bodies can cause distortion of the scan body and affect the accuracy of intraoral digital implant scans.[25] Many studies have been conducted *in vitro*, and different results have been obtained (Table 4).[17,23,29,30] No scan body design that works best for every IOS that is available may exist in this direction.[27] The scanning piece has a certain geometric shape registered in the digital library created by the manufacturer. It is stated that the scanner must be of high sensitivity to match the image of the scan piece in the digital library with the image taken during the measurement. The easy recognition and recording of the geometry of the scan piece, which was previously defined in the library in the system, affects the scanning performance of the digital impression technique.[24]

Scan Resolution and Mesh Quality

The accuracy of the scanned data may be affected by the resolution of the IOS used for digital impressions. Scanners with higher resolution can capture more precise digital impressions and more detailed information. The accuracy of the scanners may suffer from lower resolution, especially when trying to capture intricate implant geometry or fine details. In addition, mesh quality refers to the precision and resolution of the digital mesh representation created from the scanned data. The IOS software is capable of producing files with various mesh densities. However, a high mesh density for the entire tooth is meaningless because of the long computation time required. The morphological structure of indented-protruding surfaces provides dense mesh quality (i.e., high accuracy, flat surfaces create low mesh quality (i.e., low accuracy. A large number of triangles is required to precisely follow the emergence profile, whereas a small number of triangles may result in margin smoothing.[31]

Table 2. Studies evaluating the effect of scanning area on intraoral implant scanning accuracy

Study	Study type	Sample size	Arch type	Number of implant	IOS	Results
Flügge et al.[16]	*In vitro*	10	Mandible	2 and 5	- iTero, Cadent - Trios, 3Shape - True Definition of 3M ESPE	There is a difference in scanning precision between the tested IOS devices. The precision of the IOS system decreased as the distance between scan bodies increased.
Ciocca et al.[11]	*In vitro*	5	Mandible	6	- True Definition, 3M ESPE	The error rate increased as the length of the scanned arch increased.
Yilmaz et al.[19]	*In vitro*	10	Maxilla	1	- Trios 3; 3Shape A/S	Comparative partial- and complete-arch scans of anterior single implants with an intraoral scanner resulted in similar.
Donmez et al.[18]	*In vitro*	14	Maxilla	2	- Primescan, Dentsply Sirona - Trios 3; 3Shape A/S	Considering the mesiodistal angular deviations, the 3D distance and interimplant distance showed that IOSs had an effect on the trueness of the scans. The trueness of the scans was affected by IOSs when mesiodistal angular deviations, 3D distance, and interimplant distance were considered. Only 3D distance deviations were affected by the scanned region.

IOS: Intraoral scanner.

Table 3. Studies evaluating the effect of scan protocol on intraoral implant scanning accuracy

Study	Type study	Sample size	IOS	Arch type	Number of implant	The implant scan body	Scanning pattern	Results
Giménez et al.[12]	*In vitro*	5	iTero (Cadent)	Maxilla	6	PEEK (Createch Medical)	Stitching halves	The first quadrant was significantly more accurate than the second quadrant as follows:
Mandelli et al.[21]	*In vitro*	10	True Definition	Maxilla	6	NA	Stitching half technique; strategy without stitching halves (occlusalpalatal-buccal)	Stitching showed better accuracy than no stitching. A noteworthy positive association was observed between the inaccuracies and the reference length.
Mizumoto et al.[20]	*In vitro*	7	Trios 3, 3Shape A-S	Maxilla	4	DESS (Barcelona, Spain)	Stitching or unstitching of the palate, occlusalbuccal-palatal	Stitching and unstitching of the palate showed no significant differences. Implant position had a significant effect on trueness.
Wu et al.[22]	*In vitro*	160	D2000; 3A-S shape Orbscan 3, 3DShining	Maxilla	4 [16 (A) - 13 (B) - 23 (C) - 26 (D)]	IO 2C-A, ELOS MEDTECH, Gorlose, Denmark	**SP-A** O (A-D) P (D-A) B (A-D) **SP-B** O (A-B-C) O (B-C-D) The OPB sequence **SP-C** O (A-B) O (B-C) O (C-D) The OPB sequence **SP-D** Zig-zag **SP-E** O (B-C) O (C-D) O (B-A) The OPB sequence **SP-F** O (B-A) O (B-C) O (C-D) The OPB sequence **SP-G** O (B-C-D) O (C-B-A) The OPB sequence **SP-H** O (B-D) P (D-A) O (A-B) B (B-D) B (B-A)	The SPF and SPG methods demonstrated lower linear trueness

IOS: Intraoral scanner, SP: Scanning protocol, PEEK: Polyetheretherketone, O: Occlusal, B: Buccal, P: Palatinal, L: Lingual.

However, the rendering of files in a GUI often misleads about the accuracy of a scan due to the use of shaders and smoothing algorithms. Mesh quality factors such as triangle density, surface smoothness, and surface detail accuracy all affect the digital impression accuracy. Inadequate mesh quality, which is manifested by low-resolution surfaces or inconsistencies in capturing fine details, can result in inaccurate virtual models and subsequent restorations.[32]

Ambient Lighting Conditions

The amount of light (lux) in the space corresponding to the intraoral digital scan is known as the ambient illuminance conditions.[1] According to previous in vitro and clinical research, the ideal lighting conditions for scanning patients who are completely dentate vary depending on the IOS chosen.[33,34]

Most IOSs function better under 1000-lux ambient illumination conditions, also referred to as room lighting conditions, although there is no single ideal lighting condition that can maximize accuracy for all IOSs. To attain this ambient lighting condition, the dental chair light was turned off while the room ceiling light was left on. It is crucial to realize that the ambient lighting intensity in each room or facility may vary; for this reason, it is recommended to use a luxmeter to standardize ambient lighting conditions.[33,34]

Scan Body Splinting

Scan body splinting is the connection of close scan bodies to each other using a rigid material to increase scanning accuracy and facilitate scanning (Table 5).[35-38] Different splinting techniques were analyzed to improve intraoral digital implant scan accuracy. A systematic review conducted in 2021 emphasized that there are 17 different splinting techniques.[37] The best implant-scan body-splinting technique is difficult to determine as IOS technology advances daily. Therefore, the splinting method should be determined according to the IOS.

Patient-Related Factors

Implant Depth

The implant levels can be divided into bone or tissue levels, which can affect the impression accuracy. To obtain precise impressions, consideration must be given to the emergence profile and margin placement of the abutment. Incomplete capture of implant components due to subgingival margins or poor emergence profiles can result in inaccurate digital impressions. Clinical implant scan body height is correlated with implant depth (Table 6).[4,39,40] In cases in which the gingival height is high, the sensitivity of the scanner weakens as a result of the decrease in the visibility of the scanning piece. It is recommended to use long scan pieces in these cases to increase the imaging of IOS.[40]

Implant Angulation

Research has revealed that implant depth and angulation can have a negative impact on the accuracy of IOS (Table 6).[1,39,41,42] According to certain studies, implant angulation reduces digital scan accuracy compared with conventional impressions, or it reduces the accuracy of IOS scanning.[39,40] In addition, Papaspyridakos et al.[43] Study reported that there was no difference between the conventional technique and impression accuracy in cases in which the implant angle was less than 15°. It is known that with an increase in the angle, there are difficulties in recording impressions using both conventional and digital techniques.[44]

Table 4. Studies evaluating the impact of body scan design on intraoral implant scanning accuracy

Study	Study type	Sample size	Variable	Condition	IOS	Extraoral scanner	Scan body	Scan body design	Results
Althubaitiy et al.[29]	*In vitro*	140	Scan body material	Partially edentulous mandible	Trios 3, 3Shape A-S	E1; 3Shape A/S	Scan body 1: PEEK Scan body 2: metallic	Cylinder	The extraoral scanner provided the best results. Metallic scan body resulted in the best
Alvarez et al.[30]	*In vitro*	10	Scan body geometry	Partially edentulous mandible	Trios 3, 3Shape A-S	E1; 3Shape A/S	Scan body 1: PEEK Scan body 2: metallic	Cylinder	The extraoral scanner provided the best results. Metallic scan body resulted in the best
Gómez-Polo et al.[17]	*In vitro*	15	Scan body geometry	Full-arch, edentulous, and Maxilla	Trios 3, 3Shape	7Series Desktop Dental Wings	PEEK on Ti-based	Cylindric with bevels	The accuracy; implant position, angulation, and scan body geometry was affected by bevel location
Lawand et al.[23]	*In vitro*	15	Scan body geometry	Full-arch, edentulous, and Maxilla	Trios 3, 3Shape	-	PEEK	Cylinder with rounded and flat lateral sections	Subtractive modifications of scan bodies increased scanning trueness in full arch implant scans. Additive modifications to scan bodies reduced scanning trueness. The scan body geometric modifications did not affect the scanning time

IOS: Intraoral scanner, PEEK: Polyetheretherketone.

Table 5. Studies evaluating the effect of the scan body splinting technique on intraoral implant scanning accuracy

Study	Type study	Sample size	IOS	Arch type	Number of implant	The implant scan body	Scanning pattern	Results
Mizumoto et al.[36]	*In vitro*	5	Trios (hardware version unknown)	Maxilla	4	-Atlantis Intraoral FLOIO (Dentsply Sirona) -NT (Nt-Trading, Karlsruhe, Germany) -DE (DESS) -C3D (Core3D Centers, Maartensdijk, Holland) -ZI (Zimmer Biomet	OBP	Accuracy is affected by the scan technique and scan body. The ZI scan body showed better accuracy. Splinting scan bodies using floss showed lower accuracy than GB, PP, and no modification technique.
Pozzi et al.[38]	*In vitro*	30	Trios 3, 3Shape A-S	Mandibula	4	1-piece PEEK Height 9 mm Screw retained	OLB	The scan body splinting accuracy increased accuracy. Reduce the angular and linear deviations of the posterior implants
Çakmak et al.[35]	*In vitro*	14	Trios 3, 3Shape A-S	Maxilla	4	PEEK healing abutment with a screw retained and medical-grade acrylic resin scan body with friction-fitted	Conventional technique Land-marking technique Novel scanning body splinting technique	Different scanning techniques affected the trueness of the scans when the distance and angular deviation were considered. Precision was also affected when distance deviation was considered

IOS: Intraoral scanner, PEEK: Polyetheretherketone, O: Occlusal, B: Buccal, P: Palatinal, L: Lingual.

However, other studies have shown that implant angulation had no impact on the accuracy of IOS.[12]

Interimplant Distance and Interdental Space Between the Adjacent Teeth Implant and Scan Body

Only a few studies have examined the impact of interimplant distance on intraoral digital implant scan accuracy.[39,45] Studies have shown that with increasing distance between implants, similar flat gingival and crest appearances are areas that are difficult for the scanner to combine and cause distortions.[17] To address this disadvantage of the system, it has been suggested to place reference points in the inter-implant areas or splint the scan bodies (Table 6).[23,35]

The impact of the digital impression of a partial arch with missing teeth or a combination of natural teeth and implants on the accuracy of intraoral digital scans. To obtain accurate digital impressions, factors such as the position and angulation of the implants in relation to the natural teeth, accurate representation of the emergence profile, and precise details of the abutments and adjacent teeth are critical. The alignment and fit of the partial arch restoration were based on the digital impression's accuracy (Table 6).[9,46]

Palate

Few investigations have assessed the influence of palate digitization on the accuracy of maxillary intraoral digital scans in complete-arch implant digital scans in edentulous patients.[46,47] In a clinical study, when the effect of low, medium, or high maxillary palatal vault height on the accuracy of intraoral digital scans was evaluated, it was observed that the accuracy decreased as the palatal height increased, although it was not statistically significant. It also showed higher mean accuracy and precision values when the palate was not included in the intraoral digital scan.[47]

Arch Location

There are only few studies in the literature that examined the effect of whether the scanned arch is maxillary or mandibular on accuracy in digital implant scans.[48,49] In studies where the authors compared intraoral digital implant scans of the maxillary and mandibular full arch and maxillary and mandibular anterior or posterior regions, lower trueness and precision mean values of maxillary also maxillary-posterior and mandibular-posterior intraoral digital implant scans.[36,49]

Blood or Saliva

The accuracy of digital impressions can be negatively affected by blood or saliva on the implant site. Fluids can impede the scanning process, resulting in incomplete or distorted impressions. To reduce the impact of fluids on the accuracy of digital impressions, the operative field must be properly isolated and controlled.[50]

CONCLUSION

The accuracy of the digital impression directly affects the passive fit and success of restoration. The accuracy of intraoral implant scanning is affected by a variety of factors that must be carefully evaluated to provide accurate and precise outcomes. By knowing the factors that affect accuracy, dental treatments performed using digital workflows can become more predictable and reliable. Because there is not enough literature to analyze every factor, it is not possible to establish a systematic clinical recommendation. It is challenging to reach a conclusion regarding ambient light, full or partial arch scanning, scan body material or geometry, scanner used, and number of implants, and studies give contradictory results. When choosing and using a scanner, obtaining maximum performance from the scanner and knowing what factors affect the accuracy of the impression taken will enable us to use the scanner more accurately, have a higher accuracy of the impression taken, and therefore produce more successful and long-lasting restorations.

Table 6. Studies evaluating the effects of implant depth, angulation, and inter-implant distance on intraoral implant scanning accuracy

Study	Study type	Sample size	Variable	Conditior	IOS	Scan body material	Scan body geometry	Results
Gómez-Polo et al.[45]	*In vitro*	30	Implant depth	Full-arch, edentulous, and Maxilla	Trios 3, 3Shape A-S	PEEK on Ti-based	Cylindric with bevels	Accuracy was affected by inter-implant distance and scan body angulation. Greater accuracy was obtained with parallel implants.
Sequeira et al.[4]	*In vitro*	15	Implant depth	A partially edentulous cast with one implant analog at different depths (7, 6, 3 and 0 mm)	Zfx Scan III, Zfx GmbH	PEEK	Cylindric with bevels	Truness and precision were high when the implant was 0 mm deep. However, it decreased as the implant was placed subgingivally. There was no significant increase in accuracy after 3 mm submergence of the implant.
Taghva et al.[42]	*In vitro*	10	Implant depth and angulation	Four maxillary models with 2 analogs (first premolar and first molar) at depths of 1 (G1), 2 (G2), 3 (G3), and 4 (G4) mm.	Trios 3, 3Shape A-S	Titanium	Cylindric with bevels	G1 and G4 showed significantly better results
Sicilia et al.[41]	*In vitro*	15	Height of the scan body and implant angulation	Two edentulous maxillary casts with four implant abutment analogs: parallel (P) and angulated (NP) (18 degrees).	7 Series Dental Wings, Trios 4, 3Shape A-S	PEEK on Ti-based	Cylindric with bevels	An implant inclination of 18° did not significantly influence the scanning accuracy, nor did the supramucosal height of the scan body.
Gómez-Polo et al.[39]	*In vitro*	10	Interimplant distance, implant depth, and angulation	Two edentulous maxillary casts with six parallel and angulated (30 degrees) implant	Trios 3, 3Shape A-S	PEEK	Cylindric	The implant angulation and clinical implant scan body height were significant predictors of discrepancies in the angular measurement.

IOS: Intraoral scanner, PEEK: Polyetheretherketone, Ti: Titanium.

MAIN POINTS

- Ambient light, scanning distance, and scanning protocols should be applied while considering the recommendations of the manufacturer of the selected intraoral scanner.
- Blood and saliva negatively affect the scanning process and cause incomplete or distorted impressions. Therefore, the scanned area must be isolated and dry.
- With experienced operator scanning in a shorter time, less data are obtained and higher accuracy is achieved.
- Scanning devices and file formats that provide high precision and resolution allow more details to be recorded during scanning, resulting in higher accuracy.
- There is no optimal scanning protocol. Each manufacturer's recommended scanning method should be taken into consideration.

FOOTNOTES

Financial Disclosure: The author declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2025.2024-93
Cyprus J Med Sci 2025;10(1):22-32

The Therapeutic Effect of Flavan-3-Ols from Organic Extracts of *Juniperus drupacea* Fruit Against Elastase-Induced Chronic Obstructive Pulmonary Disease in Rats

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Abstract

BACKGROUND/AIMS: Chronic obstructive pulmonary disease (COPD) is a common chronic airway disease with acute exacerbations of varying frequency that is the main cause of disease morbidity and mortality. The aim of this study was to investigate the utility of extracts rich in flavanol-3-ols (85-92%) from *Juniperus drupacea (J. drupacea)* fruit in the treatment of rats with porcine pancreatic elastase (PPE)-induced COPD.

MATERIALS AND METHODS: Thirty female rats of the Wistar albino breed were randomly divided into four groups: control, PPE, PPE + methanol extract (ME), PPE + water extract. The emphysema in the lung tissues of rats and lymphocyte, [B-cells, cytotoxic T-lymphocyte, natural killer (NK) cells], cytokines [interleukin-8 (IL-8), IL-6, and tumor necrosis factor-alpha (TNF-α)], and blood gas values in blood samples were analyzed.

RESULTS: It was observed that emphysema occurred rats after PPE exposure, and the number of inflammatory cells, except for NK-cells, and IL-6, IL-8, and TNF-α cytokines in their blood increased. Among the blood gas values, $PaCO_2$ increased with emphysema, and PaO_2 decreased. The rats with PPE-induced COPD showed a decrease in the number of B-cells and NK-cells as a result of treatment with *J. drupacea* fruit extracts.

CONCLUSION: Our results showed that PPE application causes COPD, and water and ME as flavan-3-ols-rich *J. drupacea* fruit can protect against the development of elastase-induced lung injuries as an anti-inflammatory and antioxidant factor.

Keywords: COPD, flavan-3-ols, *Juniperus drupacea*, elastase, cytokines, lymphocytes

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent, avoidable, and manageable disease characterized by persistent respiratory symptoms and restricted airflow due to abnormalities in the respiratory or alveolar tracts, primarily caused by substantial exposure to harmful particles or gases.[1] COPD constitutes a range of advancing lung disorders, notably emphysema and chronic bronchitis, with many individuals experiencing both.[2] Emphysema gradually damages lung air sacs, impeding outward air movement.[3] Chronic bronchitis induces inflammation and constriction of bronchial tubes, resulting in mucus accumulation.[4] COPD is among the leading causes of chronic morbidity and mortality worldwide. COPD is a major and growing global health

To cite this article: Akbulut HF, Vatansev H, Çolak B, Özdemir H, Çelik ZE, Akbulut M. The therapeutic effect of flavan-3-ols from Organic extracts of *Juniperus drupacea* fruit against elastase-induced chronic obstructive pulmonary disease in rats. Cyprus J Med Sci. 2025;10(1):22-32

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Received: 14.10.2024
Accepted: 09.01.2025
Publication Date: 14.03.2025

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problem, which was estimated to be the third most common cause of death in the world and the fifth most common cause of disability by 2020.[5] The goals of COPD treatment are to improve symptoms, prevent disease progression, improve quality of life, increase exercise tolerance, prevent and treat complications, and reduce mortality. To achieve these goals, it is essential to reduce risk factors that decrease lung function, provide a diagnosis of COPD and educate patients, supported by pharmacological and non-pharmacological treatment.[1]

Worldwide, there may be a large number of herbs for which there is no detailed record of usage in traditional complementary medicine practices to relieve COPD. In the last few years, many herbs have been reported in the scientific literature. *Juniperus drupacea (J. drupacea)* fruits are widely used traditional medicine in Türkiye.[6] For example, *J. drupacea* fruits have been used in the treatment of helminth infections and abdominal pain,[7,8] and against hemorrhoids,[9] while the decoction product of its fresh shoots has been used in the treatment of urinary inflammation, gout, and abdominal pain, and its tar against diarrhea.[8,10] The tar of *J. drupacea* is obtained through the combustion of its stem. It is applied externally for conditions such as alopecia, eczema, and animal wounds. Internally, it is used to treat cough, cold, urinary tract inflammation, and diarrhea.[8,11] Analysis of the extracts of *J. drupacea* fruits suggested that this fruit may be a natural antioxidant supplement for food and beverages.[12] This study aimed to investigate the curative effects of flavan-3-ol rich extracts from *J. drupacea* fruit by assessing the levels of cytokines, blood gases, and lymphocytes in rats with COPD induced by PPE. Some bioactive components found in plants are better dissolved in water, while others are better dissolved in solvents such as methanol. At the same time, methanol is a solvent with a lower density and boiling point than water. With this feature, it penetrates plants faster and allows bioactive components to pass into the solvent in greater amounts. In this study, both water and methanol extracts were used with the idea that they may differ in terms of bioactive components.

The extraction yields of *J. drupacea* fruit used in this study were determined to be 25.35% for the water extract and 27.96% for the methanol extract, with the difference being statistically significant (p=0.012).

MATERIALS AND METHODS

Experimental Procedures

Animals

The protocols presented below are based on animal experiments approved by the Ethics Committee of Selçuk University Experimental Medicine Research and Application Center (approval number: 2020/52, date: 30.11.2020). In this *in vivo* research study, a total of 30 Wistar albino adult female rats aged 4-5 months and weighing 300-350 g were used. PPE (Sigma Chemical, St Louis, MO) was used to construct a model of COPD in rats. Single-sex use aims to minimize anatomical and hormonal differences that may arise from sex.

Animal Treatment and PPE Induction

The rats were randomly selected and divided into four groups. The "draw method" was preferred and used as the randomization method.[13] Six rats were selected for the control group and eight rats for each of the other groups. No procedure was applied to the negative control group. For the other three groups, a COPD model was created with the PPE. The rats were anesthetized by injecting ketamine and xylazine before intubation. The PPE mixture was prepared as 55 U/100 g by mixing with 0.5 mL NaCl following anesthesia and was administered to rats intratracheally.[14] The negative control group received only water by gavage throughout the 21-day treatment period. In the groups, the flavan-3-ols extracts from *J. drupacea* fruit were administered starting 24 h after the PPE treatment. The methanol and water extracts of *J. drupacea* fruits were separately given by gavage at a dosage of 250 mg/kg bw/day to rats for the treatment of PPE-induced COPD over 21 days. All rats in groups were housed at 21-22 °C without any water and food restrictions, under a 12-hour light/dark cycle. Food restriction was applied to all rats 12 h before anesthesia procedures. On the 21st day, the rats were anesthetized with an injection of ketamine and xylazine again before euthanizing them. Intracardiac blood was taken in accordance with euthanasia procedures, followed by the performance of the sacrifice process. Then, various tissues were collected for analysis.

Treatment start date was 04.06.2021; Ethics committee approval date was 30.11.2020. The 250 mg/kg bw/day concentration used for therapeutic purposes in this study, was determined based on the procedures described by Laouar et al.[15]

Number of Animals to be Included in the Study

The number of animals required for the animal experiment model (4 groups: group-1, control group; group-2, PPE; group-3, PPE + WE; group-4, PPE + ME), created to evaluate the effect of methanol and water extracts of *J. drupacea* fruit applied in the COPD animal model, on blood gas, histopathology (emphysema), and inflammatory markers [pH, PaO_2, $PaCO_2$, interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha (TNF-α), cytotoxic T-cells, B-cells, natural killer (NK)-cells], was determined by the resource equalitymethod.[16-18] According to this method, the number of animals to be included in each group for an experimental design consisting of 4 study groups was determined by calculating (20/4)+1=6. Considering the possible animal losses during the experimental phase, the study was planned to be conducted with 8 animals in each experimental arm, and 6 animals in the control group.

Plant Materials

Specimens of *J. drupacea* Labill., a member of the *Cupressaceae* family, were collected from the Sebil forest area in Çamlıyayla, Mersin Province, Türkiye, during July. These botanical samples were accurately identified by Prof. Dr. Osman Tugay from the Department of Pharmaceutical Botany, Faculty of Pharmacy at Selçuk University (Konya, Türkiye) and were cataloged under the herbarium record number (KNYA Herb. No: 30.115).

The Extraction Procedures

In November 2020, the fruits of *J. drupacea* at optimal maturity were collected from a forest located at an altitude of 1400 meters in the Çamlıyayla district of Mersin. After transportation to the laboratory, the fruits were thoroughly cleaned to remove any contaminants. Once broken, the fruits were ground using a hammer mill (Arzum, model AR1034, Türkiye) to produce a fine powder for extraction. For the extraction process, fifteen grams of the ground fruits were separately treated with methanol (150 mL) and distilled water (150 mL) using a Soxhlet apparatus (Electro-mag MX 425, Türkiye). The obtained extracts were collected, then the solvents were removed using a rotary evaporator (Scilogex RE100-Pro) under vacuum at 40 °C. The extracts

were then frozen at -80 °C and lyophilized. The final powdered extracts were stored at -18 °C for further analysis.

Akbulut and Akbulut[19] analyzed the phenolic compounds of water and methanol extracts of *J. drupacea* fruit used in this study and published the results. The high-performance liquid chromatography analysis revealed that flavan-3-ols, including catechin, epicatechin, epicatechin gallate, and procyanidin A2, constituted approximately 92% of the total phenolic compounds in the aqueous extract and 85% in the methanol extract.[19]

Histopathological Evaluation

The lung tissues were fixed in 10% buffered formaldehyde for 24 hours for pathological evaluation. For macroscopic sampling, 1x1 cm samples were taken from both lungs of each animal. The pieces were embedded in paraffin and 4-micron sections were taken onto slides with the help of a microtome. The slides stained with hematoxylin-eosin were evaluated under the Olympus BX53 model light microscope for the development of emphysema. The presence and extent of emphysema were evaluated by scanning the entire lung parenchyma at 40x magnification using the microscope. In the evaluation, the ratio of lung parenchyma developing emphysema to normal lung parenchyma was determined as a percentage. Accordingly, the affected area was scored as <25%, score 1; 25-50%, score 2; 50-75%, score 3; >75%, score 4. The mean of emphysema scores of each group was calculated, and the results were compared using statistical methods.[20,21]

Blood Gas Analysis (pH, pCO_2, pO_2)

For blood gas analysis, the blood was carefully drawn from the heart with a 2 mL heparin injector in the experimental rats under anesthesia. Blood gas analyses were performed on the day the study was completed. After the rats were anesthetized, blood was taken from the heart using blood gas injectors and analyzed without delay by the ABL9 blood gas analyzer (Dadiometer, Denmark). pH, arterial oxygen partial pressure (PaO_2), and arterial carbon dioxide partial pressure ($PaCO_2$) values were determined in their analyses.

Cytokine Analyzes (IL-6, IL-8, TNF-α) by ELISA Test Kit

For the analysis of cytokines, the blood taken from the heart of the rats under anesthesia, was collected in purple capped EDTA tubes and transferred to the laboratory without waiting. This blood was then centrifuged, and the plasma was kept at -80 °C until analysis. IL-6, IL-8, and TNF-α analyses were carried out using Elabscience Rat IL-6, Bioassay Technology Laboratory Rat IL-8, and Elabscience Rat TNF-α ELISA test kits, following the test procedures provided with them.

Cytotoxic T-Lymphocyte, B and NK-Cell Analysis by Flow Cytometry

The surfaces of cells were stained with monoclonal antibodies targeting anti-Rat CD8a, CD45RA, and CD161a (BD Bioscience) to assess the proportions of B-cells, cytotoxic T-cells, and NK-cells through flow cytometry. Three sample tubes were prepared: one control, one containing CD3 (APC)/CD4 (PE)/CD8 (FITC), and another with CD3 (APC)/CD45RA (FITC)/CD161a (PE). A 100 µL aliquot of rat peripheral blood was added to each tube. According to the kit instructions, the appropriate amounts of monoclonal antibodies were added to all tubes except the control. After vortexing, the tubes were incubated for ten minutes at room temperature in the dark. Next, two mL of 10X lysing solution, diluted 1:10 with distilled water, was added to lyse red blood cells. The samples were incubated for fifteen minutes in the dark at room temperature. The tubes were centrifuged at 1500 rpm for five minutes to isolate white blood cells, and the supernatant was discarded. After washing the pellet with 2 mL of phosphate-buffered saline (PBS) and centrifuging again, the supernatant was removed. The remaining cell pellet was resuspended in 500 µL of PBS. Finally, the samples were analyzed using the FACS Aria III flow cytometer (BD Bioscience), with data processed using FACS Diva version 6.1.3 software. In the generated dot plots, the cells were categorized as CD3 (+) CD8 (+) cytotoxic T-cells, CD45RA (+) B-cells, and CD161a (+) NK-cells and their respective percentages were recorded.[22]

Statistical Analysis

To statistically evaluate the histopathological, flow cytometry, blood gas, and ELISA test results obtained at the end of the study, the results were subjected to analysis of variance using the MINITAB release 16.0 (Minitab Inc., PA, USA) program. Duncan's Multiple Range Test was used to see whether the differences between group means were significant. The significance level was accepted as $p<0.05$. To differentiate between the experimental groups: control, PPE, PPE + WE, and PPE + ME, methods such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and heatmap clustering analysis were employed. To estimate the effect sizes, the partial eta squared (η^2) was chosen for this study, because it allows the calculation of variation for more than one variable.[23] In One-Way ANOVA analyses, the partial eta-squared effect size was used to determine the effect size for results that were significant between groups. Partial eta-squared tells us how large an effect the independent variable(s) has on the dependent variable. For partial eta-squared, if $\eta^2 \geq 0.14$, the effects are large; between 0.06 and 0.14, the effects are considered moderate; and when $\eta^2 \leq 0.06$, the effects are considered small.[24]

RESULTS

Lung Histopathological Evaluation

Histopathological tests are the most important indicators of whether COPD occurs in rats after PPE induction. The photomicrographs of general lung histology are presented in Figure 1. The rats without PPE comprised the control group (Figure 1A); the rats with PPE-induced COPD comprised the PPE group (Figure 1B); the rats with COPD exposed to PPE and treated by gavage with the water extract (250 mg/kg body weight/day) of *J. drupacea* fruit for 21 days comprised the PPE-WE group (Figure 1C); the rats with COPD exposed to PPE and treated by gavage with the methanol extract (250 mg/kg body weight/day) of *J. drupacea* fruit for 21 days comprised the PPE-ME group (Figure 1D).

Development of emphysema in the lung tissues of the rats with PPE-induced COPD and treated with the water and methanol extracts of *J. drupacea* fruit is shown in Figure 1E. The emphysema scores in the histopathological evaluation were determined to be 0.667±0.516, 2.400±0.548, 1.286±0.488, and 1.000±0.001 in the control group rats, the PPE group rats, the PPE + WE group rats, and the PPE + ME group rats, respectively. In the method section, detailed information is given about the development of emphysema. In this determination method, the ratio of the lung parenchyma developing emphysema to the normal lung parenchyma was determined as a percentage. Accordingly, the affected area was scored as <25%: score 1, 25-50%: score 2, 50-75%: score 3, >75%: score 4. According to the results of this evaluation, the highest emphysema development was detected in the rats with the

PPE-induced COPD (between 50-75%), while the lowest emphysema development was determined in the control group (<25%). Emphysema in the rats in the PPE + WE, PPE + ME groups decreased compared to the rats in the PPE group. This indicated that both water and methanol extracts of *J. drupacea* fruits were effective in the treatment of COPD. Emphysema in the rats treated with the methanol extracts of *J. drupacea* fruit was lower than that in the group of rats treated with its water extracts. It has been determined that the methanol extracts of *J. drupacea* fruit are more effective in the regression of emphysema. It seems that the methanol extracts of *J. drupacea* fruit may be more effective than the water extracts in the treatment of COPD.

Arterial Blood Gas Analyses in the Rat Bloods

The pH, $PaCO_2$, and PaO_2 results determined in the rats in our study are given in Table 1. As seen in Table 1, the pH was determined as 7.3950±0.0152, 7.3520±0.0045, 2.3714±0.605, 7.3529±0.340, and 7.3400±0.0185 for the rats of the control group, the PPE group, the PPE + WE group, and the PPE + ME group, respectively. The distinction in pH levels between the groups was determined statistically significant ($p<0.05$). The highest pH was determined in the rats in the Control group, followed by the PPE + WE, the PPE, and the PPE + ME groups, respectively. The pH values of the rats, to which the COPD model was applied and which either received treatment or did not, were found to be lower than those of the rats that did not receive the COPD model or any treatment. $PaCO_2$ was determined as 45.317±1.903, 51.520±2.960, 49.757±4.170, and 49.350±2.677 mmHg for the control, the PPE, the PPE + WE and the PPE + ME, respectively (Table 1). While the highest $PaCO_2$ was found in the rats in the PPE group with COPD but no treatment, the lowest $PaCO_2$ was determined in the rats in the control group. $PaCO_2$ in the rats with COPD treated with water and methanol extracts of *J. drupacea* fruits decreased with the treatment process compared to the PPE group and was found to be close to the values in the control group rats. It is seen that $PaCO_2$ values are close to each other in the rats in the PPE + WE and the PPE + ME, treated with the water and methanol extracts. The PaO^2 determined in the rats in this study was 63.800±11.52, 48.750±5.37, 51.833±6.43 and 50.857±5.18 mmHg for the control, the PPE, the PPE + WE and the PPE + ME, respectively (Table 1). The highest PaO_2 values were detected in the control group rats; the lowest PaO_2 values were observed in the PPE group rats, in which COPD was formed but no treatment was applied. In the rats with COPD, an increase in PaO_2 was observed after 21 days of treatment with the water and methanol extracts of *J. drupacea* fruits. It was determined that the increase in PaO_2 between these two groups was higher in the PPE + WE group rats than in the PPE + ME rats and statistically significant ($p<0.05$).

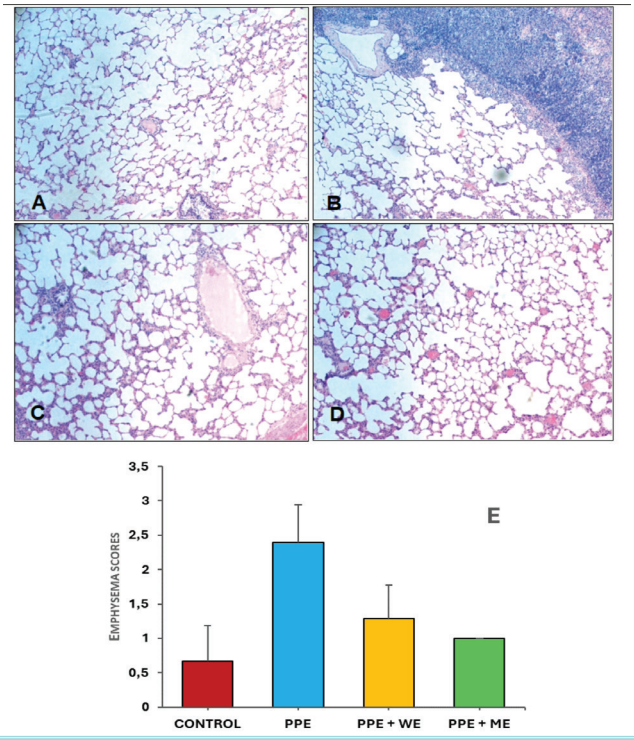


Figure 1. Photomicrographs of general lung histology. (A) Rats without PPE, (B) Rats with PPE, (C) Rats induced COPD with PPE and treated for 21 days by gavage with WE of *Juniperus drupacea* fruit, (D) Rats induced COPD with PPE and treated for 21 days by gavage with ME of *Juniperus drupacea* fruit, (E): Emphysema scores of all rat groups [the effect size (η^2)=0.698, p=0.001).

PPE: Porcine pancreatic elastase, COPD: Chronic obstructive pulmonary disease, WE: Water extract, ME: Methanol extract.

The Effects of Flavan-3-Ols Rich Extracts from *J. drupacea* Fruit on the Cytokine Production in the Rat Bloods

In the present study, IL-8, IL-6, and TNF-α cytokines were also determined, in addition to other markers, to observe the COPD status created with PPE in the rats. The treatment process involved the water and methanol extracts of *J. drupacea* fruits. IL-8, IL-6, and TNF-α were analyzed by the ELISA method. The changes between the groups are also shown in Figure 2. According to these results, IL-6 was determined as 20.633±2.53, 22.563±2.58, 20.753±9.52, and 20.598±2.235 pg/mL for the control, PPE, PPE + WE, and PPE + ME groups of rats, respectively. The highest IL-6 levels were observed in the rats with PPE-induced COPD. It is observed that IL-6 values obtained in the rats of PPE + WE and PPE + ME groups, were quite close to those of the control group. The IL-6 values of rats with COPD, which were treated using *J. drupacea* fruit extracts, decreased to the levels of normal rats without COPD. This indicates that the rats responded positively to the treatment process.

When the IL-8 measured in this study was investigated (Figure 2), it was observed that a similar situation occurred with the IL-6. However, the differences in IL-8 levels were statistically significant ($p<0.05$). The IL-8 in the control, PPE, PPE + WE and PPE + ME groups of rats was

Table 1. The changes in arterial blood5 gas results of the rats with PPE exposure and *J. drupacea* fruit extracts treatment

Groups	pH	$PaCO_2$ (mmHg)[3]	PaO_2 (mmHg)[4]
Control	7.3950±0.0152[a]	45.317±1.903[b]	63.800±11.52[a]
PPE	7.3520±0.0045[b]	51.520±2.960[a]	48.750±5.37[b]
PPE + WE	7.3529±0.0340[b]	49.757±4.170[a,b]	51.833±6.43[b]
PPE + ME	7.3400±0.0185[b]	49.350±2.677[a,b]	50.857±5.18[b]
Effect sizes (η^2)	**0.514**	**0.363**	**0.411**
p-value	**0.009**	**0.026**	**0.016**

[1]Values are expressed as "mean ± standard deviation". [2]There is no statistical difference between the values indicated with the same letter. [3]PaO_2: Arterial oxygen partial pressure, [4]$PaCO_2$: Arterial carbon dioxide partial pressure, [5]Blood gas analyses were performed on the day the study was completed, after the rats were anesthetized and blood was taken from the heart using blood gas injectors and analyzed without delay by the ABL9 blood gas analyzer (Dadiometer, Denmark) in the room at that time. PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

determined to be 174.37±3.71, 222.05±12.54, 210.52±23.49, and 169.71±22.12 ng/mL. The highest levels of IL-8, similar to those of IL-6, were found in the group of rats with PPE-induced COPD. The IL-8 of the control and PPE + ME groups of rats was found to be close to each other. It was seen that IL-8 levels in rats with COPD treated with *J. drupacea* fruit water extract (PPE + WE) were higher than the IL-8 levels in those treated with methanol extract (PPE + ME). This situation gives the result that the methanol extract of *J. drupacea* fruit is more effective at decreasing IL-8.

TNF-α for the control, PPE, PPE + WE, and PPE + ME group rats was determined to be 255.12±25.4, 417.97±108.6, 267.44±36.1, and 259.57±39.5 pg/mL, respectively. The differences in TNF-α were determined to have strong statistical significance ($p<0.05$). As with IL-6 and IL-8, the highest TNF-α was observed in the group of rats with PPE-induced COPD. It was found that the TNF-α of the control group was close to those in the PPE + WE and PPE + ME group rats. TNF-α of the rats with PPE-induced COPD increased compared to the control group, but the values decreased following treatment with *J. drupacea* extracts. These results show that both extracts of *J. drupacea* fruit are effective in decreasing TNF-α.

Effects of Flavan-3-Ols Rich Extracts from *J. drupacea* Fruit on the B, Cytotoxic T- and NK-Cells in the Rat Bloods

The results of B-cells, cytotoxic T-lymphocyte (CTLs), and NK-cells are exhibited in Figure 3. B-cells (%) for the control, PPE, PPE + WE, and PPE + ME groups were detected as 23.940±5.91, 31.980±5.12, 16.800±3.08, and 22.575±4.43, respectively. The differences between the groups in terms of B-cell counts were statistically significant ($p<0.05$). The highest number of B-cells was determined in the rats in the PPE-induced COPD group (31,980), while the lowest was detected in the rats in the PPE + WE group (16,800). B-lymphocyte counts decreased in the rats with PPE-induced COPD, treated by the water and methanol extracts.

The number of NK-cells was determined as 2.8167±1.910, 1.3200±0.286, 2.3714±0.605 and 2.3714±0.605 for the control, PPE, PPE + WE and PPE + ME groups of rats, respectively. The highest number of NK-cells was determined in the rats in the control group, followed by the PPE + WE, PPE + ME, and PPE group rats, respectively. The NK count was the lowest in the rats with PPE-induced COPD.

CTL counts were determined as 11.250±4.57, 16.720±4.52, 17.357±2.88, and 19.400±4.45 (%) for the control, PPE, PPE + WE, and PPE + ME group rats, respectively. The lowest number of CTLs was determined in the control group without COPD, while the highest number was found in the PPE + ME group, rats, (Figure 3). Although there was an increase in CTLs in the rats with PPE-induced COPD compared to the control group, no change was observed as a result of 21-day treatment, with both water and methanol extracts of *J. drupacea* fruit.

PCA, HCA and Heatmap Analyzes Regarding Inflammation Biomarker Values

In this study, statistical methods such as PCA, HCA, and Heatmap analysis were employed to visually and comprehensively evaluate the analyses from different perspectives. PCA mitigates correlations among numerous variables under investigation by reducing them to linear combinations of principal components. In our study, a technique was utilized to visualize differences in inflammation biomarkers such as IL-6, IL-8, TNF-α, B-cells, cytotoxic T-cells, and NK-cells. Figure 4 illustrates a scatter plot (Figure 2A) where points represent the control, PPE,

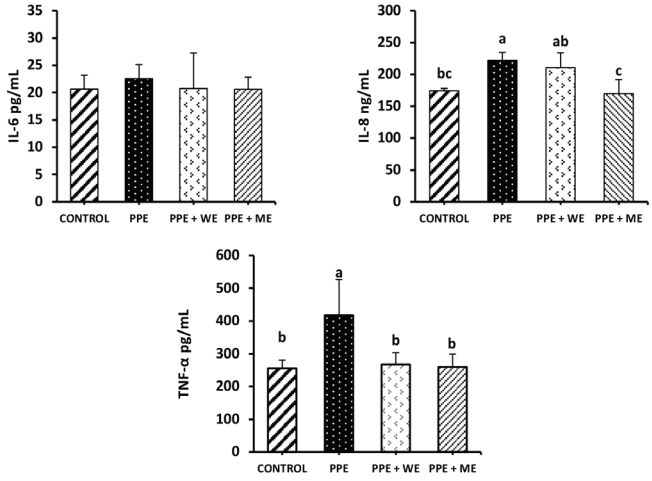


Figure 2. Effect of *Juniperus drupacea* fruit extract on the cytokines in the blood of rats. The levels of IL-8, IL-6 and TNF-α in the rat blood were detected by ELISA. Control: No procedure was applied to the control group, PPE treated, PPE + WE of *Juniperus drupacea*, PPE + ME of *Juniperus drupacea* fruit, Values are expressed as mean ± standard deviation, there is no statistical difference between the values indicated with the same letter ($p<0.05$) [IL-8, the effect size (η^2)=0.632, p=0.001; TNF-α, the effect size (η^2)=0.591, p=0.001].

IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

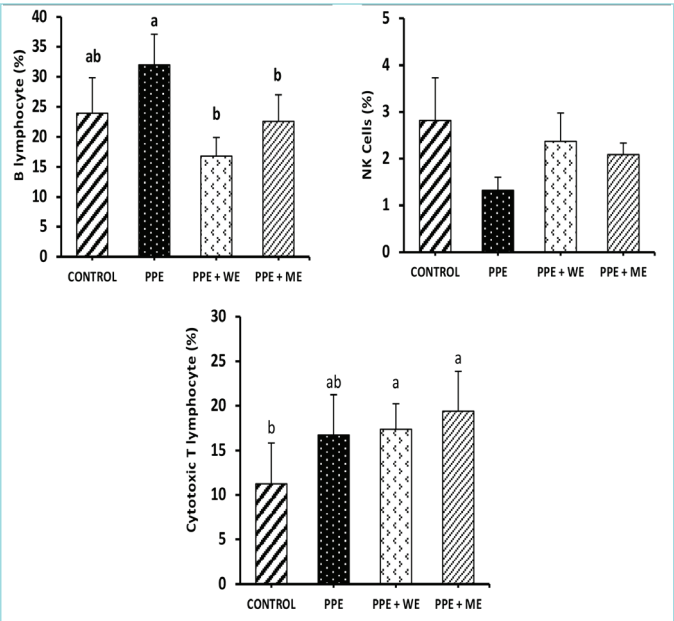


Figure 3. The effect of water and methanol extract treatment of *Juniperus drupacea* fruit on B-cells, CTLs, and NK-cells in the blood of rats. Control: No procedure was applied to the control group, PPE treated, PPE + WE of *Juniperus drupacea* fruit, PPE + ME of *Juniperus drupacea* fruit, values are expressed as mean ± standard deviation, there is no statistical difference between the values indicated with the same letter ($p<0.05$) [B-cells, the effect size (η^2)=0.314, p=0.001; TNF-α, the effect size (η^2)=0.389, p=0.032].

CTLs: Cytotoxic T-lymphocyte, NK: Natural killer, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract.

PPE + WE, and PPE + ME groups, while the vectors represent tested inflammation biomarkers. PC1 explains 61.8% of the variance, and PC2 explains 31.6%, as shown in Figure 4.

The control, PPE + WE, and PPE + ME groups showed close relationships with NK-cells, pH, and pO_2 clustering on the negative side of PC1. In contrast, the PPE group, which exhibits strong association with B-lymphocyte, IL-6, IL-8, emphysema, TNF-α, pCO_2, and CTL, were clustered on the positive right side of PC1 (Figure 4). In Figure 5, it is observed that the control, PPE, PPE + WE, and PPE + ME groups were divided into two clusters based on inflammation biomarkers tested. The first cluster consisted of the control, PPE + WE, and PPE + ME, along with IL-6, TNF-α, emphysema, IL-8, pCO_2, and CTLs. The second cluster consisted of the PPE group along with B-cells, inflammation, NK-cells, pH, and pO_2. This situation indicated that all groups could be clearly differentiated based on the inflammation biomarkers tested. It was understood that PC1 explains 61.8% and PC_2 explains 31.6% of the variance, and that PC1 and PC2 together explain 93.4% of the variance (Table 2). PC1, PC2, and PC3 vectors that contribute to this separation are seen in Table 2.

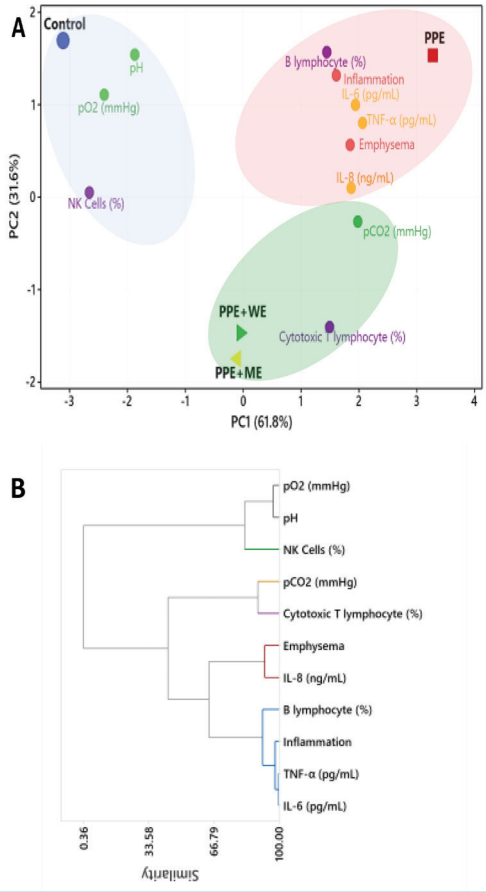


Figure 4. (A) The loading and score plot of PC1 and PC2 describing the changes among the pro-inflammatory cytokines, lymphocytes and blood gas values of the control, PPE, PPE + WE and PPE + ME group rats, (B) Dendrogram obtained through hierarchical cluster analysis.

PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract, NK: Natural killer, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, PC1: The first principal component, PC2: The second principal component.

Table 2. PCA results regarding the evaluation of the effects of water and methanol extracts of *Juniperus drupacea* fruits on inflammation biomarkers in the rats applied with a COPD model

Items	PC1*	PC2	PC3
Eigenvalue	6.80	3.48	0.72
Variance percentage (%)	61.8	31.6	6.6
Cumulative variance (%)	61.8	93.4	100
Eigenvectors			
IL-6 (pg/mL)	**0.324**	0.286	-0.031
IL-8 (ng/mL)	0.291	0.103	**-0.734**
TNF-α (pg/mL)	**0.331**	0.272	-0.003
B-lymphocyte (%)	0.210	**0.386**	**0.503**
NK-cells (%)	**-0.374**	-0.055	-0.234
Cytotoxic T-lymphocyte (%)	0.235	**-0.410**	0.233
pH	-0.270	**0.368**	-0.215
pCO_2 (mmHg)	**0.367**	-0.150	-0.086
pO_2 (mmHg)	**-0.341**	0.243	-0.045
Emphysema	**0.365**	0.148	-0.154
Inflammation	-0.065	**0.525**	0.141

PCA: Principal component analysis, COPD: Chronic obstructive pulmonary disease, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, NK: Natural killer,PC1*: The first principal component, PC2: The second principal component, PC3: The third principal component.

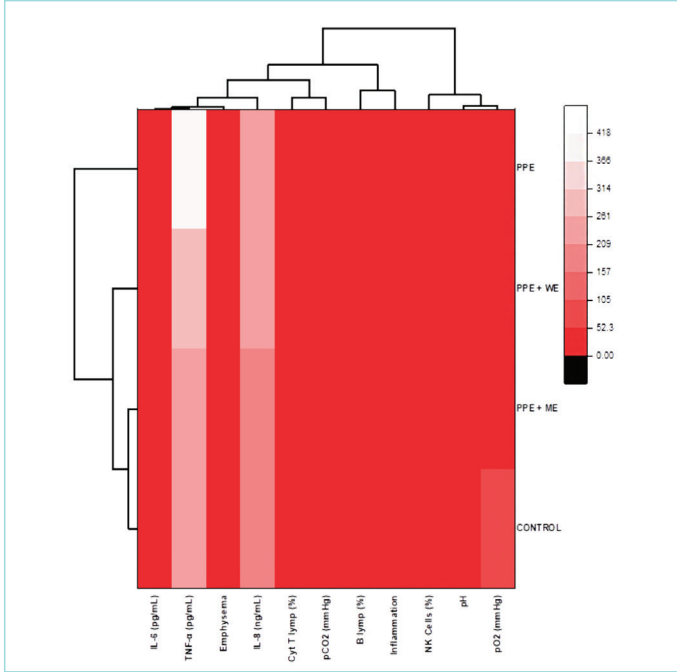


Figure 5. Heatmap obtained through the evaluation of the effects of water and methanol extracts of *Juniperus drupacea* fruits on inflammation biomarkers in rats applied with a COPD model. Heatmap shows the changes and contribution levels of the biomarkers evaluated in the monitoring of the treatment process with the water and methanol extracts of *Juniperus drupacea* fruit to the treatment process of COPD rats.

COPD: Chronic obstructive pulmonary disease, PPE: Porcine pancreatic elastase, WE: Water extract, ME: Methanol extract, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha, NK: Natural killer.

DISCUSSION

In our study, the number of B-cells and CTLs increased, and NK-cells decreased due to inflammation/emphysema in the rats with PPE-induced COPD. At the same time, the levels of IL-6, IL-8, and TNF-α increased with COPD. As a result of the 21-day treatment of the rats with PPE-induced COPD using the water and methanol extracts of *J. drupacea* fruit, the numbers of B-cells and NK-cells, as well as the levels of IL-6, IL-8, and TNF-α, returned to the levels of the control group rats. This study found that both extracts of *J. drupacea* fruit showed a protective effect against lung inflammation and alveolar deterioration caused by exposure to the PPE in rats. It was estimated that this might be due to the high levels of catechins contained in *J. drupacea* fruit extracts, and phenolic acids such as gallic acid (GA), which have been shown to have a positive effect on COPD. The *J. drupacea* fruit extracts are rich in catechins such as catechin, epicatechin, and epicatechin gallate, contain significant amounts of protocatechuic and GA.[19,25,26] Akbulut and Akbulut[19] stated that among the phenolic compounds, catechins such as catechin, epicatechin, and epicatechin gallate, appears to be the most abundant phenolic compound isolated in all extracts and the total of these catechins constitute approximately 79.7-81.5% of all phenolics in *J. drupacea* fruit extracts. In some studies, it was stated that catechins were dominant among phenolic compounds in *J. drupacea* fruit extracts,[19] and in some studies, it was stated that protocatechuic acid was the most abundant phenolic compound.[25,26] It is important to note that different distributions and concentrations of phenolic compounds in *J. drupacea* fruit may vary depending on factors such as geographical location, climatic conditions, and growth and harvest stages of the plant.[19]

Sohn et al.[27] reported that the levels of IL-1β and IL-6, known as pro-inflammatory cytokines, were reduced in the lung BAL fluids of the mice, treated with extracts from a mixture of sixteen medicinal plants (Gamijinhae-tang extract) for acute lung injury. Ahmed[28] observed that epigallocatechin 3-gallate, a catechin group phenolic, suppressed the expression of IL-6 and IL-8 *in vitro.* In the present study, the findings obtained regarding the changes in the levels of pro-inflammatory cytokines IL-6 and IL-8 in the blood of COPD rats treated with *J. drupacea* fruit extracts were consistent with the results obtained from the study of Sohn et al.[27] and Ahmed[28].

In light of the existing body of literature, the assessment of arterial blood gas (ABG) exchange is a primary measure to determine the damage to the alveolar wall and confirm the efficiency of gas exchange in the lungs, which indicates lung injury in rats.[29,30] ABG analysis is a crucial diagnostic tool for assessing the severity of emphysema.[31] Jiang et al.[31] stated that at the end of the treatment process of rats with lipopolysaccharide (LPS)-induced COPD with pyrrolidine dithiocarbamate pH and PaO_2, which are blood gas values, decreased compared to the rats in the control group, $PaCO_2$ increased. In the present study, the severity of emphysema increased significantly rats with COPD due to PPE exposure, and this severity decreased again at the end of the 21-day treatment period with the water and methanol extracts of *J. drupacea* fruit. A similar situation was seen in the blood gas analysis results of rats, as $PaCO_2$ increased and PaO_2 decreased of COPD rats exposed to PPE. However, at the end of 21-days of treatment with both extracts of *J. drupacea* fruit at a dose of 250 mg/kg body weight/day, $PaCO_2$ decreased, and PaO_2 increased, approaching the blood gas values in the control group rats. This shows that the treatment of inflammation/emphysema in the rats with *J. drupacea* fruit extracts helps alleviate the PPE-induced COPD (Table 1).

COPD is a progressive respiratory disorder characterized by permanent airflow limitation, inflammation of the airways, and respiratory symptoms such as shortness of breath, chronic cough, and sputum production. COPD primarily includes two main conditions: chronic bronchitis and emphysema.[27,32]

COPD is a long-term lung condition in which the flow of air in the respiratory system is consistently restricted. This restriction tends to worsen over time and is linked to a heightened, ongoing inflammatory reaction in the air passages and lungs, triggered primarily by harmful substances like cigarette smoke (CS). Inflammation plays a critical role in the pathogenesis and progression of COPD, leading to structural changes in the airways and lung tissue. Chronic inflammation in COPD primarily involves the small airways and lung parenchyma. In response to inhaled irritants like CS, there is an influx of inflammatory cells such as neutrophils, macrophages, and lymphocytes into the airways and lung tissue. These cells release various inflammatory mediators, including cytokines, chemokines, proteases, and reactive oxygen species (ROS), contributing to tissue damage and inflammation.[33,34]

Cytokines such as IL-1, IL-6, and TNF-α are elevated in COPD patients and contribute to the inflammatory response, tissue destruction, and recruitment of immune cells. Chemokines such as IL-8 play a crucial role in recruiting neutrophils to the airways, contributing to airway inflammation and obstruction. Matrix metalloproteinases (MMPs) and neutrophil elastase are proteases released by inflammatory cells that lead to tissue destruction and remodeling in COPD. The chronic inflammatory process in COPD also results in oxidative stress due to an imbalance between ROS and antioxidants. Oxidative stress further exacerbates inflammation and tissue damage in the lungs. Chronic inflammation in COPD triggers structural changes in the airways, including thickening of the airway wall, mucus hypersecretion, and destruction of lung parenchyma (emphysema). This remodeling further exacerbates airflow limitation and respiratory symptoms. Inflammatory responses are amplified during acute exacerbations of COPD, leading to worsened symptoms, increased airway inflammation, and accelerated decline in lung function.[33,34]

IL-8, IL-6, and TNF-α are pro-inflammatory cytokines associated with COPD. These cytokines play a significant role in the inflammatory process and contribute to the pathogenesis and progression of COPD. IL-6 is a pro-inflammatory cytokine that is elevated in COPD patients. It is produced by a variety of cells, including macrophages, T-cells, and endothelial cells. Elevated levels of IL-6 are associated with the severity of COPD and its related comorbidities, including muscle wasting and cardiovascular disease.[35] IL-8 is a chemokine that plays a crucial role in recruiting neutrophils to the airways and promoting inflammation. Increased levels of IL-8 are found in COPD patients and are associated with increased airway neutrophilia, exacerbations, and disease severity.[36] TNF-α is a pro-inflammatory cytokine involved in the regulation of immune cells and inflammation. In patients with COPD, TNF-α levels increase depending on the severity of the disease and are associated with airway inflammation, disease progression, and systemic symptoms.[37] Understanding the role of these cytokines is essential for developing targeted therapies and interventions to manage COPD and improve the quality of life for individuals with this chronic respiratory condition.

B-cells, CTLs, NK-cells, and their involvement in COPD are important aspects of the immune response and its impact on respiratory health.

However, it's important to note that the exact role and mechanisms of these immune cells in COPD may be complex and not fully understood. B-cells play a role in COPD through antibody production and involvement in the inflammatory response. They can produce autoantibodies, such as rheumatoid factor and anti-elastin antibodies, which may contribute to tissue damage in COPD. B-cell activation and antibody production may contribute to chronic inflammation and lung tissue destruction in COPD.[38] CTLs play a crucial role in cell-mediated immune responses, including targeting infected or damaged cells. In COPD, CTLs are involved in targeting and eliminating infected or damaged lung cells, but an excessive CTL response may also contribute to tissue damage and inflammation in the lungs.[39] NK-cells are part of the innate immune response and are involved in early defense against viral infections and tumor cells. In COPD, altered NK-cell activity has been observed, which may contribute to impaired antiviral defense and increased susceptibility to respiratory infections.[40]

Monitoring specific parameters like pH (acidity), PaO_2 and $PaCO_2$ is crucial in managing and assessing the severity of COPD. The pH of blood is an important indicator of the body's acid-base balance. In COPD, the blood pH can be affected due to respiratory acidosis, where there is an accumulation of carbon dioxide in the blood.[41] PaO_2 represents the pressure of oxygen dissolved in the blood. In COPD, impaired lung function often leads to decreased PaO_2 levels, resulting in hypoxemia, which can further worsen the symptoms and prognosis of COPD patients.[42] $PaCO_2$ measures the pressure of carbon dioxide dissolved in the blood. In COPD, the retention of carbon dioxide due to impaired lung function can lead to respiratory acidosis and affect overall blood gas levels.[1] Regular monitoring of these parameters through ABG analysis is critical in managing and adjusting treatment plans for individuals with COPD. It helps healthcare professionals assess the severity of the disease, optimize oxygen therapy, and make appropriate adjustments to ventilation strategies.

The primary approach for treating COPD involves using a combination of medications and non-drug-based strategies. Pharmacological treatments aim to relieve symptoms, improve exercise tolerance, reduce exacerbations, and improve overall quality of life. There are some common classes of medications such as bronchodilators, inhaled corticosteroids (ICS), phosphodiesterase-4 (PDE-4) inhibitors, methylxanthines, antibiotics, oxygen therapy, and vaccinations used in the pharmacological treatment of COPD.[43] In the treatment of inflammatory lung diseases, classical drugs, in combination with different drugs, include inhaled glucocorticosteroids, β2-adrenoceptor agonists, leukotriene receptor antagonists, methylxanthines, theophylline, and others.[27,44-46] However, bronchodilators, ICSs, and PDE-4 inhibitors, which are the classes of drugs used in this treatment, may cause side effects such as tachycardia, tremor, headache, palpitations, increased heart rate, muscle cramps, thrush (oral yeast infection), hoarseness, increased risk of pneumonia, bone density loss with long-term use, nausea, diarrhea, weight loss, depression, and insomnia.[27,47-51] That's why there is a need to develop treatment methods that are more effective, safer, and with little or no side effects. In our study, we observed that exposure to PPE induced structural and functional changes that were typical of COPD, including airway remodeling, alveolar expansion, emphysema, lung inflammation, and increased numbers of lymphocytes (B-cells, CTLs, NK-cells), and the levels of IL-8, IL-6, TNF-α in the rat blood. At the same time, there were changes in the blood gas values of pH, PaO_2, and $PaCO_2$, and as in typical COPD, a decrease in PaO_2 and an increase in $PaCO_2$ occurred. On the other hand, during the treatment process with the water and methanol extracts of *J. drupacea* fruits, the structural and functional changes in the lung caused by the PPE exposure model began to normalize. These results suggest that both extracts of *J. drupacea* fruit, especially methanol extracts, are useful therapeutic agents in preventing these structural and functional changes related to COPD.

Medicinal plants are used in traditional and complementary medicine as an alternative to modern methods, or in combination with them for addressing health problems. Many studies have reported that various extracts of some medicinal plants, such as *Nigella sativa L*. seed extracts,[52] *Myristica fragrans*, *Cinnamomum cassia*, *Camellia sinensis*, and *Curcuma longa* have anti-elastase activity. This is mainly because plant extracts are rich in bioactive compounds, especially phenolic compounds. Green tea (*Camellia sinensis*) is rich in flavonoids such as catechin and epigallocatechin, and these phenolic compounds are known to be elastase inhibitors.[53]

Bioactive phytochemicals with high antioxidant and anti-inflammatory activities may have a healing effect on impaired lung functions. It has been observed that the use of fruits, vegetables, and plants as rich sources of bioactive phytochemicals, can reduce the risk of COPD.[3,54,55] Bioactive compounds found in plants, like phenolic compounds, carotenoids, and alkaloids, inhibit DNA methylation by preventing oxidative stress and inflammation, thereby preventing the progression of COPD.[3,56]

J. drupacea, commonly known as the Syrian juniper, is a species of juniper native to the eastern Mediterranean region, including parts of Türkiye, Syria, Lebanon, Israel, and North Cyprus.[19] It is often used in traditional medicine for various purposes, including respiratory ailments.[6] The phenolic compounds present in *J. drupacea* fruits may include various classes of polyphenols, such as flavonoids, phenolic acids, lignans, and tannins. *J. drupacea* fruits include phenolic acids (GA, protocatechuic acid, p-hydroxybenzoic acid, and p-coumaric acid) and flavonoids (catechin, epicatechin, epicatechin gallate, and procyanidin A2).[19,25,26] Yaglioglu and Eser[57] determined four different phenolic compounds in the cones of four different *Juniperus* species, *J. communis*, *J. excelsa*, *J. foetidissima*, and *J. oxycedrus*, and reported that the most abundant phenolic compound was catechin. Akbulut and Akbulut[19] stated that among the phenolic compounds, catechins such as catechin, epicatechin, and epicatechin gallate, appears to be the most abundant phenolic compound isolated in all extracts and the total of these catechins constitute approximately 79.7-81.5% of all phenolics in *J. drupacea* fruit extracts. In some studies, it has been stated that catechins are dominant among phenolic compounds in *J. drupacea* fruit extracts,[19] and in some studies, it has been stated that protocatechuic acid is the most abundant phenolic compound.[25,26] It is important to note that different distributions and concentrations of the phenolic compounds in *J. drupacea* fruit may vary depending on factors such as geographical location, climatic conditions, and growth and harvest stages of the plant.[19]

Oxidative stress significantly contributes to the development and progression of COPD. Antioxidants, including those found in medicinal plants, can help combat oxidative stress and potentially mitigate the progression of COPD. (-)-epigallocatechin-3-gallate, a flavanol polyphenolic compound, is a potent natural leukocyte elastase inhibitor that can be used to reduce elastase-mediated emphysema.

This flavanol is abundant in green tea and exhibits a dose-dependent, non-competitive inhibition of leukocyte elastase at a non-cytotoxic concentration, being effective in neutrophil culture.[19,58] In a study, the use of (-)-epigallocatechin-3-gallate showed promise in reducing the severity of acute lung injury caused by LPS in mice. This was evident through several positive outcomes: improved lung injury scores; reduced total cell, neutrophil, and macrophage counts; suppressed myeloperoxidase activity; lower wet-to-dry weight ratio of lung tissues; and a decrease in the release of inflammatory cytokines TNF-α, IL-1β, and IL-6.[59] Some flavonoids exert anti-inflammatory effects through blockade of inflammasomes, such as nuclear factor kappa B and NLRP3, suppression of production of pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, TNF-α, and IL-17A, down-regulation of chemokines, and reduction of reactive nitrogen species and ROS.[60] In a study, BAL cellularity, neutrophil recruitment, and BAL MCP-1, IL-6, and TNF-α expressions, lung histological parameters, and platelet uptake increased in rats in which lung inflammation was induced by applying the intratracheal elastase model. However, on the 7th day of treatment with pomegranate peel extract (250 mg/kg body weight), it was determined that MCP-1, MMP-2, and IL-6 levels in the animals decreased to the levels of the animals in the control group, and lung TNF-α and MCP-1 expression decreased significantly.[61] GA is a naturally occurring and abundant phenolic compound in plants that is known to have antioxidant/anti-inflammatory activities. Singla et al.[62] stated that elastase and IL-6 and TNF-α cytokine levels in CS-induced COPD mice were significantly increased compared to control group mice, but after GA treatment, these factors returned to the control group level.

In our study, treatment with water and methanol extracts of *J. drupacea* fruit significantly decreased the levels of IL-6, IL-8, TNF-α, and B-cells in the blood and increased the levels of NK-cells. In particular, treatment with methanol extracts of the plant was more effective in reducing IL-6, IL-8, and TNF-α levels. This suggests that the anti-inflammatory effect of *J. drupacea* fruit methanol extracts can be attributed to the suppression of proinflammatory cytokine production in the lung. It is thought that both extracts of *J. drupacea* fruit may provide beneficial clinical effects in the treatment of COPD, but methanol extract may be more effective in this treatment due to its greater impact on inflammation biomarkers. Although in the present study, the power calculation result showed that the number of animals used was sufficient to convey statistical significance, the sample size of this study was relatively small (n=6), which might have led to misleading data interpretation. To overcome such limitations, additional studies are needed to investigate the effects of *J. drupacea* fruit extracts on lung inflammation.

J. drupacea trees are endemic plants and are widespread in high altitude forests along the Mediterranean coast of Türkiye. From ancient times to the present day, the cones (fruits) of this endemic tree have been collected by local people and used in traditional folk medicine to treat respiratory diseases such as asthma.[6,19] At the same time, its fruits are boiled for a long time to obtain a thick syrup called pekmez (molasses) and are considered a healthful food.[63] The reason why these fruits, which grow widely in forests, are widely used by the public is that they are easily accessible, sustainable and cheap.

Although herbal remedies are among traditional supportive methods used in the treatment of COPD, their natural origin does not guarantee they are safe. Herbal products contain active ingredients, and these can have both beneficial and harmful effects. The use of extracts from the fruits of *J. drupacea* in the treatment of COPD should be closely monitored in future clinical studies; particularly regarding potential side effects such as allergic reactions, including skin rashes, itching, swelling, or respiratory distress; drug interactions; gastrointestinal issues such as nausea, diarrhea, vomiting, or stomach discomfort; hormonal effects; respiratory impacts; and other related adverse effects. Therefore, the possible toxicity and side effects of herbal treatments that may carry risks in terms of individual health status and interactions with other medications should be kept in mind.[64]

The fact that no negative effects have been observed in the use of the fruits of this tree for a long time indicates that the fruits of this plant can be used in the treatment of COPD together with modern drugs. The use of plant extracts in the treatment of COPD should be considered with caution due to the potential for interactions with modern drugs. Herbal products may affect the efficacy and safety of prescription drugs through pharmacokinetic and pharmacodynamic interactions. In this respect, advanced research should be conducted to evaluate the components found in *J. drupacea* fruit extracts from a broader perspective. It is essential to study and reveal the interactions of bioactive components that may be present in the structure of *J. drupacea* fruits with modern drugs used in the treatment of COPD through *in vitro* and *in vivo* studies.

Study Limitations

The findings from animal research do not completely represent the conditions in humans. Therefore, more extensive and detailed studies are needed to assess the therapeutic effects of water and methanol extracts of *J. drupacea* fruits on COPD biomarkers.

CONCLUSION

In this study, the therapeutic effect of water and methanol extracts of *J. drupacea* fruit on PPE-induced COPD was investigated. This study sought to determine with inflammatory mediators whether COPD occurs with PPE, and whether both extracts of *J. drupacea* fruit are effective in the treatment of COPD. For this purpose, the changes in the histopathological tests (emphysema), ABG values (pH, PaO_2 and $PaCO_2$), IL-6, IL-8, TNF-α cytokines, and B-cells, CTLs, NK-cells were observed. In rats with PPE-induced COPD, emphysema increased significantly, PaO_2 decreased, and $PaCO_2$ increased. In addition, with PPE exposure, an increase in B-cells and CTLs, but a decrease in NK-cells, was observed in the blood of rats. At the same time, IL-6, IL-8, and TNF-α levels, which are among the cytokines examined in the current study, increased. This strongly indicates that COPD occurred in PPE-induced mice. In rats with COPD that were treated with water and methanol extracts (250 mg/kg bw/day) of *J. drupacea* fruit for 21 days, via gavage, there is a decrease in IL-6, IL-8, and TNF-α cytokines compared to PPE group rats. These decreases were close to those of the control group rats without COPD. The methanol extracts of *J. drupacea* fruit were determined to be more effective in decreasing IL-6, IL-8, and TNF-α cytokines. In terms of blood gas values, the water extract might be more effective, but the difference between the values is low, and both water and methanol extract affect the result to a similar extent. In addition, with PPE exposure, an increase in B-cells and CTLs, but a decrease in NK-cells, was observed in the blood of rats. According to the results of flow cytometric analysis, the water extracts of *J. drupacea* fruit were more effective on B-lymphocytes than on other cell types. In conclusion, treatment of rats with COPD using *J. drupacea* extracts, which have been determined by studies to be rich in bioactive components, shows that *J. drupacea* extracts can reduce the negative effects of COPD. This shows that *J. drupacea* fruit extracts can be used in the treatment of COPD, but more research is needed.

MAIN POINTS

- In all rats with porcine pancreatic elastase (PPE)-induced chronic obstructive pulmonary disease (COPD), emphysema in the lungs was observed from histopathological evaluation.
- As a result of treatment with water and methanol extracts of *Juniperus drupacea (J. drupacea)* fruit given to PPE-induced COPD rats, emphysema decreased to the control sample levels.
- After PPE-induced COPD, levels of IL-6, IL-8, and TNF-α in the blood, as well as B-cells and NK-cells, returned to levels similar to those in rats without COPD after extract treatment.
- Both extracts of *J. drupacea* fruit responded positively to COPD treatment.

ETHICS

Ethics Committee Approval: The protocols are based on animal experiments approved by the Ethics Committee of Selçuk University Experimental Medicine Research and Application Center (approval number: 2020/52, date: 30.11.2020).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: B.Ç., Concept: H.F.A., H.V., Design: H.F.A., H.V., B.Ç., Data Collection and/or Processing: H.F.A., H.V., M.A., Analysis and/or Interpretation: H.F.A., H.V., H.Ö., Z.E.Ç., M.A., Literature Search: H.F.A., M.A., Writing: H.F.A., M.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-77
Cyprus J Med Sci 2025;10(1):33-37

The Impact of Grand Multiparity on Perinatal and Neonatal Results in Females Over 35 Years of Age

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Abstract

BACKGROUND/AIMS: The purpose of this current research was to evaluate the perinatal and neonatal outcomes among >35-year-old grand multiparous, multiparous, and primiparous pregnant females.

MATERIALS AND METHODS: In this study, a total of 156 patients who underwent pregnancy follow-up and gave birth in the obstetrics clinic between January 2018 and January 2024 were included. The participants were divided into 3 groups based on primiparous (single birth), multiparous (2-4 births), and grand multiparous (5 or more births). The age, parity, type of birth, presence of perineal tears, blood transfusion history, presence of gestational hypertension, and gestational diabetes during pregnancy were scanned retrospectively from the hospital database of the females analyzed in the research.

RESULTS: The mean body mass index of grand multiparous pregnant females was 27.4±3.1 kg/m^2, which was considerably larger than that of the other groups (p=0.032). The gravida number of grand multiparous pregnant females was 6 (5.7) and the parity number was 6 (5.5), which were found to be higher than in the other groups (p=0.012, p=0.008, respectively). The rate of perineal laceration was considerably higher in the primiparous pregnant group than in the other groups (p=0.021). When compared with regard to pregnancy and birth-complications, estimated blood loss volume and >1000 cc bleeding rates were shown to be considerably larger in primiparous-pregnancies than in other pregnancies (p=0.012, p=0.046, respectively). Neonatal intensive care unit need was observed tobe significantly greater in the primiparous pregnant groups than in the other groups (p=0.024).

CONCLUSION: In this current research we showed that grand multiparity (GM) has complication rates similar to other groups and is not a risk factor alone. Advanced maternal age may also be associated with difficulties associated with GM. Pregnancy monitoring and birth should be performed more frequently and carefully to reduce risks in this patient group.

Keywords: Advanced maternal age, multiparity, neonatal, perinatal

To cite this article: Atlıhan U, Yavuz O, Avşar HA, Ata C, Erkılınç S, Bildacı TB. The impact of grand multiparity on perinatal and neonatal results in females over 35 years of age. Cyprus J Med Sci. 2025;10(1):33-37

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Received: 13.08.2024
Accepted: 30.10.2024
Publication Date: 14.03.2025

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INTRODUCTION

Grand multiparity (GM) is generally referred to the pregnant females having a parity of five or more.[1] Some previous studies showed that higher perinatal-mortality, postpartum-hemorrhage (PPH), cesarean section (C/S) rates, placenta-previa, antepartum-hemorrhage, diabetes mellitus, and iron deficiency-anemia, are all considerably higher in GM patients.[2-4] According to some research, there is no difference in the antepartum complication rate between grand multiparous-pregnancies and ordinary multiparous pregnancies.[5,6] Complications associated with GM are less common because GM is not common in developed countries due to socioeconomic and sociocultural factors. GM and its accompanying complications have become a problem and can cause maternal and newborn death and morbidity, especially in developing and underdeveloped countries.[7-10] Gestational diabetes mellitus (GDM), gestational hypertension (GHT), premature birth, and postpartum hemorrhage are among the most common pregnancy complications of grand multiparous females.[7-10] Studies have reported that the prevalence of these complications increases with maternal age.[11] In another study that considered maternal age as a separate risk factor in this respect, socioeconomic status and conditions that trigger cardiovascular disease were not excluded.[12] Advanced maternal age was independently linked with many GM problems.[13] The purpose of this current investigation was to compare perinatal and neonatal outcomes between grand multiparous pregnant women aged 35 years and over and multiparous and primiparous pregnant women.

MATERIALS AND METHODS

The present research was constructed in a retrospective-observational design following the principles of the Declaration of Helsinki. Informed consent documents were obtained from each patient for the current investigation. This research was initiated after receiving ethics committee approval from Buca Seyfi Demirsoy Training and Research Hospital's Ethics Committee (approval number: 2024/292, date: 29.05.2024). A total of 156 pregnant women, whose pregnancy follow-up was performed in the Buca Seyfi Demirsoy Training and Research Hospital, Clinic of Gynecology and Obstetrics and who gave birth in our clinic, from January 2018 to January 2024, were included in the current research. Depending on their parity, the subjects were split into 3 groups: grand multiparous (5 or more births), multiparous (2-4 births), and primiparous (single births). Fifty-two patients in each of the primiparous, multiparous, and grand multiparous groups were analyzed in the research. Females aged >35 years and having a history of singleton pregnancy were included in the study. No perinatal or neonatal risks were detected in the previous pregnancies of the women included in this study. Those <35 years of age, females who had not given birth for >24 weeks, females with multiple-pregnancies, females with consanguineous marriages, females with coagulation disorders, and females whose information was incomplete were not included in the research. The age of the females in the research, gravida, body mass index (BMI) at the time of pregnancy, parity, number of abortions, smoking status, gestational age, type of birth, presence of perineal tear, prenatal and postpartum-hemoglobin and hematocrit scores, postpartum hysterectomy background, hospital stay, blood transfusion background, presence of GHT, and GDM were scanned retrospectively from the hospital database and patient files. Regular cigarette use was defined as 10 cigarettes per day.[14] Fetal congenital anomaly, 1st and 5th minutes Apgar scores, neonatal intensive care unit (NICU) history, birth weight data, and intrauterine fetal death history were evaluated. GDM was constructed according to the criteria established by the American Diabetes Association.[15] GDM is confirmed if one or more of these plasma glucose levels meet or exceeds the specified thresholds.: Fasting: 92 x mg/dL (5.1 x mmol/L), 1h: 180 x mg/dL (10.0 x mmol/L), 2h: 153 x mg/dL (8.5 mmol/L). A 75 g oral glucose tolerance tests (OGTT) test is conducted at 24-28 weeks in pregnant women who have never had diabetes before, and plasma glucose levels are evaluated during the first and second hours of fasting. After a minimum of eight hours of fasting overnight, the OGTT should be performed in the morning. Based on the most recent recommendations from the American-College of Obstetricians and Gynecologists bulletin,[15] GHT was diagnosed.[16] Proteinuria and hypertension together are diagnostic criteria for preeclampsia. After the 20th week of pregnancy, in females with formerly normal-blood pressure, GHT was defined as a systolic-blood pressure of no less than 140 mmHg or a diastolic-blood pressure of no less than 90 mmHg tested four hours apart or more. A systolic-blood pressure of 160 mmHg or greater or a diastolic-blood pressure of 110 mmHg or greater is considered severe hypertension. For the diagnosis of preeclampsia, females with hypertension must also exhibit proteinuria, which is defined as the presence of a minimum of 300 mg of protein in a 24-hour urine collection. Patients who satisfied the requirements for hypertension associated with preeclampsia but did not exhibit proteinuria or any severe additional complications were diagnosed with GHT.[16] Hemoglobin values <11 g/dL were used to diagnose anemia.[17] Delphicriterion was used to diagnose fetal growth restriction.[18] A fetus's death at >24 weeks' gestation was referred to as fetal mortality. Estimated blood loss volume, postpartum-hemoglobin score, and presence of blood transfusion were evaluated in the evaluation of peripartum-hemorrhage. The expected blood loss of >1000 mL was considered excessive. Control hemoglobin levels were measured in all females approximately 12 hours after birth. The estimated volume of blood loss was measured by utilizing the pregnant females's height, weight, and prenatal and postpartum hematocrit values.[19] Blood transfusion indications were determined in terms of vital signs, estimated blood loss volume, and postpartum hemoglobin value <8 g/dL.[19]

Statistical Analysis

Statistical analysis was performed using SPSS 26.0 (IBM-Inc-Chicago-IL-USA). The distribution normality was measured with the Kolmogrov-Smirnov test and Shapiro-Milk test, ANOVA, or Wallis test on the basis of whether the data demonstrated normal-distribution. The Fisher's exact and chi-square tests were employedin the categorical-data analysis. The quantitative-data of the patients were presented as mean ± standard deviation (minimum-maximum). The results were evaluated at a 95% confidence interval. The p-value, <0.05, was regarded as statistically significant. The post-hoc power analysis was performed using the G*Power 3.1 tool (Erdfelder-Faul-Buchner-Düsseldorf-Germany). A total of 144 was determined to be the required sample size.

RESULTS

The average age of the participants for this present research was found to be 38.1±2.5 and the average BMI was 26.2±3.8 kg/m^2. The average BMI of the grandimultiparous patients was; 27.4±3.1 kg/m^2, which was significantly higher than the BMI mean of the primiparous and multiparous patient groups (p=0.032). The mean gravida of the grand multiparous patients was 6 (5.7), and the parity mean was found to be 6 (5.5), which was significantly higher than the mean gravida and parity of the primiparous and multiparous patients (p=0.012, p=0.008; respectively) (Table 1).

The high rate of C/S in multiparous and grand multiparous pregnant females was attributed to the increase in the rate of previous C/S indications. In the primiparous group, one patient had cleft lip and palate, and one patient had umbilical hernia. In the multiparous group, one patient had cleft lip and palate, and one patient had syndactyly. In the grand multiparous group, one patient had trisomy 21. Two patients underwent hysterectomy due to uterine atony after vaginal birth. The rate of perineal laceration was significantly greater in the primiparous group than in the grand multiparous patients (p=0.015). ΔHb level was significantly greater in the primiparous group than in the other groups (p=0.016). When analyzed by comparing the pregnancy and birth complications, primiparous pregnancies had an estimated blood loss volume that was substantially larger than pregnancies that were multiparous (p=0.012). The >1000 cc bleeding rate was significantly greater in the primiparous patient group than in the grand multiparous patients (p=0.046) (Table 2).

The need for NICU was significantly greater in primiparous patients than in grand multiparous patients (p=0.024). Among primiparous patients, 8 (72.7%) patients requiring NICU had a history of vaginal birth. No significant differences were observed across the groups on the basis of Apgar (1st minute) and (5th minute) scores, birth weights, or fetal mortality rates (Table 3).

DISCUSSION

There were no notable differences observed in grand multiparous-pregnant females on the basis of perinatal and neonatal risks compared with other groups in the present study. The presence of perineal laceration estimated blood loss volume and NICU need were significantly higher in primiparous pregnant women. The mean BMI of grand multiparous pregnant women was significantly higher than that of the other groups. Many previous studies have evaluated the effects of GM in pregnancy. However, GM was not evaluated as an

Table 1. Intergroup comparison of demographic data

	Primiparous (n=52) Mean ± SD	Multiparous (n=52) Mean ± SD	Grand multiparous (n=52) Mean ± SD	p
Age (year)	38.0±2.5	38.1±2.6	38.2±2.4	0.860
BMI (kg/m²)	24.4±2.7	25.2±2.6	27.4±3.1	0.032
Smoking	12 (23%)	10 (19.2%)	13 (25%)	0.780
Gravida	1 (1.3)	3 (3.7)	6 (5.7)	0.012
Parity	-	3 (2.6)	6 (5.5)	0.008
Abortion	0 (0.2)	0 (0.1)	0 (0.2)	0.560
Gestational week	38.9±1.5	38.7±1.8	38.8±1.7	0.790

Values are expressed as frequency or percentage. Values are expressed as mean ± standard deviation. Pearson's chi-square test was used. Fisher's exact test was used. BMI: Body mass index, SD: Standard deviation.

Table 2. Intergroup comparison of perinatal outcomes

	Primiparous (n=52) Mean ± SD	Multiparous (n=52) Mean ± SD	Grand multiparous (n=52) Mean ± SD	p
GHT	3 (5.7%)	4 (7.6%)	5 (9.6%)	0.380
Preeclampsia	3 (5.7%)	3 (5.7%)	4 (7.6%)	0.690
GDM	3 (5.7%)	6 (11.5%)	4 (7.6%)	0.160
Vaginal birth	40 (76.9%)	35 (67.3%)	37 (71.1%)	0.480
C/S	12 (23.1%)	17 (32.7%)	15 (28.9%)	
Premature birth	4 (7.6%)	6 (11.5%)	7 (13.4%)	0.410
Perineal laceration	5 (12.5%)	2 (5.7%)	1 (2.7%)	0.021
Anemia	12 (23%)	10 (19.2%)	13 (25%)	0.720
Preoperative-Hb	12.2±1.5	12±1.7	11.9±1.9	0.780
Postoperative-Hb	10.9±1.8	11.3±1.8	11.1±1.7	0.140
ΔHb (preop-postop)	1.3±0.8	0.8±0.6	0.9±0.7	0.016
Estimated volume of blood loss	472 (240-725)	265 (90-520)	350 (125-620)	0.012
>1000 cc bleeding	8 (15.3%)	4 (7.6%)	2 (3.8%)	0.046
Blood transfusion	5 (9.6%)	4 (7.6%)	4 (7.6%)	0.880
Hospital stays	1.4±0.5	1.3±0.7	1.3±0.6	0.710
Hysterectomy	0 (0.0)	1 (1.9)	1 (1.9)	0.560
Congenital anomaly	2 (3.8%)	2 (3.8%)	1 (1.9%)	0.840

Values are expressed as frequency or percentage. Values are expressed as mean ± standard deviation. Pearson's chi-square test was used. Fisher's exact test was used. GDM: Gestational diabetes mellitus, GHT: Gestational hypertension, C/S: Cesarean section, Hb: Hemoglobin, SD: Standard deviation.

Table 3. Intergroup comparison of neonatal outcomes

	Primiparous, (n=52) Mean ± SD	Multiparous, (n=52) Mean ± SD	Grand multiparous, (n=52) Mean ± SD	p
Apgar (1st minute)	8.1±0.9	8.2±0.7	8.1±0.8	0.850
Apgar (5th minute)	8.8±1.15	8.7±1.1	8.7±1.2	0.770
Birth weight (gr)	3100±520	3130±440	3180±500	0.660
NICU	11 (21.1%)	6 (11.5%)	6 (11.5%)	0.024
Fetal death	1 (1.9%)	0 (0.0)	1 (1.9%)	0.560

Values are expressed as frequency or percentage. Values are expressed as mean ± standard deviation. Pearson's chi-square test was used. Fisher's exact test was used. NICU: Neonatal intensive care unit, SD: Standard deviation.

independent risk factor in these studies but was evaluated together with many risks, including age, socioeconomic-status, and smoking status.[20-22] Only women aged >35 years were included in the present study in all groups because the majority of issues observed in grand multiparous pregnancies might be linked to older mothers. Since all patients were >35 years old, we believe that age differences between the groups had little bearing on pregnancy problems, even if a substantial difference in gravida and parity was seen between them. It is particularly important to evaluate the increased risks of GM and prenatal and postnatal complications, independent of age, smoking, socioeconomic status, and ethnic background. Previous studies have reported that the prevalence of complications considered to be linked with GM, (e.g., placenta-previa, preeclampsia, and PPH) increases as maternal age increases.[11,12] It is difficult to distinguish whether the complications in these patients are associated with advanced age or GM because grand multiparous females are likely to be older.[12,23] In previous research, Alsammani et al.[21] reported that many complications were reduced in young grands multiparous females compared with older grand-multiparous patients, and many complications increased when compared with primiparous and multiparous patients who were of the same age. To make this distinction, a preliminary study was conducted on women >35 years of age in the current investigation, and the risks associated with the age factor that might occur between the groups were eliminated. Thus, a suitable environment is provided to evaluate only the risks associated with parity. Studies have reported that a low socioeconomic status is linked to more births.[5,24] Many previous studies investigating the relationship between GM and pregnancy outcomes have reported a lack of prenatal care.[25,26] The socioeconomic status and prenatal care parameters of the patients could not be evaluated in this study due to insufficient data. In the literature, several studies have reported that low birth weight newborns are more likely to have a history of GM.[5,21] There were no noticeable differences in the rates of low birth weight infants between the groups in the present study. Previous studies have shown that the prevalence of hypertensive pregnancy disorders as a pregnancy complication is elevated in grand multiparous patients compared with other patients.[27] No increased risk of GHT and preeclampsia was detected in grand multiparous patients compared with the other groups in the present study. It has been reported in the literature that postpartum bleeding is a common complication in grand multiparous patients.[28,29] It is generally believed that an increase in the number of births causes uterine atony, leading to postpartum hemorrhage.[30] Unlike the literature data, the estimated blood-loss volume in primiparous pregnancies was substantially larger than that in multiparous pregnancies in the current research when evaluated in terms of pregnancy and delivery problems. The bleeding rate of >1000 cc was significantly greater in the primiparous group than in the grand multiparous group. The reason for this was considered to be a significantly higher rate of perineal laceration in primiparous pregnant females in the present investigation and the bleeding caused by this. Previous studies in the literature have shown that the postpartum hemogram values of grand multiparous patients are lower.[27,29] Regarding postoperative hemoglobin levels, no statistically significant differences were observed between the groups in this study. Studies in the literature have reported that the Apgar score is lower in grand multiparous females.[3,21] Al-Shaikh et al.[31] conducted a study on grand multiparous patients and reported that they had similar perinatal and neonatal-risks when compared to other groups after adjusting for age. No significant differences were detected in this present investigation between the 1st and 5th-minute Apgar scores between the groups. However, the requirement for NICU was significantly higher in the primiparous patient group. It is considered that this might have occurred because of fetal distress secondary to prolonged labor in primiparous patients. Because of the age group selected in this study, eliminating the effect of age risk, which is known to be a significant risk factor for perinatal and neonatal outcomes, and focusing only on parity might be regarded as this current study's strength.

Study Limitations

The most important limitation of this study was that the data collected were restricted to what could be found in the records of patients because it was a retrospective study. Another limitation was that no data were available on factors that affect pregnancy outcomes, such as socioeconomic status and pre-pregnancy care.

CONCLUSION

Our study showed that GM pregnancy is not an independent risk factor; rather, perinatal and neonatal complication rates are comparable to those of nulliparous and multiparous pregnancies. We believe that poor prenatal care, low socioeconomic status, and advanced age may be linked to a number of GM-related complications. Pregnancy monitoring and birth should be performed more frequently and carefully to reduce the risks in these patients.

MAIN POINTS

- Grand multiparity is not an independent risk factor, and perinatal and neonatal complications are similar to nulliparous and multiparous pregnancies.
- We believe that a number of Grand-multiparity complications may be related to low socioeconomic-status and advanced age.

- To reduce risks in grand multiparous patients, pregnancy follow-up should be performed more frequently and carefully.

ETHICS

Ethics Committee Approval: This research was initiated after receiving Buca Seyfi Demirsoy Training and Research Hospital's Ethics Committee approval (approval number: 2024/292, date: 29.05.2024).

Informed Consent: Informed consent documents were obtained from each patient for the current investigation.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: U.A., S.E., Concept: O.Y., C.A., T.B.B., Design: U.A., H.A.A., Data Collection and/or Processing: U.A., S.E., Analysis and/or Interpretation: U.A., O.Y., Literature Search: C.A., T.B.B., Writing: U.A., O.Y., S.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2025.2024-68
Cyprus J Med Sci 2025;10(1):38-43

Gender Authorship Trends of Review Articles in the Ophthalmology Literature from 2000 to 2022

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Abstract

BACKGROUND/AIMS: To determine the gender distribution of authors and the change in this distribution between 2000 and 2022 in review articles published in the ophthalmology literature.

MATERIALS AND METHODS: The PubMed database was scanned using "Review", "Systematic Review", and "Meta-Analysis" as filters. Articles published in 71 major ophthalmology journals between 2000 and 2022 were included in the study. Genders of the first and last authors, and the countries of their institutions were extracted using the gender application program interface (https://gender-api.com) and MATLAB data analysis software.

RESULTS: A total of 16,711 review articles were published from 2000-2022, and 64,419 authors were evaluated within the scope of our study. Of these, 5,578 (33.4%) first authors and 4,081 (24.5%) last authors were female. In 2000, 8.6% of first authors and 6.0% of last authors were women. By 2022, this percentage had increased to 39.8% and 30.6%, respectively. The increasing trends in the rate of females becoming both first and last authors were statistically significant, and the difference between the slopes of the regression curves by analysis of covariance was so as well ($R=0.861$, $p<0.001$ for first authors and $R=0.781$, $p<0.001$ for last authors, respectively). In addition, there was a significant relationship between the gender identity of the first and last authors ($p<0.001$).

CONCLUSION: Our study reveals a trend towards resolving gender inequality in the field of ophthalmology. This novel finding is encouraging; however, we believe these developments are insufficient.

Keywords: Gender, gender disparity, authorship, ophthalmology, review

INTRODUCTION

Throughout the world, women may face unfair and unequal treatment simply because of their gender due to social prejudices and conservative perspectives. Unfortunately, the female gender still poses an obstacle to professional and academic progress in the medical field. However, recent studies present promising results, that reveal this situation is changing for the better in the field of medicine, especially in ophthalmology. In a study conducted in 2019 for the first time in the history of the United States (US), the rate of female students exceeded the rate of male students, reaching 50.5%. In addition, the proportion of female ophthalmology residents increased from 25% in 2017 to 41%.[1-3]

Despite these encouraging developments, 90% of department chairs of ophthalmology remain male, and just 28.0% of ophthalmology faculty members are women.[4,5] Although there has been an increase in the proportion of female ophthalmology doctors in recent years, the proportion of women editors in ophthalmology journals still lags significantly.[6,7] Moreover, in ophthalmology publications, female authors are considerably less than male authors.[8-10] Revealing this gender inequality and understanding its causes is crucial in changing the current situation and ensuring gender equity.

To cite this article: Özcan D. Gender authorship trends of review articles in the ophthalmology literature from 2000 to 2022. Cyprus J Med Sci. 2025;10(1):38-43

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Received: 09.07.2024
Accepted: 17.01.2025
Publication Date: 14.03.2025

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Despite publications examining gender inequality among major ophthalmology journals, and more comprehensive publications examining many such journals, in the literature, none of these has examined review articles.[8-16] Review articles are typically invited, and such invitations are limited to prominent names in the field and prestigious positions in the clinic, both of which are dominated by males. Thus, for the first time, in the current study, we specifically aimed to show the gender distribution of authors and the change in this distribution over time in review articles published in the ophthalmology literature between 2000 and 2022. We believe that examining review articles is imperative to truly understand gender inequity in the ophthalmology field.

MATERIALS AND METHODS

The PubMed database was scanned using the selected filters: "Review", "Systematic Review", and "Meta-Analysis". Articles published in 71 major ophthalmology journals, with the highest impact factor according to Web of Science metrics, between 2000 and 2022 were included in the scope of the study (Supplementary Table 1).

MATLAB (version R2020a) (MathWorks, Natick, MA) was used to download articles from PubMed. Using the program, the names of the first and last authors and the countries of their institutions were determined, and the total numbers of authors and articles were evaluated. The Gender application program interface (API) (https://gender-api.com) was used to determine the genders of first and last authors based on their first names. Gender-API returns female, male, or undetermined for each given first name. This algorithm is the most accurate gender assignment program with over 98% accuracy. Authors with unknown gender were excluded. When only one author was listed, they were assigned to the first author cohort and excluded from the last author group.[17,18]

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics (version 28.0). Descriptive statistics, including the mean ± standard deviation, median (interquartile range), and median (minimum-maximum), are presented in terms of frequency distributions. The assumption of normal distribution of data was tested by the Kolmogorov-Smirnov test. Independent sample t-tests were performed for comparison of the data. Relationships between authors' genders were examined with Pearson's chi-squared test. Linear regression analysis was used to examine the trend in the proportion of female authors over the years. The slope of the regression curves was compared by analysis of covariance (ANCOVA). The significance level was considered at 0.05 in the analyses, and results with $p<0.05$ were interpreted as statistically significant.

RESULTS

A total of 17,225 review articles with 64,419 authors, published between 2000 and 2022, were evaluated within the scope of our study. The gender of the first author was assigned in 16,711 articles, where it was determined that only 5,578 (33.4%) of the first authors were female. Of the 16,669 review articles in which the gender of the last author could be determined, only 4,081 (24.5%) last authors were female.

Table 1. Relationship between the first and last authors' gender

Gender of first author	Gender of last author		p
	Male	Female	
Male	8,853 (71.8%)	2,054 (51.4%)	<0.001*
Female	3,480 (28.2%)	1,942 (48.6%)	

*Pearson chi-square test.

As shown in Table 1, 51.4% of first authors were male if the last author was female, but only 28.2% of first authors were female if the last author was male. The relationship between the first and last authors' genders of the articles was statistically significant ($p<0.001$).

In addition, when we compared the average number of authors based on the gender of the first author, we found that the average number of authors in publications with male first authors (3.6±2.8) is statistically significantly lower than that in those with female first authors (4.0±3.0) ($p=0.02$).

When the distribution of authors by gender was examined over time, we found an increase in the number of first and last female authors in parallel with an increase in the number of publications. While the rate of females becoming first authors was 8.6% in 2000, this rate has increased over the years and reached 39.8% in 2022. When gender distributions were examined by the last authors, the proportion of females increased from 6.0% in 2000 to 30.6% in 2022 (Figure 1).

The increasing trends in the numbers of females as both the first author and the last author are statistically significant, and there is a significant difference between the slopes of the regression curves ($R=0.861$, $p<0.001$, and $R=0.781$, $p<0.001$, respectively, ANCOVA) (Figure 2).

The five countries that published the most review articles with female first and last authors and the highest proportion of female authors are shown in Table 2, Figure 3. Considering both the total number of authors and the number of female authors, the US ranked first, and the United Kingdom (UK) ranked second regarding the number of first and last authors. Although Chinese and Indian institutions published fewer ophthalmology review articles, these reviews had a slightly higher female-to-male gender ratio for the first and last authors than reviews from the US and the UK.

DISCUSSION

Women face many inequalities in both their social and professional lives in almost every country in the world simply because of their gender. Unfortunately, this remains an issue for female doctors. Especially in surgical specialties such as ophthalmology, women's professional advancement is hindered because of both the demanding working conditions and the historically male-dominated hierarchy.[19]

Despite promising findings indicating increased women's dominance in ophthalmology, studies from 2019 and 2021 in the US revealed that professional women's expectations for promotion are significantly lower than those of men.[20,21] Although there are many reasons for this, the most important reasons may include the continued male dominance in leadership positions, an inability to find female mentors, ongoing male gender-related pressures in surgical specialties, a bias towards hiring men, and the demands of child care necessitating time away from the clinic.[22-25] As a woman, one needs tremendous effort to progress in a field that requires sustained practice and productivity over an extended period to become a skilled surgeon or to advance as an academic. However, despite these difficulties, studies show that women are more considerate and accommodating than men. Many studies conducted between 1989 and 2014 demonstrate that women prioritise patient care

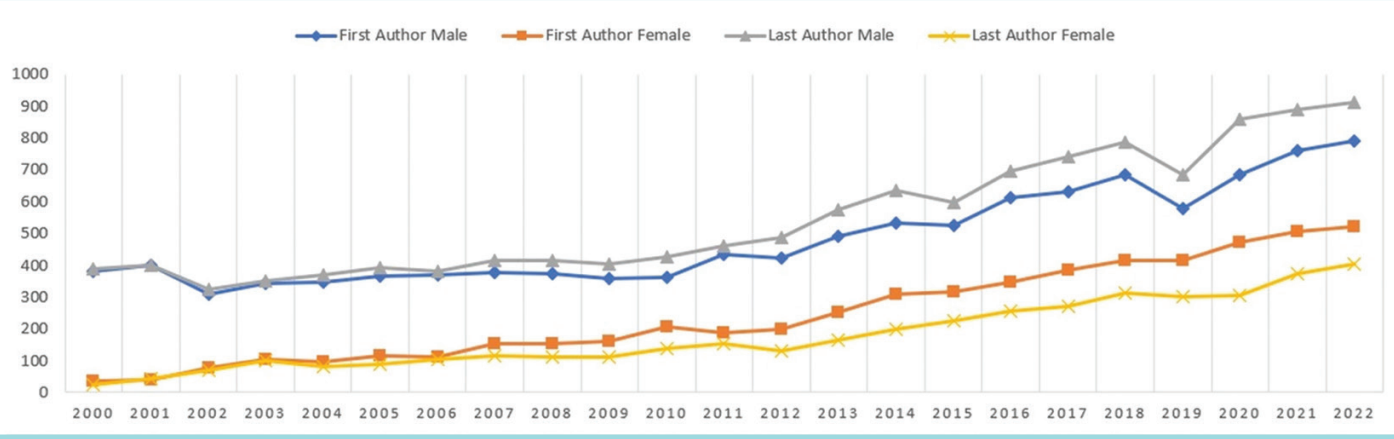


Figure 1. Change in the number of the first and last authors by gender over the years.

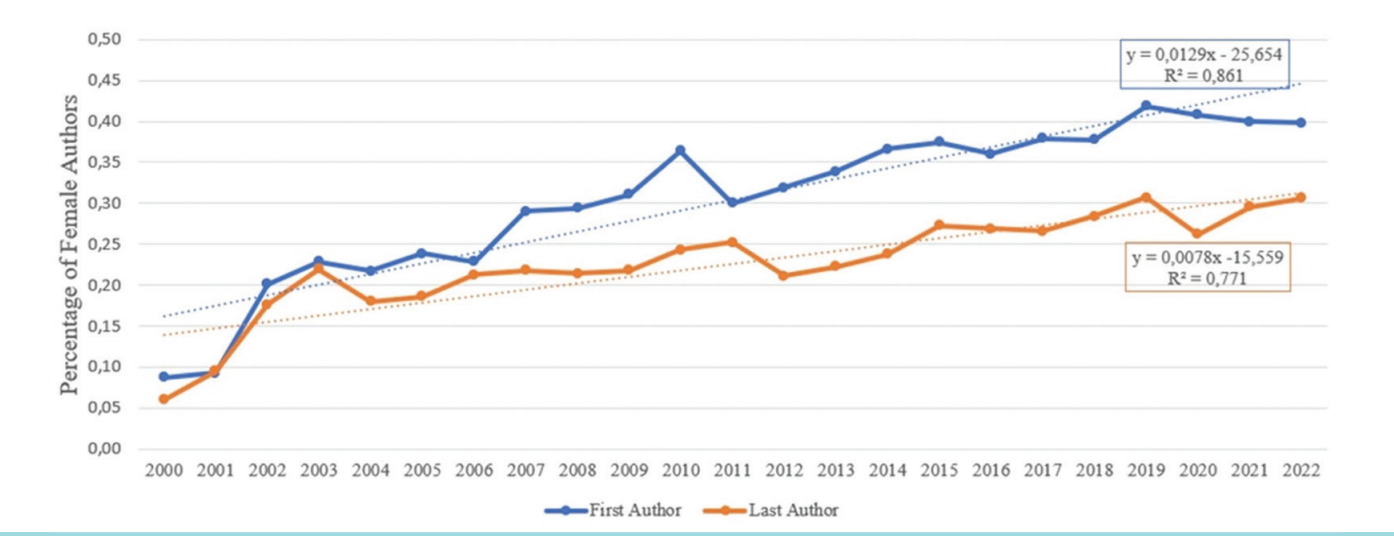


Figure 2. Ratio of female authors over the years.

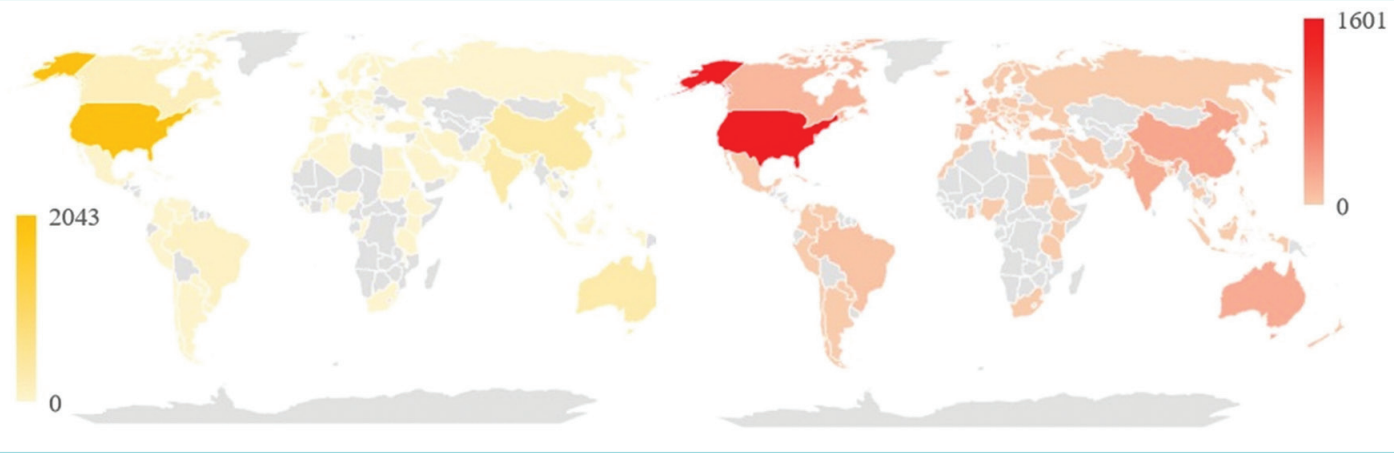


Figure 3. Distribution map of female authors by countries (yellow indicates the first authors, red indicates last authors).

Table 2. Top 5 countries list by number of the first and last female authors

Country	Article count, (n)	Female first author, (n, %)	Article count, (n)	Female last author, (n, %)
United States	6,176	2,043 (33.1%)	6,378	1,601 (25.1%)
United Kingdom	1,470	431 (29.3%)	1,513	295 (19.5%)
China	1,053	413 (39.2%)	960	279 (29.1)
Australia	934	329 (35.2%)	956	241 (25.2)
India	831	325 (39.1%)	794	228 (28.7)

and trainees' education, over academic progress, and consider their family and social responsibilities equally important to their progress in their professional lives.[25-28] In addition, a 2011 study by Reed et al.[26] shows that women's academic production increases significantly after the early stages of their professional lives. Unfortunately, the demands of surgical and academic life cause this accommodating attitude of women to hinder their professional advancement. Many current studies examining gender inequality in the ophthalmology literature have found promising results indicating that the number and proportion of female authors are increasing.[8-16] These studies that evaluated changes in first and last author genders over time were conducted using similar methods to our study. Although the studies show that the number and ratio of female to male both first and last authors are increasing, the increase in the ratio of female to male last authors is significantly lower than the increase in the ratio of female to male first authors in all studies. In addition, two comprehensive studies that scanned publications from 2000-2009 and from 2015-2019 showed a significant correlation between the gender of the first and last author. The number of publications in which both the first and last author were male was significantly higher than those with female first and last authors.[9,10]

Typically, the first authors are young physicians, whereas the last authors usually hold senior positions in the clinic, are established in the field, and are predominantly male. Consistent with this, studies investigating gender inequality in the ophthalmology literature revealed that, although the increase in the proportion of female first authors is encouraging, the change in the proportion of female last authors is less impressive. Our study was based on the assumption that writing an ophthalmology review article requires a journal invitation and experience, i.e., working in the field for a long time and holding a leadership position in the clinic. Thus, to further our understanding of gender inequality in the field of ophthalmology, we specifically analyzed the authorship gender trends of review articles from 2000 to 2022. We evaluated 17,225 articles written by 64,419 authors, published in 71 major ophthalmology journals, and found that the percentage of female first authors was 33.4%, similar to previous results in the literature, whereas that of female last authors was only 24.5%. In addition, we detected a high degree of correlation between male individuals serving as both first and last authors. However, the fact that we identified a statistically significant increase in the proportion of female authors from below 10% on average in 2000 to over 30% in 2022 gives us hope. Despite the significantly increasing number of female ophthalmologists, as reflected by the increase in the number of female authors of review articles, the high percentage of male clinical leaders and journal editors indicates continued bias and inequality. In addition, the fact that articles with female first authors included more authors overall, articles with female last authors more often have male first authors, shows that the accommodating attitude of women continues despite all the difficulties they experience. If this injustice persists for ophthalmologists, who are among the most intelligent and hard-working individuals, receive the highest level of education, and as professionals, communicate with all segments of society, it is clear that gender inequity desperately needs to be addressed at all levels of society.

Study Limitations

Our study had several limitations: It was limited to accessible articles, and those articles not in online indexes could not be evaluated. Our study only included articles from 2000; data older than that were left unanalysed. Owing to the study design and the lack of analyses, such as the independent and comparative evaluation of general ophthalmology journals and subspecialty specific ophthalmology journals, we could not draw any firm conclusions about the significance of the prevalence and correlations described. Moreover, the fact that the proportion and number of women working in ophthalmology clinics are not known exactly may have caused incomplete evaluation of our results. Although our study excluded publications in which we could not determine the gender of the authors from their first and last names, our results are similar to those published in the literature. However, if the author changed their last name during the academic career, they might be considered two different authors and may have caused errors in our results. In addition, scanning articles from various countries may have caused our results to be affected by economic and cultural differences.

CONCLUSION

In conclusion, we believe that our results are crucial because our study is the first to specifically examine the gender of authors of review articles in the ophthalmology literature, as well as the changes in gender over the past two decades. Although the results of our study revealed promising developments regarding gender equality in the field of ophthalmology, we believe that more progress is required. We conclude that our study yields clinically important results that may serve as the basis for further studies in this field.

MAIN POINTS

- Review articles are typically invited, and such invitations are limited to prestigious individuals and prestigious clinical positions, both of which are dominated by males.
- In the current study, we specifically aimed to show the gender distribution of authors and the change in this distribution over time in review articles published in the ophthalmology literature between 2000 and 2022, considering that examining these articles is imperative for understanding gender inequity in the ophthalmology field.

- Although the results of our study revealed promising developments regarding gender equality in the field of ophthalmology, we believe that more progress is required.

ETHICS

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

FOOTNOTES

Financial Disclosure: The author declared that this study had received no financial support.

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Supplementary Table 1. Journals reviewed in alphabetical order
1. Acta Ophthalmologica
2. Advances in Ophthalmology
3. American Journal of Ophthalmology
4. Annals of Eye Science
5. Annual Review of Vision Science
6. Arquivos Brasileiros de Oftalmologia
7. Asian Journal of Ophthalmology
8. Asia-Pacific Journal of Ophthalmology
9. BMC Ophthalmology
10. BMJ Open Ophthalmology
11. Canadian Journal of Ophthalmology
12. Chinese Journal of Ophthalmology
13. Clinical and Experimental Ophthalmology
14. Clinical Experimental Optometry
15. Clinical Ophthalmology
16. Contact Lens & Anterior Eye
17. Cornea
18. Current Eye Research
19. Current Ophthalmology Reports
20. Current Opinion in Ophthalmology
21. Documenta Ophthalmologica
22. European Journal of Ophthalmology
23. Experimental Eye Research
24. Eye
25. Eye & Contact Lens
26. Eye and Vision
27. Graefe's Archive for Clinical and Experimental Ophthalmology
28. Indian Journal of Ophthalmology
29. International Journal of Ophthalmology
30. International Journal of Ophthalmology
31. International Journal of Retina and Vitreous
32. International Ophthalmology
33. International Ophthalmology Clinics
34. Investigative Ophthalmology and Visual Science
35. JAMA Ophthalmology
36. Japanese Journal of Ophthalmology
37. Journal of Cataract and Refractive Surgery
38. Journal of Current Ophthalmology
39. Journal of Eye Movement Research
40. Journal of Glaucoma
41. Journal of Ocular Pharmacology and Therapeutics
42. Journal of Ophthalmic Inflammation and Infection
43. Journal of Ophthalmic Vision Research
44. Journal of Ophthalmology
45. Journal of Refractive Surgery
46. Journal of the American Association for Pediatric Ophthalmology and Strabismus
47. Journal of Vision

Supplementary Table 1. Continued
48. Korean Journal of Ophthalmology
49. Middle East African Journal of Ophthalmology
50. Molecular Vision
51. Ocular Immunology and Inflammation
52. Ocular Surface
53. Oman Journal of Ophthalmology
54. Ophthalmic Epidemiology
55. Ophthalmic Genetics
56. Ophthalmic Plastic and Reconstructive Surgery
57. Ophthalmic Research
58. Ophthalmic Surgery, Lasers Imaging Retina
59. Ophthalmologica
60. Ophthalmology
61. Ophthalmology and Therapy
62. Optometry and Vision Science
63. Orbit
64. Pakistan Journal of Ophthalmology
65. Perception
66. Progress in Retinal and Eye Research
67. Retina
68. Seminars in Ophthalmology
69. Survey of Ophthalmology
70. The British Journal of Ophthalmology
71. Turkish Journal of Ophthalmology

RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-45
Cyprus J Med Sci 2025;10(1):44-50

Comparison of the Effects of Different Fixation Methods on Fragments and Temporomandibular Joint in Sagittal Split Ramus Osteotomy Applied to Patients with Mandibular Asymmetry Using Three-Dimensional Finite Element Analysis

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Abstract

BACKGROUND/AIMS: Fixation of the mobilized bone fragments is of importance for the healing of the patients and stabilization of the osteotomy in the postoperative period. In our study, models with different degrees of asymmetry were fixed with different fixation methods and the results were evaluated.

MATERIALS AND METHODS: Models with right rotations of 2, 5, and 10 mm were fixed with three bicortical screws and two miniplates with four monocortical screws, and the results were compared with those of finite element analysis (FEA). Tension and compression stresses in the bone segments and temporomandibular joint of two different fixation methods were compared.

RESULTS: FEA showed that the tensile and compressive stresses in the buccal, lingual, and temporomandibular discs were higher with bicortical screws.

CONCLUSION: It was predicted that stabilization problems would increase with increasing motion in sagittal split osteotomy. When selecting the most stable fixation method, the least stress to the surrounding tissues should be taken into consideration.

Keywords: Three-dimensional finite element analysis, fixation systems, orthognathic surgery, sagittal split ramus osteotomy

INTRODUCTION

Mandibular osteotomies are surgical procedures performed on the ramus, corpus, and symphysis to treat mandible anomalies. Split osteotomy, which consists of osteotomies that separate the medial and lateral cortices of the ramus in the mandible.[1] Sagittal split ramus osteotomy (SSRO) was first described by Trauner and Obwegeser[2]. Dalpont modified the technique in 1961 by positioning the buccal osteotomy behind the 2nd molars to increase the contact between the bone segments. In 1968, Hunsuck described the osteotomy procedure that is commonly used today, which involves terminating the lingual osteotomy just behind the lingula, extending to the posterior border of the ramus.[1,2]

In SSRO, the mandible is segmented into two independent fragments, the proximal fragment and the distal fragment. In this technique, the distal bone fragment, which becomes free, is moved in a 3-dimensional plane. With a pre-prepared guide plate (occlusal splint) placed on the

To cite this article: Özlü M, Altundoğan S, Öztürk Kavuncu S. Comparison of the effects of different fixation methods on fragments and temporomandibular joint in sagittal split ramus osteotomy applied to patients with mandibular asymmetry using three-dimensional finite element analysis. Cyprus J Med Sci. 2025;10(1):44-50

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Received: 23.05.2024
Accepted: 16.11.2024

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maxilla, the mandible is positioned in the required position and fixed with an appropriate fixation method.[3,4] Osteosynthesis methods in rigid internal fixation used in maxillofacial surgery consist of titanium miniplates and monocortical and bicortical screws.[5]

The most important factors affecting the success of SSRO are stabilization, fixation instruments, and passive fusion of the bone fragments. As the movement speed increases, the changes in neighboring tissues and the stress on the tissues and fixation instruments also increase. Increased stress can also lead to changes in the sizes of miniplates, screws, and tissues.

Patients with mandibular asymmetry are treated with SSRO by changing the facial view, occlusion, and masticatory mechanics. With changes in the position of the mandible, the temporomandibular joint disk changes over time. The fixation methods used in osteotomy of the mandible lead to stress in the mandibular fragments and the temporomandibular joint. The stress on the mandible and temporomandibular joint increases with an increase in the amount of movement, which may lead to postoperative relapse, resorption, and temporomandibular joint disorders. The lack of a definite consensus on fixation methods directs surgeons to perform fixation based on observation and experience during the operation.[6]

Three-dimensional finite element analysis (FEA) and two-point biomechanical testing are the most important *in vitro* study designs developed to examine the reliability and effectiveness of fixation techniques. The FEA technique is a research method that can simulate the complex biomechanical analysis of the mandible close to reality and can change the direction and intensity of the forces applied on the model.[7] In studies conducted with three-dimensional FEA, both time and cost savings are achieved, and the results obtained in simulations are reported to be compatible with clinical studies.[8]

In this study, models with different degrees of asymmetry will be fixed with different fixation methods using the Hunsuck method with SSRO and right rotation of the mandible model. The results will be evaluated by performing static linear analysis with the three-dimensional finite element method on models with 2 mm, 5 mm, and 10 mm right rotation in three different cases. This study aimed to compare the stress caused by different fixation methods on the bone segments and temporomandibular joint in different magnitudes of rotation movements compared with the FEA method.

MATERIALS AND METHODS

This study was conducted at the Ankara University. The Declaration of Helsinki was complied with, and approval was obtained from Ankara University Faculty of Dentistry Ethics Committee (approval number: 36290600/42/2023, date: 19.06.2023). This study was designed in the format of FEA, and the materials used in the study were provided through the Human Visible Project. No volunteer data were used, and no consent form was required.[9]

In this study, six three-dimensional models with SSRO were fixed with two bilateral, four-monocortical screws and flat miniplates and three bicortical screws after 2 mm, 5 mm and 10 mm rotation movements. The effects on the buccal and lingual segments and the temporomandibular joint were then examined using three-dimensional modeling and FEA.

In this study, the tensile and compression forces on various regions of the mandible caused by the plate and screw systems applied for fixation were evaluated. The analysis focuses solely on the forces and changes resulting from the plates and screws without any external forces being applied to the mandible.

The tomography images needed to create the three-dimensional models in the study were obtained from the Human Visible Project conducted by the US National Library of Medicine.[9] The tomography images obtained from the Visible Human Project were scanned with a cross-sectional interval of 1 mm, and the ".stl" files of the three-dimensional toothed mandible model were transferred to 3D-Doctor (Able Software Corp., MA, USA) software in DICOM 3.0 format.[9] After simplification and reformatting processes were applied to the images with 3D-Doctor software, the images were transferred to Rhinoceros 4.0 (3670 Woodland Park Ave N, Seattle, WA 98103 USA) 3D modeling software for 3D modeling. In the Rhinoceros program, osteotomies in accordance with the Hunsuck technique were simulated on the mandible model and 2 mm, 5 mm and 10 mm rotation movements were made to the distal segment. After creating the models of the plates and screws, the models were placed in the correct coordinates in three-dimensional space, and the modeling process was completed. The models created using Rhinoceros software were converted into geometric models, and a mesh structure was created using VR Mesh Studio (Virtual Grid Inc. Bellevue City, WA, USA) software to prepare them for analysis. Rhinoceros 4.0 (3670 Woodland Park Ave N, Seattle, WA 98103 USA) 3D modeling software, VRMesh Studio (VirtualGrid Inc, Bellevue city, WA, USA), and Algor Fempro (ALGOR, Inc. 150 Beta Drive Pittsburgh, PA 15238-2932 USA) analysis software was used for editing and homogenizing the 3D mesh structure, creating the 3D solid model, and finite element stress analysis.

The physical properties of the modeled cortical bone (Erkmen et al.[14]), spongiose bone (Erkmen et al.[14]), tooth (Ammoury et al.[15]), Ti-6 Al-4V (Bataineh and Janaideh[16], Shu et al.[18]), zygomatic process (Mirow et al.[17]), articular cartilage (Mirow et al.[17]), temporomandibular ligament (Li et al.[19]), and disc (Li et al.[19]) are shown in Table 1.

Statistical Analysis

Evaluation of Finite Element Stress Analysis Results

Since the values obtained as a result of finite element stress analysis are the result of mathematical calculations without variance, statistical

Table 1. Physical properties of the materials

	Young module (Mega Pascal)	Poisson ratio	References
Cortical bone	13,700	0.3	Erkmen et al.[14]
Spongious bone	1,370	0.3	Erkmen et al.[14]
Tooth	20,000	0.3	Ammoury et al.[15]
Ti-6 Al-4V	116,000	0.34	Bataineh and Janaideh[16]
Zygomatic bone	1,000	0.3	Mirow et al.[17]
Articular cartilage	0.79	0.49	Shu et al.[18]
	Matrix material constant	**Compression module**	
Temporomandibular ligament	6	0	Li et al.[19]
Disc	0.770562	1.41	Li et al.[19]

analysis cannot be performed. The precise evaluation and interpretation of the amount and distribution of stress in cross-sectional images and nodes is important.

In the analysis results, positive and negative values indicate tensile and compressive stresses, respectively. If the absolute value of a stress type is greater for a stress element, the stress element is under the influence of that stress type and should be evaluated.

The models with fixation with three linearly placed bicortical screws and two double-sided mini-plates with four monocortical screws and flat features are shown in Figure 1.

RESULTS

The maximum principal stress (tensile) values in the cortical bone are shown in the images on a scale ranging from red to blue, and the minimum principal stress (compression) values in the cortical bone are shown on a scale ranging from blue to red, as they are negative values.

The tensile and compressive stresses in the bone, which were rotated 10 mm to the right and fixed with two mini plates with bilateral, four-hole, and flat features, are shown in Figure 2, Graph 1, Table 2.

The tensile and compressive stresses in the bone, which were rotated 10 mm to the right and fixed with bicortical screws, are shown in Figure 3, Graph 2, Table 3.

DISCUSSION

SSRO is a popular osteotomy method for the correction of maxillofacial deformities and esthetic and functional incompatibilities of the mandible and maxilla. Fixation is important for the success of osteotomy to ensure the stabilization of bone fragments and healing.



Figure 1. Models fixed with 3 linearly placed bicortical screws and 2 mini plates with 4 monocortical screws.

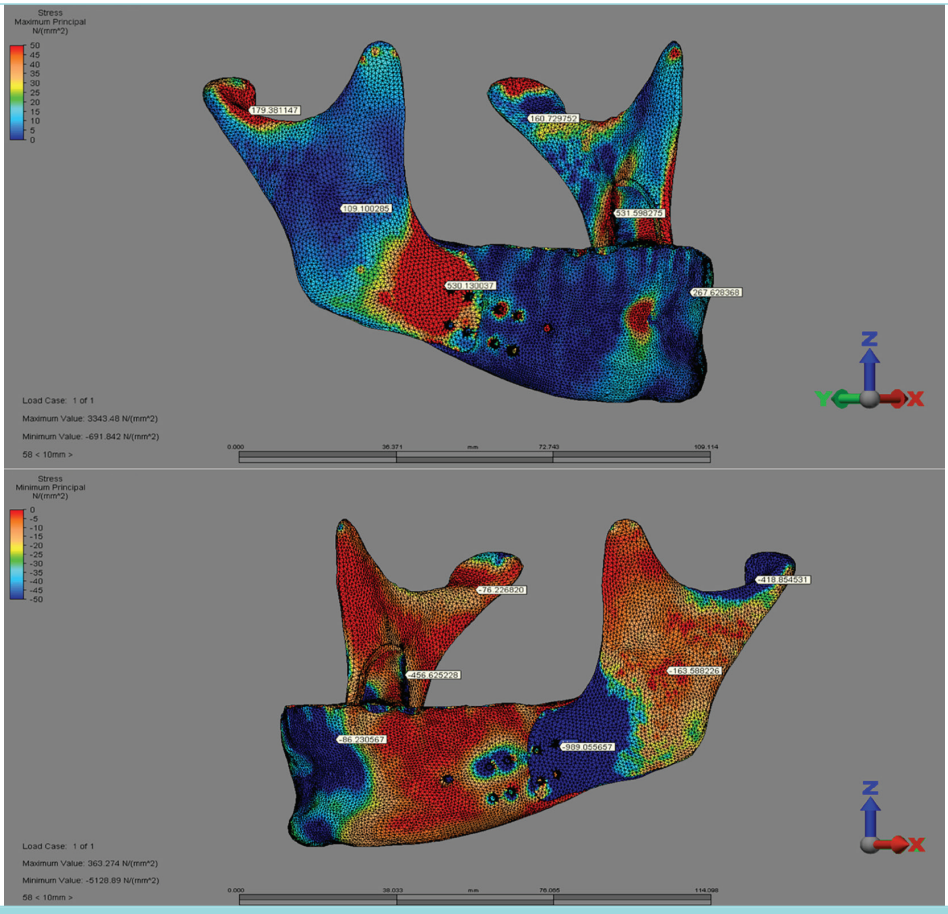
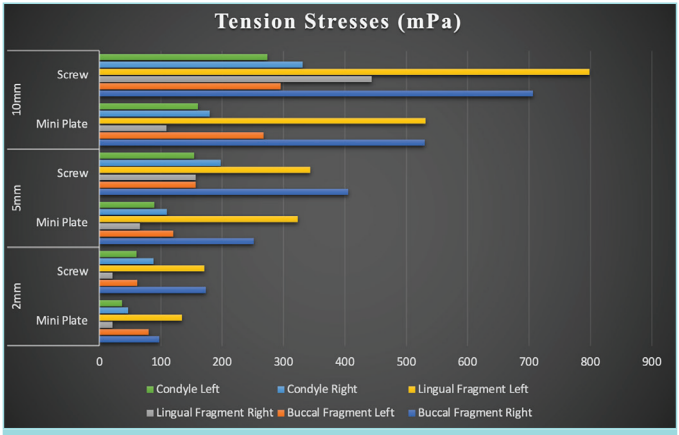


Figure 2. Stresses in bone rotated 10 mm to the right and fixed with 2 mini plates with 4 holes; (a) tension (b) compression.

The ideal fixation should provide the highest stability between the bone fragments, provide adequate resistance to displacement forces at the osteotomy site, and place less stress on the surrounding tissues to ensure proper healing.[6] As a result of the studies, the rigid internal fixation method has become the standard method, thus enabling patients to regain their postoperative functions more easily, and stabilization and relapse problems have been greatly reduced. In the rigid internal fixation class, postoperative complications are minimized using bicortical screws, monocortical screws, or combinations of the two, and fragment stabilization can be achieved.[10,11] Miloro[13] demonstrated in their study that the use of three bicortical screws for the fixation of SSRO was stable, with an average relapse rate of 0-8%. Sigua-Rodriguez et al.[11] reported that when two separate mini-plates are positioned in the tension and compression areas during fixation, the system is sufficiently resistant to displacement, resulting in lower stress at the osteotomy sites. In our study, we aimed to comparatively evaluate the fixation methods of three bicortical screws, which are considered more stable in the literature, and two mini-plates, which have been shown to generate less stress, using FEA.[11-13] Although rigid fixation with mini-plates and lag screws has several advantages, disadvantages such as nerve damage due to compression and displacement of the condyle from the fossa have also been reported.[6] In order to avoid these disadvantages of rigid fixation, there are also studies that mention semi-rigid fixation.[12] Mavili et al.[12] investigated the short- and long-term stability of their semi-rigid fixation method in 23 patients who underwent maxillofacial surgery and 12 patients who underwent mandibular regression surgery. It was reported that the semi-rigid fixation method consisting of two bicortical screws with a diameter of 2 mm for mandibular fixation provided adequate stability and no recurrence in the short- and long-term. While fixation was achieved with bicortical screws, care was taken to maintain the gap between the fragments to avoid loading on the temporomandibular joint, and bone grafts were used when necessary. To stabilize semi-rigid fixation, jaw movements of the patients were restricted with maxillomandibular elastics for 2-4 weeks after 48 hours postoperatively. In our study, three bicortical screws and two miniplates, which are rigid fixation methods, were compared. The graft model was not placed in the opening between the fragments as the mandible moved. The fragments could approach each other under the forces applied by the screws. It was observed that as the amount of movement of the mandible increased, the gap between the fragments also increased. It was observed that the bicortical screws, while providing rigid fixation on the one hand, tended to close the gap between the buccal and lingual segments more with the compression force applied, thus creating more stress.



Graph 1. Tensile stresses applied by bicortical screws and mini plates on fragments and condyle at different amounts of motion (Mega Pascal).

SSRO is a frequently preferred osteotomy method for the correction of maxillofacial deformities and esthetic and functional incompatibilities of the mandible and maxilla. Fixation is important for the success of osteotomy to ensure the stabilization of bone fragments and healing. The ideal fixation should provide the highest stability between the bone fragments, provide adequate resistance to displacement forces at the osteotomy site, and place less stress on the surrounding tissues to ensure proper healing.[6] In our study, the stress and compression values of bicortical screws were higher than those of miniplate fixation models. In cases in which the bone fragments are flimsy and thin, it may be recommended to prefer plates because unwanted fractures may occur during surgery with the stress caused by bicortical screws. In the long term, resorption and temporomandibular joint problems are more likely to occur in patients who are fixed with bicortical screws than with miniplate. Sato et al.[6] used five different rigid fixation techniques with miniplate and bicortical screws in a clinical setting. They fixed bone segments using rigid fixation methods in models that were advanced 5 mm with sagittal split osteotomy. They compared the stabilization values obtained by applying a force on the first molar tooth and evaluated these results using FEA. They reported that FEA is a numerical method for evaluating biomechanical problems and a powerful research tool that can provide precise information about the stress behavior of the mandible affected by mechanical forces. They reported that the mechanical connections between the distal and proximal segments, fixation materials, and stress in adjacent areas can be measured using this method. In the study, it was reported that the bicortical screw had higher stability than the mini plate. They explained that bicortical screws provide better three-dimensional stabilization than mini-plates because they attach to both bone segments. The mini-plates provide stabilization with a structure of bridge between the screws that provides force transmission, thus allowing flexion against external torsional forces. A force fracture transmits a lower compressive force to

Table 2. Tensile stresses applied by bicortical screws and mini plates on fragments and condyle at different amounts of motion (Mega Pascal)

		2 mm		5 mm		10 mm	
		Mini plate	Screw	Mini plate	Screw	Mini plate	Screw
Buccal fragment	Right	97,622	173,353	251,317	405,024	530.13	706,419
	Left	80,074	61,609	120,317	156,881	267,628	294,963
Lingual fragment	Right	21,325	21,241	65,784	156,699	109.1	443,458
	Left	134,445	170,955	322,753	343,631	531,598	798,638
Condyle	Right	46,901	88,231	109,482	197,787	179,381	330,987
	Left	36,792	60,602	89.49	154,062	160,729	273,456

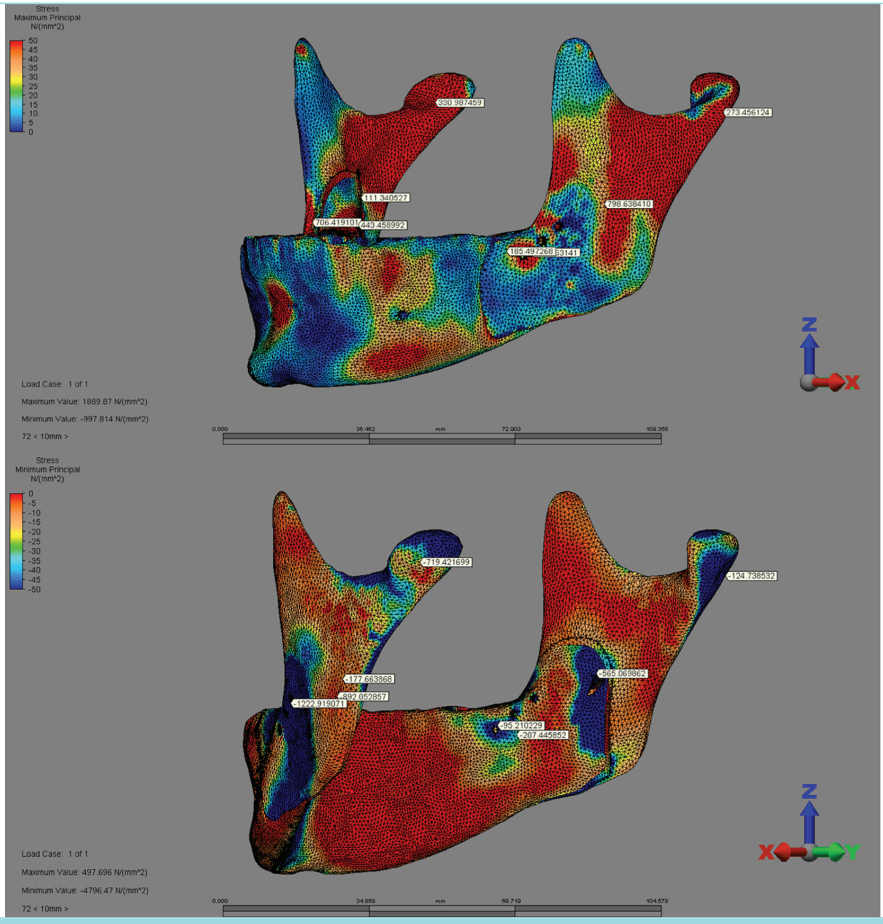
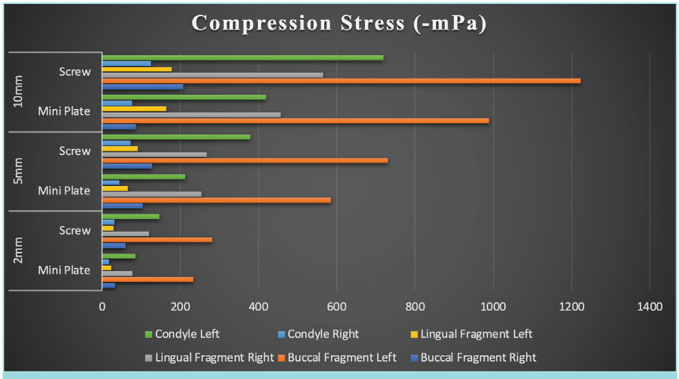


Figure 3. Stresses in bone rotated 10 mm to the right and fixed with 3 bicortical screws; (a) tension (b) compression.



Graph 2. Compressive stresses applied by bicortical screws and mini plates on fragments and condyle at different amounts of motion (Mega Pascal).

the bone fragments and lower torque force to the temporomandibular joint. Therefore, mini-plates have been reported as an alternative to bicortical screws in terms of biomechanical strength and transmitted stresses in sagittal split osteotomies. In the present study, we aimed to evaluate the stresses that may occur between segments using different fixation methods using FEA. It was found that the compression and tensile forces in the models fixed with the miniplate used were lower than bicortical screws. This may be explained by the fact that the force-transmitting plate allows flexion and force fracture. The stresses caused by the transmission of lower torque force to the temporomandibular joint were also lower in models with mini-plate fixation. Our results support the view that mini-plates are a good alternative to bicortical screws because of their lower stress generation.

FEA is a powerful in vitro method that can provide highly accurate information regarding the biomechanical behavior of the mandible, which exhibits diverse and complex properties. This method allows for the definition of different material characteristics and the modification of the magnitude and direction of the applied forces in the designed models. Three-dimensional FEA reflects the stress behavior on the models in a manner that is closer to reality than other in vitro methods, taking into account the complexities of clinical conditions.[20] The objective of studies conducted using the FEA method is to predict the behavior of designed biomaterials and existing systems, thereby guiding surgeons' clinical decisions in a more predictable manner. In this study, we chose to employ this method for the aforementioned reasons.

Study Limitations

There are some limitations to this study, including the FEA. Although the mandibular bone model created by three-dimensional FEA is defined as isotropic, homogeneous, and linear elastic, the mandible is anisotropic and heterogeneous. Anatomically, each mandible has a different cortical bone density, spongiosis bone density, and masticatory-occlusive mechanics. The deformation characteristics and resorption patterns of each patient's mandible differ according to external effects.

Table 3. Compressive stresses applied by bicortical screws and mini plates on fragments and condyle at different amounts of motion (Mega Pascal)

		2 mm		5 mm		10 mm	
		Mini plate	Screw	Mini plate	Screw	Mini plate	Screw
Buccal fragment	Right	-33,341	-59,195	-103,833	-127.6	-86.23	-207,445
	Left	-233,365	-280,726	-584.12	-730,419	-989,055	-1222.919
Lingual fragment	Right	-77,269	-119,708	-253.33	-267,014	-456,625	-565,069
	Left	-22,858	-29,251	-65,483	-91,513	-163,588	-177,663
Condyle	Right	-17,531	-31,503	-43,639	-72,204	-76,226	-124,738
	Left	-84,762	-146,74	-212,205	-378,342	-418,854	-719,421

A standardized and homogeneous mandible can be projected by FEA. In spite of these disadvantages, it is often preferred in scientific research due to its advantages, such as imitating biomechanically complex structures as close as possible to reality, changing the intensity and direction of the forces to be applied, and defining different material properties. The results obtained in this study need to be supported by clinical studies.

CONCLUSION

After sagittal split osteotomy, the mandibular models were rotated 2 mm, 5 mm, and 10 mm to the right and fixed using two different methods: three bicortical screws in a linear position and two mini plates with four screws. After fixation, the tension and compression stresses on the buccal and lingual bone segments on the right and left sides of the mandible and condyle were analyzed. The stress on the condyle and bone fragments increased as the movement speed increased. When we compared the two different fixation techniques, the stresses on the bone segments and condyle were higher in fixation with bicortical screws.

It was predicted that stabilization issues would increase with increasing the amount of motion during sagittal split osteotomy. When selecting the most stable fixation method, the least stress to the surrounding tissues should be taken into consideration.

MAIN POINTS

- Stabilization of the fixation is an important factor affecting the success of sagittal split osteotomy.
- Bicortical screws, monocortical screws, and plates are commonly used for rigid internal fixation.
- Bicortical screws used during fixation may cause higher tensile and compressive stresses than monocortical screws and plates.

ETHICS

Ethics Committee Approval: The Declaration of Helsinki was complied with, and approval was obtained from Ankara University Faculty of Dentistry Ethics Committee (approval number: 36290600/42/2023, date: 19.06.2023).

Informed Consent: Not applicable.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: M.Ö., Concept: M.Ö., S.A., Design: M.Ö., S.A., Data Collection and/or Processing: M.Ö., S.Ö.K., Analysis and/or Interpretation: M.Ö., Literature Search: M.Ö., S.Ö.K., Writing: M.Ö., S.Ö.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-3
Cyprus J Med Sci 2025;10(1):51-57

Association Between Handgrip Strength and Fatigability and Cognitive Performance in Adults Aged 65 and Older

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Abstract

BACKGROUND/AIMS: To explore the relationship between handgrip strength, fatigability, and cognitive function in older adults.

MATERIALS AND METHODS: This cross-sectional study included 89 adults aged 65-85 who were receiving services from a physical therapy and rehabilitation center. Handgrip strength and fatigability were assessed using the Biopac Student Laboratory, and cognitive function was evaluated using the Standardized Mini-Mental State Examination (SMMSE).

RESULTS: Handgrip strength was moderately correlated with cognitive function. There was no relationship between handgrip fatigability and cognitive function. The regression analysis indicated that an increase in grip strength would lead to a 0.59-point increase in the SMMSE score. It can be stated that the average SMMSE score of an individual with a secondary school education is 2.28, high school graduate is 2.94, and university graduate is 3.45 points higher than an individual with only a primary school education. These increases were found to be statistically significant.

CONCLUSION: Our findings indicate that the decline in cognitive functions associated with aging should be considered alongside motor functions like muscle strength, and various individual factors.

Keywords: Handgrip strength, fatigability, cognitive function, older age

INTRODUCTION

Age-related deteriorations in the central nervous and peripheral musculoskeletal systems in elderly individuals cause a decrease in motor and cognitive abilities; accordingly, weak muscle function constitutes a risk factor for poor senescence.[1,2]

Grip strength, a measure of body function, is predicted as a biological marker of aging and has been widely researched as an indicator of a person's current state and as a predictor of their future states.[3] Studies have shown that general health status is associated with weak grip strength, which is associated with morbidity, functional disability, and early mortality. In addition, weak grip strength was shown to be a stronger parameter of mortality than systolic blood pressure.[4-7]

The study also examined the increase in fatigue with age. Muscle motor fatigue is defined as a reduction in muscle power production.[8] Various factors can contribute to muscle fatigue, including the accumulation of metabolites in muscle fibers and inadequate motor command generation in the motor cortex.[8] Individual factors such as sex, age, body mass index (BMI), pain levels, overall health, active muscle groups, and task-specific characteristics (static or dynamic) influence fatigue and related performance declines in adults.[8]

Age-related functional decline may occur in motor function as well as in cognitive, motor, social, and psychological functions.[9] Elderly individuals often exhibit increased anxiety, poor memory and attention, slow processing speed, decreased motor ability and learning capacity, and variable behavioral changes.[9,10] It is known that there is a decrease

To cite this article: Gürsoy Karaman N, Koç E. Association between handgrip strength and fatigability and cognitive performance in adults aged 65 and older. Cyprus J Med Sci. 2025;10(1):51-57

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Received: 29.01.2024
Accepted: 25.11.2024
Publication Date: 14.03.2025

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in neural behavioral functionality due to aging in individuals, and this effect is particularly evident in adults aged 65 years and over.[11]

Cognitive function plays a key role in motor skills. Studies have shown a strong relationship between decreased motor cognitive control and motor performance.[12] However, the association between low grip strength and regressed cognitive function remains unclear.[4] Cognitive demand for motor tasks, such as manual dexterity, increases with age. While these abilities are supported by the musculoskeletal and nervous systems, they also play a decisive role in grip strength.[5]

Age-related studies have shown that individuals experience both motor and cognitive decline. In some studies, the relationship between grip strength and cognitive function in elderly individuals has been investigated.[5,13] In our study, in addition to grip strength and cognitive functions, the relatively less evaluated fatigue during grip strength was also evaluated, and the relationship between motor and cognitive findings was investigated.

MATERIALS AND METHODS

This research is cross-sectional and was carried out in accordance with the Declaration of Helsinki. Ethics Committee approval was received from Dr. Burhan Nalbantoğlu State Hospital Scientific Research and Publication Ethics Committee (approval number: YTK. 1.01, date: 06.01.2021). The study was performed in accordance with the Good Clinical Practice guidelines.

Data Collection

The study population consisted of individuals between the ages of 65 and 85 who received health care services from the Department of Physical Therapy and Rehabilitation, Dr. Burhan Nalbantoğlu State Hospital, Nicosia, North Cyprus. The study was conducted with the participation of 89 volunteers who applied to the department between April and June 2021 and did not have any orthopedic or neuromuscular problems affecting upper extremity function.

All participants were informed about the study, and an informed consent form was signed by each participant who agreed to participate prior to being included in the study. The physical and sociodemographic characteristics of the participants were recorded by mutual interview. In the study, grip strength and fatigability were measured using the Bio-Pac handgrip dynamometer and "Student Lab" software. The validity and reliability of the data collection tool have been demonstrated in other studies.[14,15]

The participants were positioned with their shoulders in the adduction neutral position, elbows at 90º flexion, forearms in the neutral position, and wrists in 0º to 30º extension, and were instructed to grip the dynamometer. Initially, a trial test was conducted, and the test was randomized. Subsequently, the patient was instructed to squeeze the dynamometer with maximum force upon command. Each measurement was repeated three times with a 30 s rest interval between each measurement. The maximum voluntary contraction exerted by the participant was used in the data analysis.

In this study, motor fatigue was measured by calculating the Fatigability index. The BioPac "Student Lab" measurement device was used for the measurements. During the measurement, the electrodes were placed as follows: negative electrode on the medial side of the proximal forearm, positive electrode on the lateral side of the distal forearm, and ground electrode on the medial side of the distal forearm.

The measurement was performed in the grip strength measurement position. Accordingly, the participants were positioned with their shoulders in the adduction neutral position, elbows at 90º flexion, forearms in the neutral position, and wrists in 0º to 30º extension, and were instructed to grip the dynamometer. Participants were asked to squeeze the dynamometer with maximum force and to maintain contraction for 30 s. When the maximum voluntary contraction time reached 30 s, the participant was prompted to end the contraction. The Fatigability index was calculated using the equation shown below (Figure 1):

$$\text{fatigability index} = 1 - \frac{\text{real area}}{\text{maximal voluntary contraction} * \text{time}} * 100\%$$

(Lou[15], 2012).

In this equation, the region referred to as the actual area represents the area under the curve during the period during which the participant can maintain maximum contraction. The Fatigability index can be calculated by subtracting from one the result obtained by dividing the real area by the product of maximum voluntary contraction and time and then taking the percentage. An increased Fatigability index indicates increased physical fatigue. The measurement was repeated for both hands.

Cognitive function was evaluated with the "Standardized Mini Mental State Examination", the validity and reliability of which has been proven in the diagnosis of mild dementia in the Turkish population.[16] The test comprises five main sections: orientation, registration, attention and calculation, recall, and language. The test is evaluated from a total of 30 points. Conducting tests according to standardized test manuals can increase consistency among administrators.

Statistical Analysis

The research data were analyzed with SPSS version 21.0 for Windows. Whether grip strength, fatigue, and cognitive function differ in terms of individual characteristics was tested using the t-test, one-way ANOVA when the assumptions of normal distribution were met, and Wilcoxon

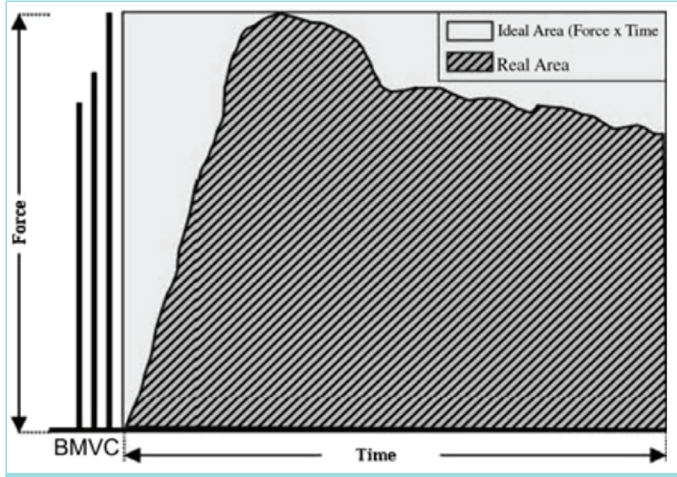


Figure 1. Fatigability index.

test, Mann-Whitney U test and Kruskal-Wallis test when the assumptions were not met, and the results were reported.

The relationship between grip strength, grip fatigue, and cognitive function was investigated using Pearson's correlation analysis. Regression analysis was performed using the independent variables of grip strength, fatigue, and individual characteristics, which are thought to explain the change in cognitive function, the dependent variable. The statistical significance level was set as $p<0.05$ in the study.

RESULTS

The average age of the participants was 72.25±5.05 and their average BMI was 28.53±4.27 (Table 1).

Eighty-three participants were right-handed, while 6 were left-handed. The Wilcoxon test revealed no statistically significant difference in grip strength between the dominant and non-dominant hands ($Z=-0.897$; $p>0.05$). Consequently, the grip strength of the dominant hand was used in the analysis.

Intergroup comparison methods were used to evaluate whether the mini-mental test showing the level of cognitive function and its sub-headings differed according to individual characteristics. The level of cognitive function represented by the SMMSE differed according to gender, education, and employment status. In the analysis, which evaluated sub-headings in terms of individual characteristics, it was determined that the orientation subheading differed in terms of gender and age, while the attention calculation sub-heading differed in terms of gender, education, and employment status. The language sub-heading differed only in terms of education status. On the other hand, the subheading recall and registration did not show a statistically significant difference in terms of any characteristics (Table 2).

Table 1. Descriptive characteristics of the participants

Variables		
Gender	**n**	**%**
Female	43	48.30
Male	46	51.70
Age		
Youngest old (65-74 years)	59	66.30
Middle old (75-84 years)	28	31.50
Oldest old (≥85 years)	2	2.20
BMI		
Normal (<25)	16	18.00
Overweight (25< BMI <30)	47	52.80
Obese (>30)	26	29.20
Martial status		
Single	15	16.90
Married	73	83.10
Education status		
Primary school	37	41.60
Secondary school	7	7.90
High school	22	24.70
University	23	25.80
Employment status		
Never worked	20	22.50
Retired	67	75.30
Working	2	2.20
Total participants	**89**	**100.00**

BMI: Body mass index.

The grip strength assessment between the groups revealed a statistically significant difference in handgrip strength according to gender and employment status. In contrast, grip strength did not differ significantly according to age, BMI, and educational status (Table 3).

The association between the mini-mental test and its subcomponents, which assess grip strength and cognitive function, was examined through correlation analysis. This analysis revealed a statistically significant but moderate correlation between the overall SMMSE score and grip strength ($r=0.31$; $p<0.01$). Additionally, a statistically significant moderate correlation was observed between grip strength and attention and calculation subtest ($r=0.34$; $p<0.01$). No statistically significant correlations were observed between grip strength and the other sub-components (Table 4).

There was no statistically significant relationship between Fatigability index values and general scores or SMMSE subheadings.

The SMMSE score used to evaluate cognitive function was included in the regression equation as a dependent variable. The analysis, in which all independent variables were included, showed that the regression model established with the dominant handgrip strength and education variable, which had statistically significant coefficients, was all significant. Examination of the corrected R^2 value showed that only 37% of the variance in the mental test was explained by the variables of grip strength and education. The grip strength coefficient was observed to be statistically significant, and a unit change in grip strength resulted in an increase of 0.59 points in the mini-mental test score.

From the model, it can be stated that the average SMMSE score of an individual with a secondary school education is 2.28, that of a high school graduate is 2.94, and that of a university graduate is 3.45 points higher than that of an individual with only a primary school education. These increases are found to be statistically significant which is a categorical variable, with primary school graduation as the reference category.

DISCUSSION

Age-related deterioration is known to occur in the central nervous and musculoskeletal systems.[1] Age-related neurological decline has cognitive and non-cognitive consequences.[17] Although decreases in muscle strength in aging adults are attributed to physiological changes in the muscular system, previous studies have shown that it is a product of decreased nervous system functioning.[17] Grip strength is an easy-to-use, inexpensive, and applicable screening tool for evaluating age-related muscle weakness.[4] The main purpose of this study was to evaluate the relationship between cognitive functionality and non-cognitive parameters such as handgrip strength and fatigue due to aging.

The decline in cognitive function in elderly individuals leads to decreased individual independence and dementia, affects public health, and imposes a serious economic burden.[18] A considerable number of studies have shown the correlation of handgrip strength with cognitive

Table 2. Individual characteristics and cognitive functioning parameters

Variables		Orientation				Registration				Attention and calculation				Recall				Language				SMMSE			
Gender	**n**	**Min.**	**Max.**	**Med.**	**Z**	**Min.**	**Max.**	**Med.**	**Z**	**Min.**	**Max.**	**Med.**	**Z**	**Min.**	**Max.**	**Med.**	**Z**	**Min.**	**Max.**	**Med.**	**Z**	**Min.**	**Max.**	**Med.**	**Z**
Female	43	9	10	10	**-1.034**	3	3	3	-0.967	9	10	10	**-4.013****	0	3	2	-1,298	5	9	8	-1.523	19	30	23	-3.184*
Male	46	10	10	10		1	3	3		10	10	10		0	3	2		7	9	8		19	30	26	
Age		**Min.**	**Max.**	**Med.**	**KW-H[1]**	**Min.**	**Max.**	**Med.**	**KW-H**	**Min.**	**Max.**	**Med.**	**KW-H**	**Min.**	**Max.**	**Med.**	**KW-H**	**Min.**	**Max.**	**Med.**	**KW-H**	**Min.**	**Max.**	**Med.**	**KW-H**
Younger old (65-74 years)	59	10	10	10	**43,500****	3	3	3	2,179	10	10	10	0.938	0	3	1.50	1,631	10	10	10	0.248	19	30	24	0.256
Middle old (75-84 years)	28	10	10	10		1	3	3		10	10	10		0	3	1.50		10	10	10		19	30	24	
Oldest old (≥85 years)	2	9	10	10		3	3	3		9	10	10		1	2	1.50		9	10	10		21	22	21.50	
BMI																									
Normal	16	10	10	10	0.504	1	3	3	2,938	10	10	10	0.933	0	3	1	2,938	6	9	8	0.933	19	28	25	2.938
Overweight	47	10	10	10		3	3	3		10	10	10		0	3	2		5	9	8		19	30	25	
Obese	26	9	10	10		3	3	3		9	10	10		0	3	2		6	9	8		21	30	24	
Edu. status																									
Primary school	37	10	10	10	0.704	3	3	3	0.704	0	5	1	**22,958****	0	3	2	2,955	5	9	7	**25,835****	19	29	23	**31,746****
Secondary school	7	10	10	10		3	3	3		0	5	1		1	3	2		7	9	8		23	29	24	
High School	22	10	10	10		3	3	3		0	5	5		0	3	2		7	9	8		22	30	25.50	
University	23	10	10	10		3	3	3		1	5	5		0	3	2		6	9	9		23	30	27	
Emp. status																									
Never worked	20	9	10	10	3,450	3	3	3	0.328	0	5	0	**24,165****	0	3	2	1,371	5	9	7.50	4,694	19	29	23	**15,819****
Retired	67	10	10	10		3	3	3		0	5	4		0	3	2		6	9	8		19	30	25	
Working	2	10	10	10		3	3	3		5	5	5		2	2	2		8	9	8.50		28	29	28.50	
Total	**89**																								

KW-H[1]: Kruskas-Wallis H test statistic. *p<0.05, **p<0.0, BMI: Body mass index, Min.: Minimum, Max.: Maximum, Med.: Median, SMMSE: Standardized Mini-Mental State Examination, Edu.: Educational, Emp.: Employment.

functions and health conditions that pose a risk of cognitive decline in middle-aged and older adults.[4] While some studies are cross-sectional in nature, there are also significant longitudinal studies.[17,19-21] Our study initially focused on the evaluation of the correlation between grip strength and SMMSE over the total score, while its correlation with the sub-titles of SMMSE was evaluated later. According to the results obtained, there is a moderate positive correlation between grip strength and SMMSE total score. While there was a moderate positive correlation between attention, calculation, and grip strength between grip strength and SMMSE sub-headings; no significant correlation was found between orientation, recall, memory, and language and grip strength. In a cross-sectional pilot study conducted by Klawitter et al.[19] in 2020, the relationship between different grip strength measurements and cognitive function in elderly individuals was evaluated. It was stated that elderly individuals with cognitive impairment had weaker grip strength measurements, but no statistically significant differences were observed between these measurements and cognitive functions. Shaughnessy et al.[4] conducted a systematic literature review on this topic in 2020. In a cross-sectional study included in this review, 70 elderly individuals with a mean age of 70±4.7 years participated in the study, and a significant relationship was found between grip strength and cognitive function.[20] In another study, the relationship between grip strength and cognitive function in elderly Americans was investigated longitudinally.[17] According to this study, the relationship between decreased grip strength and decreased cognitive function was determined, and it was suggested that with each increased amount of 5 kg grip strength, cognitive dysfunction that would occur in the future would decrease by 3%. In a four-year longitudinal study that included 1,514 and 1,223 women, a 0.233 point increase in SMMSE was predicted for every 6.14 kg grip strength increase in men, while a 0.197 SMMSE score increase was predicted for every 4.12 kg grip strength increase

in women.[22] In their study, Alfaro-Acha et al.[23] monitored 2,160 elderly Mexican Americans for 7 years and concluded that the SMMSE scores of individuals in the lowest grip strength range showed a decline of 1.28 within 7 years. In a prospective cohort study by Turusheva et al.[21], unlike other studies, no relationship was found between grip strength and cognitive function in elderly individuals.

Decreased attention, memory, and processing speed are observed in older individuals. It is known that behavioral selection, particularly a more active lifestyle, reduces the negative effects of aging on motor and cognitive decline.[9] In our study, it was determined that according to employment status, attention and calculation, which are the subtitle of the SMMSE, differantiated. From this perspective, the ways in which the cognitive functions of elderly individuals with active working life are affected should be investigated in more detail. Furthermore, according to the results of the study, attention and calculation subheading differ in terms of education level. During the aging process within the life cycle, changes occur in individuals' functional capacity. These functional changes depend on individuals' personal genetic differences, lifestyles, motivations, sociocultural backgrounds, exercise, and learning experiences.[9] In light of this information, it is believed that these distinct characteristics of individuals with active working life and advanced education have a positive reflection on cognitive function. In this study, in addition to education and employment status, attention and calculation subheadings of the SMMSE differ according to gender. Gender can affect dementia risk and cognitive function.[17] Again, in the study of McGrath et al.[17], while evaluating the relationship between grip strength and cognitive functions, gender differences were noted. At this point, health care providers should consider the role of gender when examining the relationship between grip strength and cognitive function.

Table 3. Individual characteristics and handgrip strength

Variables		Handgrip strength			
Gender	**n**	**Min.**	**Max.**	**X̄ ± SD**	**t**
Female	43	5.78	47.92	13.68±4.38	**-7.827****
Male	46	4.22	21.44	25.53±9.23	
Age		**Min.**	**Max.**	**Med.**	**KW-H**
Youngest old	59	4.22	47.92	16.60	3,072
Middle old	28	5.78	36.46	21.39	
Oldest old	2	10.49	12.20	11.34	
BMI					
Normal (<25)	16	5.78	35.47	17.75	2,248
Overweight (25< BMI <30)	47	5.96	42.06	19.47	
Obese (>30)	26	4.22	47.92	16.22	
Education status					
Primary school	37	4.22	47.92	15.92	3,478
Secondary school	7	9.29	27.37	19.65	
High school	22	5.78	35.75	18.85	
University	23	7.60	42.06	21.44	
Employment status		**Min.**	**Max.**	**X̄ ± SD**	
Never worked	20	7.25	19.65	13.40	**12,976****
Retired	67	4.22	47.92	21.74	
Working	2	14.77	21.53	18.15	
Total	**89**				

BMI: Body mass index, Min.: Minimum, Max.: Maximum, SD: Standard deviation, Med.: Median, X̄:Mean, KW-H: Kruskas-Wallis H test.

The decrease in strength with aging causes instability during muscle contractions. This instability is also associated with fatigue.[19] The Fatigability index and the relationship between statically evaluated results and SMMSE scores were investigated. According to the results obtained, no correlation was found between the Fatigue index and cognitive function. While evaluating the relationship between grip strength and cognitive function in elderly individuals, the hand grip strength was evaluated under various conditions, and its relationship with fatigue was investigated. As a result of this study, no correlation was found between hand fatigability and SMMSE scores, which evaluate cognitive functions. Although the fatigability of the hand is frequently evaluated in the literature, studies evaluating its correlation with cognitive functions are limited.

In this study, grip strength, fatigue level, and cognitive function were analyzed using a multiple regression model. Accordingly, approximately only the 37% of the variance in the mental test can be explained by the dominant handgrip strength and education level variables. The length of formal education years completed by an individual is positively associated with cognitive function throughout adulthood and is predicted to reduce the risk of dementia later in life.[24] The observations obtained as a result of multiple cohort studies and meta-analyses have led to the suggestion that prolonging education may affect cognitive ability and reduce aging-related declines in cognitive functions.[24] The results obtained in the regression model of this study also support these propositions. In addition to these, although the relationship between grip strength and cognitive function has been mentioned many times, it was also found in the regression model.

Our findings indicate that the decline in cognitive functions associated with aging should be considered alongside motor functions like muscle strength, and various individual factors.

In this study, we observed a relationship between grip strength and cognitive function in elderly individuals. Continuing to unravel the relationship between muscle strength, nervous and muscular system integrity, and cognitive functioning will assist in the provision of new resources for future research. In line with the novel information to be obtained, a guide should be created for healthcare professionals to maintain muscle strength and cognitive abilities in aging adults.

Table 4. Correlations between dominant handgrip strength measurements and cognitive functioning parameters

		SMMSE	Orientation	Registration	Attention and calculation	Recall	Language
Dominant handgrip strength	r	**0.31****	0.12	-0.02	**0.34****	-0.07	0.16
	p	**0.004**	0.261	0.847	**0.001**	0.500	0.11

SMMSE: Standardized Mini-Mental State Examination.

Determining measurable indicators of cognitive impairment in healthy older adults will facilitate the early identification and potential prevention of pathological cognitive decline, such as mild cognitive impairment.

Therefore, intervention programs that aim to manage cognitive processes and contribute to an independent aging process can be created.

Study Limitations

Our study was a cross-sectional study, and as the next step, a long-term evaluation and observation of the changes that may occur in muscle strength, fatigue, and cognitive functions over time will provide us with valuable information. In this study, the relationship between cognitive function and grip strength, a physiological and measurable parameter, was investigated. Additionally, numerous studies have shown that various factors, such as the general health status of elderly individuals, genetic and environmental factors that can influence aging, and cardiometabolic diseases, also affect cognitive function. From this perspective, the causality and underlying mechanisms of the relationship between muscle strength and cognitive function require further investigation. In addition, fatigue was evaluated statically. In future studies, incorporating dynamic fatigue into the study and measuring the fatigue of elderly individuals during activities will contribute to our understanding of the problems experienced in daily life activities.

CONCLUSION

Our results show that educational attainment has a positive effect on cognitive function in the later years of life by contributing to individual differences in cognitive skills that emerge in early adulthood but continue at later ages. Therefore, the positive effect of extensive educational development on cognitive aging is a factor that should be emphasized for healthy cognitive aging and public health.

MAIN POINTS

- There is a significant relationship between the level of education, attention and computation in aged people.
- Cognitive functions due to aging are closely related to motor functions such as muscle strength.
- Educational attainment will have a positive effect on cognitive function in elderly people.

ETHICS

Ethics Committee Approval: Ethics Committee approval was received from Dr. Burhan Nalbantoğlu State Hospital Scientific Research and Publication Ethics Committee (approval number: YTK. 1.01, date: 06.01.2021).

Informed Consent: All participants were informed about the study, and an informed consent form was signed by each participant who agreed to participate prior to being included in the study.

Footnotes

Acknowledgements

This study was presented as a poster communications in the 47th National Turkish Society of Physiological Sciences Congress in Antalya, 2022.

Authorship Contributions

Concept: N.G.K., E.K., Design: N.G.K., E.K., Data Collection and/or Processing: N.G.K., Analysis and/or Interpretation: N.G.K., Literature Search: N.G.K., E.K. Writing: N.G.K., E.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-54
Cyprus J Med Sci 2025;10(1):58-61

The Role of Contact Allergens on Facial Seborrheic Dermatitis

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Abstract

BACKGROUND/AIMS: Seborrheic dermatitis (SD) is an inflammatory skin condition characterised by scaly erythematous patches. The causes of SD include inflammatory reaction to Malassezia species, genetic predisposition and hormonal factors; however, any association with contact allergens has not been established. The purpose of this study is to investigate the role of contact allergens on facial SD.

MATERIALS AND METHODS: Thirty patients with symptoms of, or a prediagnosis of, facial SD along with 30 healthy individuals as the control group were included in this study. Two different sets of patch tests (international standard series IS-1000 and cosmetic series C-1000) were used in this study. Allergens were placed in small chambers and applied to the patients' backs. They were then evaluated 48 hours, 72 hours, and 1 week after the patches were removed. The results were classified according to the International Contact Dermatitis Research Group.

RESULTS: Positive reactions to standard and cosmetic series patch test allergens in the patient and control groups were not statistically significant.

CONCLUSION: It is not possible to establish an association between contact allergens and facial SD.

Keywords: Contact allergens, patch test, seborrheic dermatitis

INTRODUCTION

Seborrheic dermatitis (SD) is a persistant and recurring inflammatory skin condition characterised by erythematous patches with varying degrees of scales. This condition usually affects seborrheic areas like the scalp, face, chest, back, axilla and inguinal region.[1] Facial SD occurs in areas of face rich in sebaceous glands, including nasolabial folds, preauricular/postauricular regions, eyebrows and eyelids. Males are more commonly affected than females, with a peak incidence in the third and fourth decades of life.[2]

SD usually arises as an inflammatory reaction to Malassezia species, although a causal relationship has not been established. In addition, intrinsic host factors such as genetic predisposition, defective epidermal barrier, hormones, increase or change in sebaceous gland activity and host immune response, are important factors in SD pathogenesis.[2,3]

MATERIALS AND METHODS

Thirty patients who presented to the outpatient dermatology clinic, with symptoms or a prediagnosis of facial SD along with 30 healthy individuals as the control group, were included in this study. Two different sets of patch tests (international standard series IS-1000 and cosmetic series C-1000) were used in this study.

For SD patient group, data such as age, gender, occupation, education level, marital status, disease duration, treatments received, stress level, location of SD on the face and whether it showed seasonal variation, were recorded. Patients were subjected to international standard series

To cite this article: Ünsal G, Kömürcügil İ, Karaosmanoğlu N. The role of contact allergens on facial seborrheic dermatitis. Cyprus J Med Sci. 2025;10(1):58-61

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Received: 27.06.2024
Accepted: 12.12.2024
Publication Date: 14.03.2025

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containing 30 allergens and cosmetic series containing 63 allergens, as patch tests. Allergens were placed in small chambers and applied to the patients' back. They then were evaluated 48 hours, 72 hours and 1 week after the patches were removed. The results were classified according to the International Contact Dermatitis Research Group as follows: 0 (no reaction), +/- (erythema, suspicious result), + (erythema and infiltration), ++ (erythema, infiltration, papule, vesicle), +++ (erythema, infiltration, bulla). Pregnant women, those with active dermatitis, those who used medications such as antihistamines, leukotriene antagonists or topical steroids in the past week or systemic steroids in the last month, were excluded from the study. Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Ankara Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 1312/2023, date: 06.09.2023).

Statistical Analysis

In descriptive statistics related to continuous data, mean, standard deviation, median, minimum and maximum values were provided. The Shapiro-Wilk test was used to examine the normal distribution fit of continuous data (such as age, duration of illness). Independent samples t-test was utilized for comparing the ages between patient and control groups. Nominal variables' group comparisons (in contingency tables) were conducted using chi-square and Fisher's exact tests. The diagnostic accuracy of the cosmetic series patch test was evaluated using sensitivity, specificity, positive predictive value, and negative predictive value. Statistical analyses were performed using IBM SPSS for Windows 20.0 (SPSS Inc., Chicago, IL), with a significance level set at $p<0.05$.

RESULTS

In the patient group, the median duration of illness was found to be 36 months. Treatment with topical corticosteroids was observed in 76.7% of patients, while shampoo was administered to 36.7% and immunomodulators to 10%. Among the patients, the usage rate of cosmetic moisturizers was 53.3%, cosmetic soaps stood at 50%, and other cosmetic products were used by 23.3%. Additionally, the rate of disease exacerbation due to stress was 60%, while seasonal changes contributed to an increase of 63.3%, with a rise of increases of 43.3% in winter and 16.7% in summer (Table 1).

In the patient group, the involvement rate in the eyebrow area was 60%, while it was 86.7% in the ala of the nose. The involvement rate in the auricular area was 30%; it was 16.7% in the chin area. Upon examination of the patients' involvement areas, it was noted that the highest involvement (86.7%) was observed in the ala of the nose. Additionally, it was found that 20% of patients had accompanying dermatological conditions including macular amyloidosis, acne, hirsutism, and pernio.

Table 1. Features of the patient group

	(n=30)
Disease duration (month); median (min.-max.)	36 (1-240)
Treatments	
Topical corticosteroid treatment, n (%)	23 (76.7)
Shampoo treatment, n (%)	11 (36.7)
Immunmodulator treatment, n (%)	3 (10.0)
Cosmetic moisturiser, n (%)	16 (53.3)
Cosmetic soap, n (%)	15 (50.0)
Other cosmetic products, n (%)	7 (23.3)
Seasonal variation	
Winter-related increase, n (%)	13 (43.3)
Summer-related increase, n (%)	5 (16.7)

min.: Minimum, max.: Maximum.

In 46.7% of the patient group, suspicious reaction was detected in the standard series patch test, while in the control group, suspicious reaction was observed in 13.3% of the participants. The difference in the rates of suspicious reactions in the standard series patch test results between the patient and control groups was statistically significant ($p<0.01$). In 76.7% of the patients, a suspicious reaction was observed in the cosmetic series patch test allergens, while no suspicious reaction was detected in the control group. There was a significant difference in the rates of suspicious reactions in the cosmetic series patch test results between the patient and control groups ($p<0.001$) (Table 2). The number of suspicious reactions to standard series patch test allergens in individuals using topical corticosteroids was found to be statistically significantly higher ($p<0.05$). Analysis of adverse reactions to standard series patch test allergens revealed no statistically significant differences between users of medical shampoos, immunomodulators, cosmetic moisturizers, soaps, other cosmetics, and non-users ($p>0.05$) (Table 3).

In 20% of the patient group, a positive reaction to standard series patch test allergens was detected, while 13% of the control group showed a positive reaction. The allergens that caused positive reactions in the patient group included textile dye mix, lanolin alcohol, colophonium, fragrance mix, mercapto benzothiazole, and cobalt chloride. The difference between the positive reactions to the standard series patch test in the patient and control groups was not statistically significant. In 13% of the patient group, a positive reaction to cosmetic series patch test allergens was detected, while none of the control group showed a positive reaction. The allergens that resulted in a positive reaction among the patient group included stearyl alcohol, hydroxyethyl alcohol, DMDM hydantoin, and octyl gallate. The difference between the positive reactions to the cosmetic series patch test in the patient and control groups was not statistically significant.

DISCUSSION

SD is a multifactorial inflammatory skin condition characterized by erythematous patches with scales, commonly affecting areas rich in sebaceous glands, especially the face, scalp, trunk, and body folds.[2] SD shows a bimodal age distribution: infantile and adult types. Infantile type, which manifests as scales on the scalp and erythematous plaques in the body folds, usually occurs within the first 3 months of life and resolves spontaneously within the first year. Adult type has a chronic course and affects patients' quality of life. While its etiology involves

Table 2. Comparison of standard and cosmetic series patch test results between the patient group and the control group

	Patient, (n=30)	Control, (n=30)	p
Standard allergen n (%)			
Suspicious reaction	14 (46.7)	4 (13.3)	0.005[b]
No reaction	16 (53.3)	26 (86.7)	
Cosmetic allergen n (%)			
Suspicious reaction	23 (76.7)	0	<0.001[b]
No reaction	7 (23.3)	30 (100)	

[b]Chi-square test.

Table 3. Comparison of standard series test results with given treatments in the patient group

Standard allergen	Suspicious reaction	No suspicious reaction	p
Topical steroid treatment n (%)			
Yes	8 (34.8)	15 (65.2)	0.031[b]
No	6 (85.7)	1 (14.3)	
Shampoo treatment n (%)			
Yes	3 (27.3)	8 (72.7)	0.105[b]
No	11 (57.9)	8 (42.1)	
Immunmodulator treatment n (%)			
Yes	0	3 (100)	0.105[b]
No	14 (51.9)	13 (48.1)	
Cosmetic moisturiser n (%)			
Yes	5 (31.2)	11 (68.8)	0.070[b]
No	9 (64.3)	5 (35.7)	
Cosmetic soap n (%)			
Yes	7 (46.7)	8 (53.3)	1.000[b]
No	7 (46.7)	8 (53.3)	
Other cosmetic products n (%)			
Yes	2 (28.6)	5 (71.4)	0.399[b]
No	12 (52.2)	11 (47.8)	

[b]Chi-square test/Fisher's exact test.

factors like Malassezia species, genetic predisposition, and hormonal influences, the contribution of contact allergens is still unknown.[4] This study aimed to investigate the role of contact allergens, particularly in facial SD, by conducting patch tests on patients with facial SD and on healthy controls.

This study included 30 patients with facial SD and 30 healthy individuals as the control group. The patients' disease characteristics, treatments and reactions to patch test allergens were recorded and analysed. The results revealed a greater number of suspicious reactions to both standard and cosmetic series patch tests in the SD patient group compared to the control group, indicating a potential association between contact allergens and facial SD. This suggests that patients with SD may exhibit greater sensitivity to certain allergens compared to healthy individuals. However, positive reactions to standard and cosmetic series patch tests in the patient and the control group, were not statistically significantly different from each other. Therefore, it is not possible to establish a direct association between contact allergens and SD.

In a case-control study by Ljubojevic et al.[5], 66% of SD patients, treated with topical corticosteroids, 34% of SD patients with no prior topical corticosteroid treatment, and 10% of the control group, demonstrated a positive reaction for baseline series allergens. Additionally, 3% of SD patients who had previously used topical corticosteroids exhibited a positive reaction to the individual corticosteroid series, whereas none of the SD patients without prior topical corticosteroid treatment or any individuals in the control group showed a positive reaction.[5] Their study highlighted that chronic corticosteroid use not only complicates SD but also predisposes patients to sensitization to baseline allergens and individual corticosteroid preparations. These results suggest that long-term topical treatments may exacerbate allergic sensitivities, further complicating SD management. These findings support the results of our study, as a statistically significant increase in suspicious reactions to standard series patch test allergens was observed in individuals who had used topical corticosteroids.

Moreover, the study by the North American Contact Dermatitis Group (2001-2016), highlights the co-occurrence of SD and allergic contact dermatitis. Patients with SD exhibited distinct allergen profiles, with the most common allergens including nickel sulfate, fragrance mix I, methylisothiazolinone, and Myroxylon pereirae resin. Interestingly, SD patients referred for patch testing demonstrated a lower rate of allergic contact dermatitis compared to non-SD patients. However, they still exhibited significant allergic sensitivity, particularly to fragrance components. Nickel sulfate was the most prevalent allergen, likely due to its widespread presence in everyday items like jewelry.[6] These findings align with our study's results of increased suspicious reactions to patch tests in SD patients, suggesting that exposure to cosmetic and topical products may exacerbate SD.

In a case-control study including children with atopic dermatitis and SD, a low prevalence of contact allergy was found in children with SD, with positive patch tests in only 6.7% of cases, predominantly to nickel sulfate. In comparison, children with atopic dermatitis showed a significantly higher rate of contact allergy. In fact, the odds ratio for developing a delayed-type hypersensitivity reaction was 11.5 times significantly lower in SD patients than in children with atopic dermatitis, indicating a significantly reduced risk of contact allergies in the SD group.[7]

Interestingly, while the rate of positive reactions to cosmetic allergens in our study was not statistically significant, the higher prevalence of suspected reactions, suggests a potential subclinical sensitivity. The prevalence of suspicious reactions to the cosmetic series patch test allergens in the patient group suggests that cosmetic products may trigger or exacerbate facial SD. Moreover, a statistically significant difference in adverse reactions between patients who used topical

corticosteroids and those who did not is observed, implying a potential link between topical corticosteroids and allergic reactions in facial SD patients. Ljubojevic et al.[5] findings reinforce the importance of patch testing in persistent or treatment-resistant SD cases, advocating for the inclusion of both standard allergens and patient-specific cosmetic or corticosteroid formulations in the diagnostic process. This approach could identify allergens that might otherwise go undetected using a standard series alone.

CONCLUSION

This study's approach to assessing patients' characteristics, treatments, and allergic reactions provides insight into the role of contact allergens. Overall, while this study emphasizes the relevance of ambiguous reactions to patch tests in SD, the absence of significant differences in positive reactions necessitates cautious interpretation. It is not possible to establish an association between contact allergens and SD. Larger-scale, controlled studies with a longitudinal design are necessary for further exploration of the effect of contact allergens on SD.

MAIN POINTS

- Higher rates of suspicious reactions to both standard and cosmetic series patch tests in the patient group indicate a potential association between contact allergens and facial seborrheic dermatitis (SD).
- Positive reactions to standard and cosmetic series patch test allergens in the patient, and control group were not statistically significant differences observed. It is not possible to establish an association between contact allergens and SD.
- The prevalence of suspicious reactions to the cosmetic series patch test allergens in the patient group suggest that cosmetic products may trigger or exacerbate facial SD.
- Greater numbers of adverse reactions in patients who used corticosteroid cream treatment, implies a potential link between topical corticosteroids and allergic reactions in facial SD patients.

ETHICS

Ethics Committee Approval: Ethical approval for this study was obtained from the University of Health Sciences Türkiye, Ankara Training and Research Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 1312/2023, date: 06.09.2023).

Informed Consent: It wasn't obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.Ü., N.K., Concept: G.Ü., İ.K., Design: G.Ü., N.K., Data Collection and/or Processing: G.Ü., İ.K., N.K., Analysis and/or Interpretation: İ.K., N.K., Literature Search: G.Ü., İ.K., Writing: İ.K., N.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2025.2024-52
Cyprus J Med Sci 2025;10(1):62-69

Questioning the Awareness of Partially Edentulous Patients About Dental Implants and Implant Supported Dental Prostheses

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Abstract

BACKGROUND/AIMS: To measure and evaluate the level of awareness of a specific patient population with partial edentulism about dental implants, implant-supported prostheses, and the general treatment processes related to these.

MATERIALS AND METHODS: A total of 130 patients were included in the present study. Patients with local or systemic conditions considered as contraindications, those with a history of previous implant surgery, and illiterate patients were excluded. Prior to their participation, the patients were given informed consent forms and these were read to them before signing. Written survey forms consisting of 13 questions were given to the patients, and they were instructed to read the questions carefully the questions and mark the options with which they aligned most closely. Statistical correlation between age and the total scores and sub-dimensions of the scale were evaluated using Spearman's rho test, while statistical comparisons according to gender and education were evaluated using the Mann-Whitney U and Kruskal-Wallis tests.

RESULTS: It was found that 58.9% of the patients who participated had awareness about dental implants. Only 39.6% had received information about dental implants from their dentists or doctors, and 63.6% indicated that dental-implant treatment is more costly than alternative options. There is no statistically significant correlation between age and the total scores and sub-dimensions of the scale ($p>0.05$).

CONCLUSION: Individuals with higher levels of education tend to exhibit greater awareness and more positive attitudes towards dental implant treatments. The high cost of implant treatment stands out as the most significant disadvantage of this approach.

Keywords: Dental implant, implant supported dental prosthesis, oral health

INTRODUCTION

Tooth loss is a pervasive condition that has a significantly negative effect on an individual's quality of life by affecting their ability to eat, speak, and smile with confidence. It not only hampers functionality but also diminishes personal aesthetics and social aspects. Tooth loss not only affects oral health and functionality but also has profound psychological and social consequences. Individuals with missing teeth often experience reduced self-esteem, social withdrawal, and impaired quality of life due to aesthetic concerns and difficulties in communication.[1] Edentulism still ranks among the top 100 global health issues.[2] With advancements in health care, however, there has been a decrease in the prevalence and incidence of tooth loss.[3] Tooth loss can be attributed to various factors, including systemic and oral diseases, aging, socioeconomic disparities and limited access to health care services.[3,4]

To cite this article: Güzelce Sultanoğlu E, Dökülmez B, Hürbağ M. Questioning the awareness of partially edentulous patients about dental implants and implant supported dental prostheses. Cyprus J Med Sci. 2025;10(1):62-69

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Received: 08.06.2024
Accepted: 28.02.2025
Publication Date: 14.03.2025

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According to research conducted by the Turkish Dental Association, tooth loss rates in Türkiye exhibit variations across different age groups and regions. The findings from a 2018 study revealed an alarming overall edentulism rate of 69.3% in the country; notably, this study observed a slightly higher edentulism rate of 71.7% among women, whereas men have a 66.8% rate.[5]

When strategizing the rehabilitation of missing teeth in patients, several factors come into play, including the location and extent of tooth loss, bone condition in the affected area, occlusal relationships with the opposing jaw, and the overall systemic health of the patient.[2] A notable approach for restoration is the utilization of "bridge veneers", which involves preparing and reducing the adjacent teeth to serve as abutments and subsequently veneering them. For the success of bridge veneers, it is essential that healthy periodontal conditions exist in the abutment teeth to ensure they can effectively withstand occlusal forces. A comprehensive consideration of these factors contributes to a well-informed, sophisticated treatment plan for patients with missing teeth. Bridge veneers are not an ideal treatment option, as it is necessary to damage healthy teeth during the construction phases and is not recommended for the treatment of edentulous spaces with a single terminal tooth.[6]

When Breine et al.[7] introduced the concept of osseointegration to the scientific world in 1964, the use of dental implants had become an alternative treatment option for missing teeth. After years of extensive research and empirical evidence, it can be confidently stated that dental implants offer the most ideal treatment option; this is primarily due to the remarkable ability thereof to enhance the retention, stability, and functional effectiveness of prosthetic restorations.[8,9] Dental implants have emerged as a transformative solution, addressing both the functional and aesthetic deficits caused by tooth loss. By restoring a natural appearance and improving oral functionality, implants play a crucial role in enhancing patients' self-confidence, social interactions, and overall well-being.[10]

Studies comparing bridge veneers and implant-supported veneers in patients with partial edentulism show that implant-supported veneers provide a higher survival rate and functional ease-of-use than bridge veneers.[9,11]

While dental implants are considered an optimal treatment option for edentulous spaces, conventional bridge prostheses are used more commonly in current practices. The predominant factors contributing to the preference for conventional bridge prosthesis over implants include patients' lack of sufficient awareness about implants and implant-supported prostheses, as well as cost-related issues.[12] Studies examining the awareness and preferences of specific populations regarding implants have reported that a significant proportion of elderly patients claim to possess awareness about dental implants; it is worth noting, however, that this information is often inaccurate, resulting in dental implants not being perceived as the ideal treatment option within this population.[13,14] Moreover, some studies have stated that patients prefer minimally invasive procedures, rather than surgical interventions.[13,14]

Although there are several studies[11-14] in the literature in which patients' awareness about implant treatments was investigated, no study has been found with a similar patient population and survey questions. The aim of this study is to evaluate the awareness levels about dental implants and implant-supported prostheses of partially edentulous patients who applied to University of Health Science Faculty of Dentistry, Department of Prosthodontics due to edentulousness.

MATERIALS AND METHODS

This study was conducted with the approval of the University of Health Sciences Türkiye, Hamidiye Scientific Research Ethics Committee (approval number: 2/4, date: 27.01.2023). A total of 130 patients were included in the present study. Patients with local or systemic conditions regarding contraindication, those with a history of previous implant surgery and/or illiterate patients were excluded. The exclusion was primarily due to the reliance on a written survey format, which required participants to read and understand the questionnaire independently.[15] This exclusion may impact the generalizability of our findings, particularly in regions with lower literacy rates.[16] As a preliminary step, informed consent forms were presented to the eligible patients who would be enrolled in this study, and they were provided with an explanation of the study before we obtained their signatures. Written questionnaires comprised of 13 questions were distributed to the patients, who were instructed to carefully read the questions and select the response option that best reflected their views. The 13 questions in the questionnaire were divided into four groups. Responses ranged from 1 (no awareness) to 5 (high awareness). The question grouping was as follows (Table 1):

Group 1: General level of awareness about implant therapy.

Group 2: Information resource on dental implants.

Group 3: Advantages of dental implants.

Group 4: Disadvantages of dental implants.

The inclusion criteria for this study required that each patient applying to University of Health Sciences Türkiye, Faculty of Dentistry, Department of Prosthodontics due to edentulism be considered. Exclusions from the study included individuals with complete edentulism (i.e., total tooth loss) and those who were only missing their third molars (i.e., their wisdom teeth) with no other tooth loss.

Statistical Analysis

Instead of using power analysis to determine the sample size in our study, we followed a method based on the proposal to determine sample size as several times, preferably 10 times or more, the number of variables, and included 130 participants for 13 questions.[17] The Cronbach's alpha coefficient was used to determine internal consistency (Cronbach's alpha=0.798). The split-half reliability method was used to evaluate between-class consistency, and the Spearman-Brown correlation coefficient was calculated (Spearman-Brown coefficient r=0.722).

Survey Validity

An exploratory factor analysis was conducted to assess the validity of the survey. Prior to performing the factor analysis, several preliminary tests were conducted. The Kaiser-Meyer-Olkin (KMO) criterion was examined for sample adequacy. The KMO index compares observed correlations and partial correlations. In this study, the KMO criterion was calculated to be 0.740, which indicated that the sample size was suitable for factor analysis.

Bartlett's test was employed as a statistical tool to determine the adequacy of a correlation matrix for multivariate normal distribution, specifically evaluating whether the correlation matrix exhibited a diagonal consisting of ones and off-diagonal elements of zeros for multivariate normality assumptions. In this study, the Bartlett's test yielded a pvalue of <0.001 at a significance level of 0.05, which confirmed that the population correlation matrix was not an identity matrix; this suggested that a factor analysis could be conducted. The diagonal values of the anti-image correlation matrix range from 0.503-0.857, which indicate that the sample size is appropriate for factor analysis.

A principal component analysis was used to determine the structure of the factors. The percentages of explained total variances are presented in Table 2. In this study, four factors account for 65.7% of the total variance. According to the exploratory factor analysis, the survey consists of 4 subscales. Since the difference in factor loadings for questions 1 and 6 was less than 0.10, the questions were removed from the survey. Factor 1 includes questions 4, 5, 7, and 8. Factor 2 includes questions 11, 12, and 13. Factor 3 includes questions 2 and 3. Factor 4 includes questions 9 and 10.

Validity/Confirmatory Factor Analysis

A confirmatory factor analysis (CFA) is a specific approach within the broader framework of structural equation modeling, which is widely recognized as a distinct research method. In the CFA model, observed variables (i.e., scale items) are represented by rectangles, latent variables (i.e., sub-dimensions) are ovals, and the letter *e* denotes the error or unexplained variance. Maximum-likelihood estimation (MLE) assumed a normal distribution for the item scores. Fit indices-including χ^2, GFI, CFI, IFI, and RMSEA-were utilized to evaluate the model fit (Figure 1).

Table 1. List of questions asked to participants and the evaluated groups

Questions	Evaluated group
1- How familiar are you with "dental implants," which are one of the treatment options for edentulous areas?	1
2- Did you acquire most of your information about dental implants through the internet/social media?	2
3- Did you acquire most of your information about dental implants through your circle of friends?	2
4- Did you acquire most of your information about dental implants through your dentist?	2
5- One of the advantages of dental implant treatment is that it allows achieving more aesthetic results.	3
6- One of the advantages of dental implant treatment is that it does not require damaging the existing teeth.	1, 3
7- One of the advantages of dental implant treatment is it reduces bone loss in the edentulous area.	1, 3
8- One of the advantages of dental implant treatment is that it provides a more permanent solution.	3
9- One of the disadvantages of dental implant treatment is that it may be aesthetically less satisfying.	4
10- One of the disadvantages of dental implant treatment is that it has a lengthy process.	1, 4
11- Another disadvantage of dental implant treatment is that it is costlier.	1, 4
12- One of the disadvantages of dental implant treatment is the need for surgical intervention.	1, 4
13- One of the disadvantages of dental implant treatment is the potential complications that may occur afterward.	4

Model Output

The output of the model that was estimated using the MLE method was as follows; notably, the model was found to be statistically significant ($p<0.05$) (Table 3).

Statistical Comparison of Survey Results

In this study, Spearman's rho correlation analysis was used to analyze the relationship between two continuous variables that do not conform to a normal distribution. The comparison of two independent and non-normally distributed variables was made with the Mann-Whitney U test. The comparison of continuous variables belonging to more than two groups that do not conform to normal distribution was conducted using the Kruskal-Wallis test. Spearman's rho correlation analysis was used to analyze the relationship between two continuous variables that do not conform to a normal distribution. The statistical significance level was determined as 0.05.

Table 2. Rotation sums of squared loadings

	Variance percentage	Cumulative %
Factor 1	20,942	20,942
Factor 2	15,280	36,222
Factor 3	14,985	51,207
Factor 4	14,540	65,747

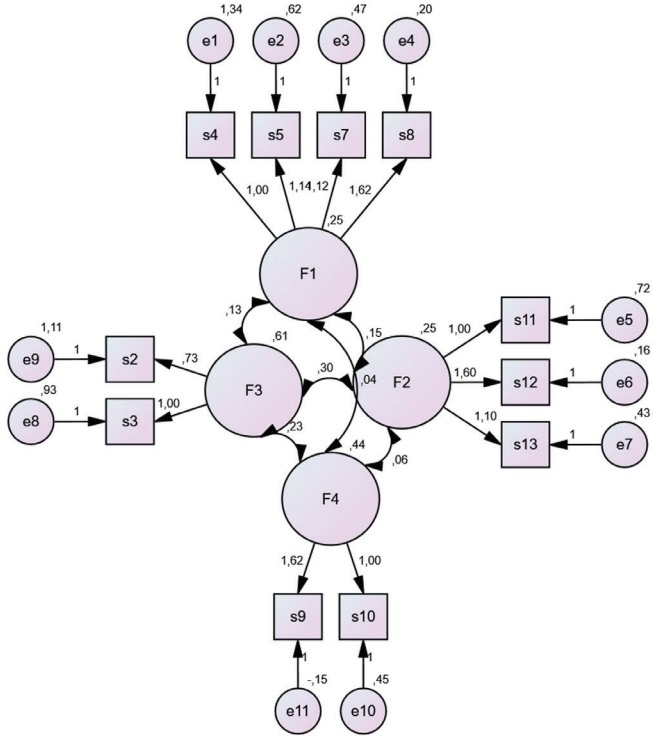


Figure 1. Modified CFA model according to EFA.

CFA: Confirmatory factor analysis, EFA: Exploratory factor analysis

RESULTS

The survey included 52 male participants (40%) and 77 female participants (59%), who ranged from 19-96 years of age with a median age of 50 years. The age distribution of the participants was as follows: 18% were younger than 30, 45% were 30-50 years of age, and 37% were older than 50. Among the participants, 65% had completed their primary education, 23% had completed their secondary education (i.e., middle school), 8% had completed high school, and 3% had attained a university degree (Table 4).

There is no statistically significant correlation between age and participants' awareness when analyzing the total scores and sub-dimensions of the scale (Spearman's rho $p>0.05$) (Table 5). There is a statistically significant difference in the distribution of the 4th dimension between participants' dental implant treatment awareness according to gender (Mann-Whitney U $p<0.05$) (Table 6). The mean is higher for men. There is no statistically significant difference according to education (Kruskal-Wallis $p>0.05$) (Table 7).

The survey revealed that 58.9% of respondents reported being aware of dental implants. However, subsequent analysis of their responses to other survey questions indicated that these patients did not have a sufficient level of accurate understanding. Furthermore, 39.6% of the participants indicated that they received information about dental implants from their dentist or doctor. 63.6% mentioned that dental-implant treatment was costlier than alternative options. 10.1% believed that dental implants were a treatment option that reduced bone loss. 53.5% did not consider dental implants to be a permanent treatment option (Table 8).

Table 3. Model output

	Chi-square	Degrees of freedom	p
Model	71,821	38	0.001

Table 4. Distributions of demographics

		Average	Median (minimum-maximum)
Age		50.73±12.67	50 (19-96)
		n	%
Gender	Male	52	40.3
	Female	77	59.7
Education	Primary education	84	65.1
	Middle school	30	23.3
	High school	11	8.5
	University	4	3.1

Table 5. Correlation analysis with age

		Age
F1	r	-0.101
	p	0.253
F2	r	0.113
	p	0.200
F3	r	-0.096
	p	0.279
F4	r	0.030
	p	0.734
Total	r	-0.045
	p	0.612

DISCUSSION

The objective of this study was to examine the awareness and attitudes of patients toward dental-implant treatment. Specifically, the research focused on a sample of partially edentulous patients who had not undergone dental-implant rehabilitation in the past. By exploring the participants' understanding and perspectives, this study aimed to gain insights into patient-awareness levels and attitudes regarding dental implants within this specific population. In addition to evaluating the participants' awareness, we also investigated their perceptions of the dental-implant treatment; this involved gathering information about the overall impact of dental implants on oral health and quality of life, the sources from which the participants acquired information about implants, and any concerns or misconceptions they had. Through an analysis of the collected data, researchers can gain valuable insights into the level of patient awareness regarding dental-implant treatment. These findings can be utilized to enhance patient education programs, develop targeted informational materials, and improve communication between dentists and patients.

The study was conducted in a region with a low level of education in İstanbul.[18] Consistent with this finding, 65% of the participants in our study were primary-school graduates, 23% had completed middle school, 8% had completed high school, and 3% were university graduates. Although we did not find a significant statistical relationship between dental implant awareness and education in our study ($p>0.05$), when we evaluated the awareness level, we observed that 60% of the study participants had no awareness. This is consistent with the studies conducted by Barot et al.[19] and Gayathri[20] on populations with low socioeconomic levels. Notably, however, these findings contradict a study conducted by Tepper et al.[21], in which 72% of the patients reported having awareness about implants. In our opinion, the socioeconomic level of the sample participating in our survey may be the primary reason for this discrepancy.

According to our survey findings, a significant majority (75%) of patients who were indicated for implant-supported restorative treatment reported that their primary source of information regarding dental-

Table 6. Comparisons according to gender

	Male	Female	
	Mean ± SD Med. (min.-max.)	Mean ± SD Med. (min.-max.)	p
F1	3.61±0.7 3.5 (1.75-5)	3.39±0.77 3.5 (1-5)	0.175
F2	3.64±0.66 3.67 (2-5)	3.66±0.77 3.67 (1-5)	0.624
F3	2.89±1.08 3 (1-5)	2.97±0.93 3 (1-5)	0.582
F4	3.02±0.87 3 (1-5)	2.65±0.92 2.5 (1-5)	**0.024**
Total	3.29±0.59 3.14 (1.69-4.75)	3.17±0.56 3.19 (1-4.44)	0.488

Min: Minimum, Max: Maximum, SD: Standart deviation.

Table 7. Comparisons according to education

	Primary-school	Middle school	High school	University	
	Mean ± SD Med. (min.-max.)	Mean ± SD Med. (min.-max.)	Mean ± SD Med. (min.-max.)	Mean ± SD Med. (min.-max.)	p
F1	3.43±0.79 3.5 (1-5)	3.54±0.73 3.5 (2-5)	3.64±0.45 3.5 (3-4.5)	3.38±0.32 3.38 (3-3.75)	0.857
F2	3.61±0.81 3.67 (1-5)	3.74±0.56 3.67 (2.67-5)	3.58±0.5 3.67 (3-4.33)	4±0.47 4.17 (3.33-4.33)	0.528
F3	2.88±1.04 3 (1-5)	3.02±0.97 3 (1-4.5)	3.18±0.78 3 (2-5)	3±0 3 (3-3)	0.839
F4	2.85±0.92 3 (1-5)	2.53±0.93 2.75 (1-4)	3.27±0.68 3 (2-4)	2.5±0.58 2.5 (2-3)	0.116
Total	3.19±0.66 3.13 (1-4.75)	3.21±0.34 3.14 (2.31-3.98)	3.42±0.39 3.54 (2.75-4.19)	3.22±0.15 3.18 (3.08-3.44)	0.278

Min: Minimum, Max: Maximum, SD: Standart deviation.

Table 8. List of questions asked to participants and percentage of given answers

Questions	Answers	n	%
1- How familiar are you with "dental implants," which are one of th treatment options for edentulous areas?	1	44	34.1%
	2	32	24.8%
	3	23	17.8%
	4	24	18.6%
	5	6	4.7%
2- Did you acquire most of your information about dental implants through the internet/social media?	1	20	15.5%
	2	39	30.2%
	3	28	21.7%
	4	32	24.8%
	5	10	7.8%
3- Did you acquire most of your information about dental implants through your circle of friends?	1	15	11.6%
	2	32	24.8%
	3	26	20.2%
	4	39	30.2%
	5	17	13.2%
4- Did you acquire most of your information about dental implants through your dentist?	1	30	23.3%
	2	21	16.3%
	3	28	21.7%
	4	37	18.7%
	5	13	10.1%
5- One of the advantages of dental implant treatment is that it allows for achieving more aesthetic results.	1	6	4.7%
	2	7	5.4%
	3	54	41.9%
	4	42	32.6%
	5	20	15.5%
6- One of the advantages of dental implant treatment is that it does not require damaging the existing teeth.	1	5	3.9%
	2	6	4.7%
	3	56	43.4%
	4	42	32.6%
	5	20	15.5%

Table 8. Continued

Questions	Answers	n	%
7- One of the advantages of dental implant treatment is that it serves as a treatment option that reduces bone loss in the edentulous area.	1	2	1.6%
	2	11	8.5%
	3	74	57.4%
	4	23	17.8%
	5	19	14.7%
8- One of the advantages of dental implant treatment is that it provides a more permanent solution.	1	4	3.1%
	2	7	5.4%
	3	36	27.9%
	4	59	45.7%
	5	23	17.8%
9- One of the disadvantages of dental implant treatment is that it may be aesthetically less satisfying.	1	10	7.8%
	2	41	31.8%
	3	54	41.9%
	4	14	10.9%
	5	10	7.8%
10- One of the disadvantages of dental implant treatment is that it has a lengthy treatment process.	1	8	6.2%
	2	40	31.0%
	3	58	45.0%
	4	15	11.6%
	5	8	6.2%
11- Another disadvantage of dental implant treatment is that it is more costly.	1	45	34.9%
	2	37	28.7%
	3	37	28.7%
	4	7	5.4%
	5	3	2.3%
12- One of the disadvantages of dental implant treatment is the need for surgical intervention.	1	2	1.6%
	2	7	5.4%
	3	38	29.5%
	4	56	43.4%
	5	26	20.2%
13- One of the disadvantages of dental implant treatment is the potential complications that may occur after the treatment.	1	2	1.6%
	2	10	7.8%
	3	70	54.3%
	4	31	24.0%
	5	16	12.4%

implant treatments was their dentist. Consistently, our results revealed that dentists were the most prominent source of information, followed by friends and relatives, the Internet, magazines, television, and general physicians, when ranked in order of importance. These findings align with the findings of Pommer et al.[22], who similarly highlighted dentists as the primary source of information for patients regarding dental implants and other dental treatments, accounting for 74% of cases. This is also in line with a study conducted by Yao et al.[23], the aim of which was to explore patients' awareness levels, perceptions, and expectations regarding implant treatment; they concluded that dentists were the most frequently consulted source of information. The results of numerous similar studies investigating the sources of information about dental implants also support and reinforce these findings.[24-27]

The study by Berge[28] in Norway found that individuals aged 45 and older, with a higher level of education, had better awareness of dental implants, which aligns with the findings of Choudhary et al.[29] in India. However, there are also surveys suggesting an inverse relationship between the level of awareness about dental implants and the age of the individual.[17,27] In this study, although individuals aged 50 and over gave more accurate answers about dental implant treatment than other age groups, no statistically significant relationship was found between age and awareness of the participants ($p>0.05$).

According to the analysis of the questions on the reliability of dental implants, only 18% of the participants evaluated implants as being more reliable than natural teeth; this indicates that the majority believed natural teeth are more reliable than implants. When considering treatment options for missing teeth, the longevity of the treatment is an important factor.[6] According to the analysis of the data obtained from our study, 96.93% of the patients stated that implant treatment was not a lifelong permanent treatment option. In contrast to our findings, in a survey study conducted by Insua et al.[30], 70.4% of the participants stated that implants are a lifelong treatment; conversely, a 2021 survey conducted by Küçük et al.[31] concluded that only 20% of the participants believed that dental implants represent a lifelong permanent treatment. The significant discrepancy in these findings may be attributed to variations in the evaluated populations across different geographical regions and with differing levels of education.

The high cost of an implant-supported prosthesis is a primary factor contributing to negative perceptions of implant treatment.[14,22,32,33] In a study conducted by Satpathy et al.[32], among patients who were awarenessable about implant treatment and had indications for dental implants, 58.79% opted for alternative treatments due to the high cost. Similarly, in a research study by Sinha et al.[33], 76% of participants expressed that implant treatment is expensive. Consistent with these findings, the current study also revealed that 86% of participants consider implant treatment to be costly. Studies focusing on populations with a lower socioeconomic status have reported that participants prefer conventional fixed and removable partial treatments over implant treatments due to the high cost associated with implants.[17,18] To address the cost barrier to implant adoption, public health initiatives and financial assistance programs should be considered. These programs can offer subsidized dental treatments or financial support to individuals from lower-income backgrounds.[34] Additionally, promoting cost-effective alternatives, such as simplified implant systems or preventive care programs, can help make dental implants more accessible.[35]

In the subsequent phase of the present study, an evaluation was conducted on patient information concerning the surgical procedure and potential complications associated with dental-implant treatment. Approximately 60% of the participants expressed their readiness for dental-implant surgery. Notably, individuals with higher levels of education displayed greater acceptance of surgical procedures. In line with similar previous studies, these collected data indicated a positive correlation between age and awareness levels regarding complications and potential failures.[27,36]

A study conducted by Atagün and Kalyoncuoğlu[27] in a different region of Türkiye reported that 95.5% of elderly participants were familiar with implant treatment, but only 21.5% possessed accurate awareness thereof. Dentists should also strive to enhance motivation and education among patients before beginning treatment.[13,31]

Study Limitations

It is important to note that the study has some limitations. Firstly, it only represents a certain segment of the population. Secondly, the survey focused solely on evaluating the patients' awareness without investigating their treatment preferences or the reasons behind their choices. Finally, the survey questions were selected from a pool of suitable questions obtained by reviewing previous studies and were optimized for the study.

CONCLUSION

In conclusion, younger participants with higher education levels tend to have better awareness of dental-implant treatment, and have a more positive attitude toward rehabilitating toothless spaces with dental implants. The most important disadvantage of an implant treatment is the high cost thereof. Considering that dentists are the most important source of information, they should take more responsibility to raise public awareness. Further studies are needed to evaluate this topic in greater depth.

MAIN POINTS

- Partially edentulous patients possess a limited awareness of dental implant treatments.

- There is no significant relationship between dental implant treatment awareness and the age of the individual.

- One of the most significant reasons why patients decline implant treatment may be that they perceive the treatments to be expensive due to illness.

ETHICS

Ethics Committee Approval: This study was conducted with the approval of the University of Health Sciences Türkiye, Hamidiye Scientific Research Ethics Committee (approval number: 2/4, date: 27.01.2023).

Informed Consent: Informed consent forms were presented to the eligible patients who would participate in this study.

Footnotes

Acknowledgement

I would like to express my gratitude to Büşra Keleş and Berk Yüzbaşıoğlu for their valuable contributions in conducting this study.

Authorship Contributions

Surgical and Medical Practices: E.G.S., Concept: E.G.S., Design: E.G.S., Data Collection and/or Processing: B.D., Analysis and/or Interpretation: B.D., Literature Search: M.H., Writing: M.H.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2025.2024-57
Cyprus J Med Sci 2025;10(1):70-78

Relationship Between Antioxidant Enzyme Chains and Trace Elements and Electrolytes Levels and Potency of Melatonin on Sepsis-Induced Small Intestine Injury

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Abstract

BACKGROUND/AIMS: It was intended to determine the impacts of melatonin (Mel) on chitinase-3-like-1 protein, an early sepsis marker, and antioxidant enzymes, trace elements, and electrolytes in the small intestine tissue treated with sepsis with lipopolysaccharide (LPS) in Sprague Dawley.

MATERIALS AND METHODS: Control, LPS (20 mg/kg i.p.), Mel (10 mg/kg i.p.x3) and LPS + Mel groups were created from Sprague Dawley rats, with 8 individuals in each group. For the LPS group to be used as a sepsis model, the rats were decapitated at the 6th hour following LPS administration, and blood samples and small intestines were taken. In serum samples, antioxidant enzymatic defense molecules such as glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD), early sepsis marker YKL-40 were evaluated by enzyme-linked immunosorbent method. For statistical analysis, ANOVA and post hoc Tukey's tests were used.

RESULTS: The concentrations of GR, GSH-Px, SOD, and YKL-40 were decreased in LPS group. Antioxidant enzyme levels in the LPS + Mel groups were found to be close to the control. Also, trace element and electrolyte levels were significantly affected by LPS induced sepsis and Mel treatment returned them to control values. In the sepsis group, small intestine's images were shown damaged tissue sign and with decreased crypt depths-villus lengths in histological examinations. The images of the other groups are similar to the control group.

CONCLUSION: We suggest that Mel application improves serum antioxidant enzyme activity and trace element-electrolyte levels in small intestine tissue with sepsis, and may protect intestinal permeability and absorption by having a preventive effect on the intestinal barrier.

Keywords: Sepsis, trace elements, anti-oxidants, YKL-40, melatonin

To cite this article: Ateş G, Yorulmaz H, Özkök E, Tamer Ş, Yalçın İE, Olgaç V. Relationship between antioxidant enzyme chains and trace elements and electrolytes levels and potency of melatonin on sepsis-induced small intestine injury. Cyprus J Med Sci. 2025;10(1):70-78

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Received: 24.06.2024
Accepted: 11.02.2025
Publication Date: 14.03.2025

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INTRODUCTION

Sepsis is a syndrome characterized by physiopathological disorders resulting from an increased and irregular inflammatory response. Severe sepsis can lead to multiple organ dysfunction syndrome (MODS), which arises from tissue and organ damage.[1] The pathogenesis of severe sepsis involves a cytokine storm, which is characterized by the release of inflammatory molecules, leading to oxidative stress due to an increase in prooxidant molecules and a failure to reduce or eliminate damage to the cell microenvironment.[2]

The pathogenesis of the disease is believed to be caused by a cytokine storm, which is characterized by the release of inflammatory mediators, an increase in reactive oxygen species radicals (ROS), and the creation of pro-oxidant molecules.[2] It has been established that the small intestines may serve as a source of MODS due to their role in facilitating bacterial translocation across the intestinal barrier. This process is compromised due to tissue damage resulting from inflammation and elevated oxidative stress.[3,4]

Melatonin (Mel), previously recognized as a neurohormone exclusively produced by the pineal gland during nocturnal hours, has been demonstrated to influence the regulation of circadian rhythm. However, recent studies have revealed that Mel possesses significant antioxidant properties.[5] It has been demonstrated that Mel reduces the occurrence of oxidative damage via lipopolysaccharide (LPS) by inducing antioxidant-enzyme expression and has anti-inflammatory effects as well as mitochondrial protection effects.[6, 7] In experimental studies, the exogenous application of Mel has been observed to increase the survival rate in sepsis induced by LPS.[7] However, the precise protective mechanism of sepsis remains to be fully elucidated. LPS is a glycolipid micromolecule derived from the cell wall of gram-negative bacteria. It has been demonstrated that LPS instigates the secretion of inflammatory molecules and reactive oxygen and nitrogen derivatives, which bind to Toll-like receptor-4. Consequently, it has been employed in experimental sepsis models.[8] Increased oxidative stress has been identified as a significant contributor to bacterial transmission observed in sepsis, resulting in enhanced intestinal permeability and impaired intestinal barrier function.[9,10] Consequently, Mel, a well-known antioxidant, has been identified as a potential protective agent against oxidative damage to the intestinal barrier and the subsequent increase in permeability.[3]

It has been established that trace elements play a pivotal role in maintaining metabolic continuity, a process that encompasses vital functions such as digestion and absorption, a wide range of essential bodily functions, and homeostasis. They act as cofactors for various metabolic enzymes, regulate gene transcription, and bolster the body's defense against oxidative stress and inflammation.[11] It has been proposed that trace elements play an essential role in the prognostication and therapeutic management of sepsis, given their function as cofactors in numerous enzymatic reactions involved in the oxidant/antioxidant system and pro/anti-inflammatory balance. These elements modulate immune responses through their antioxidant properties and the regulation of cytokine production, which is crucial in the context of sepsis. Antioxidant enzymes, such as superoxide dismutase (SOD), are contingent upon trace elements for their biological functions.[11,12] Alterations in trace element and electrolyte levels have been documented in numerous tissues and organs, particularly in serum, in the context of sepsis.[12,13] However, hypotension resulting from electrolyte imbalance in sepsis can lead to shock and signifies a poor prognosis. The primary treatment objective in shock is to regulate the disrupted fluid-electrolyte balance. This is essential for ensuring adequate perfusion of tissues and organs.

YKL-40, with its glycoprotein structure, chitinase 3-like protein 1, is released from many tissues such as macrophages and neutrophils and is a protein with a glycoprotein structure that is activated in many processes, including cancer, as well as in acute and chronic inflammatory events. Their levels are important in determining the prognosis of the disease. Therefore, it is recommended to be used as a marker in inflammatory diseases.[14,15]

The aim of this study was to determine how Mel due to its antioxidant and anti-inflammatory effects- affects antioxidant enzymes and YKL-40 in serum, small intestinal morphology and tissue trace element-electrolyte levels in LPS-induced sepsis in rats.

MATERIALS AND METHODS

Experimental Groups

Ethical approval for the research was received from İstanbul Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (approval number: 2016/62, date: 30.05.2016).

Adult male rats Sprague Dawley weighing around 200-250g were commercially supplied with a diet and tap water ad libitum. Rats were kept in an experimental environment where 12h light/12h dark, humidity (55-60%), and temperature constant at 22±2 °C. Our study consisted of control, LPS, Mel, LPS + Mel groups, with 8 rats in each group. Sample size was calculated with the power analysis method.

Experimental Procedures

We did not apply any treatment to the rats that constitute the controls. In the LPS, the rats were applied 20 mg/kg of LPS i.p. as a single dose, prepared in 1 mL sterile saline (*Escherichia coli* O127:B8, Sigma-Aldrich, Product No: L5668). Mel group was administered intraperitoneally 10 mg/kg at 2-hour at intervals, by dissolving total 30 mg/kg (Mel, Sigma Aldrich, Product No: M5250) in 2.5% mL ethyl alcohol. In the LPS + Mel group, other doses of Mel (10 mg/kg, i.p.) were executed 30 minutes before LPS application and at the 2nd and 4th hours thereafter.

Six hours after the first injection, the rats were cut off under ketamine-xylazine anesthetic conditions. (i.p. route) and blood samples were removed into yellow-dry tubes from the heart. Serum was taken at 3000 rpm for 5 minutes by centrifugation. The small intestinal tissue samples were cleaned with physiological saline, and some of them were frozen in liquid nitrogen for analyses of trace element and electrolyte levels such as sodium (Na^{+}), potassium (K^{+}), magnesium (Mg^{++}), calcium (Ca^{++}), iron (Fe^{++}), copper (Cu^{++}), zinc (Zn^{++}), selenium (Se^{++}). Also, other parts of intestinal tissues were taken in 10% formaldehyde for histological examinations, and stored at -80 °C with the serum samples to determine YKL-40, and antioxidant defense enzymes such as glutathione reductase (GR), glutathione peroxidase (GSH-Px), and SOD levels.

Analyzing YKL-40

Serum YKL-40 was measured by double antibody sandwich technique using antibody-coated plates and enzyme-linked immunosorbent (ELISA) technique (China, Lot: YHB20170315843) at 450 nm.

Analyzing Antioxidant Enzyme Chains (GSH-Px, GR, SOD enzymes)

The levels of serum GR (China, Lot: YHB20171106220), GSH-Px (China, Lot: YHB20171106219), and SOD (China, Lot: YHB20171106218) were assayed with ELISA test at 450 nm.

Analysis of the Levels of Trace Element and Electrolyte

The inductively coupled plasma-optical emission spectroscopy (ICP-OES) (PerkinElmer-Optima 7000 DV) was used the levels of Fe, Se, Zn, Cu, Na, Ca, K, Mg (PerkinElmer-Optima 7000 DV) in small intestine tissue samples. Approximately 0.200/0.250 g of small intestinal tissue samples were weighed; it was treated in 10 mL of 20% HNO3 for 5 minutes at 145 °C, 5 minutes at 165 °C and 20 minutes at 175 °C.

The test tubes warmed at ambient temperature were filtered through Whatman; it was filled to 50 mL in bottles using ultrapure water. The samples were determined at ICP-OES and had a standard concentration of 50 ppb-1 ppm. Results were calculated as mg/kg.

Quality Control, Assurance

To quantitatively calculate the levels of each element in small intestine tissue, calibration standard vials were done to diluted 1000 mg/L ICP standard solution of multi-element (Merck). Calibration charts belonging each element were prepared changing from low to high calibration standard concentrations; a robust R2 level excessive 0.999 was obtained.

The accuracy and precision of the assays were verified with repeated calculations of calibration solutions of known concentrations. Limit of detection and limit of quantification values for each element were calculated by analyzing blank solutions (Table 1).[12,15]

Statistical Analysis

A power analysis using G*Power for this study on Serum GR levels, based on a similar design, indicated a required sample size of 32, consisting of 4 groups with 8 animals each. The effect size was 1.02, α=0.05, and power=0.95. The critical F-value was 3.09 (df=20.3), resulting in an actual power of 0.978. Given the sufficiency of serum and homogenate for all parameters, the samples were subjected to two replicates.

The Statistical Package for the Social Sciences 22.0 Software Programme (SPSS, Inc., Chicago, IL, USA) was performed as blindly. The conformity of the data to normal distribution was evaluated with the Shapiro-Wilk test. For the Shapiro-Wilk test, if the significance value was p>0.5, it was accepted that the distribution was normal. The significancy was assessed using ANOVA and following post hoc Tukey's between groups. The obtained results were shown as the mean±standard deviation. The statistical significance limit was accepted as p<0.05.

RESULTS

Serum Antioxidant Enzyme Chain Findings

Serum GR was significantly lower in the LPS group than control, LPS + Mel (p<0.05, both) and Mel (p<0.001) groups. Moreover, GR was found significantly increased in Mel compared to control, LPS + Mel (p<0.01, both), and LPS (p<0.001) groups. There are no differences between control and LPS + Mel (Figure 1-a).

Table 1. Summary of instrument settings

Element	Spectral line, (nm)	LoD, (µg kg^{-1})	LoQ, (µg kg^{-1})	RSD, (%)	R^2
Ca	317,933	47.52	158.40	0.96	0.999983
Cu	327,393	1.22	4.07	0.70	0.999905
Fe	238,204	12.79	42.63	0.86	0.999894
K	766,490	76.94	256.47	0.92	0.999916
Mg	285,213	21.64	72.13	0.83	0.999932
Na	589,592	63.81	212.70	0.67	0.999897
Se	196,026	0.88	2.93	0.94	0.999879
Zn	213,857	6.79	22.63	0.90	0.999928

Ca: Calcium, Cu: Copper, Fe: Iron, K: Potassium, Mg: Magnesium, Na: Sodium, Se: Selenium, Zn: Zinc, LoD: Limit of detection, LoQ: Limit of quantification, RSD: Relative standard deviation, R^2: Determination coefficient.

Table 2.1. Comparison of GR (ng/mL) values according to groups

Groups	n	Mean	F	p
Control	8	26.9148±0.51		
LPS	8	17.7015±0.13		
Mel	8	39.3503±0.91	16.95	0.00
LPS + Mel	8	26.8193±0.69		
Total	32	27.6964±0.57		

GR: Glutathione reductase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.2. Comparison of GSH-Px (mU/mL) values according to groups

Groups	n	Mean	F	p
Control	8	165,797±18.24		
LPS	8	151,575±12.11		
Mel	8	287,143±11.92	6,808	0.0026
LPS + Mel	8	199,042±16.23		
Total	32	200,889±14.45		

GSH-Px: Glutathione peroxidase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.3. Comparison of SOD (ng/mL) values according to groups

Groups	n	Mean	F	p
Control	8	32.6342±0.76		
LPS	8	38.7994±1.13		
Mel	8	34.7321±1.91	8,274	0.0025
LPS + Mel	8	38.7150±1.95		
Total	32	35.9592±2.97		

SOD: Superoxide dismutase, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 2.4. Comparison of YKL-40 (pg/mL) values according to groups

Groups	n	Mean	F	p
Control	8	19.012±0.32		
LPS	8	13.9289±1.10		
Mel	8	16.3200±0.80	11,934	0.0175
LPS + Mel	8	22.2171±1.93		
Total	32	17.8695±.077		

LPS: Lipopolysaccharide, Mel: Melatonin.

Serum GSH-Px was higher in the Mel compared to the results obtained in the control and LPS significant ($p<0.01$). There are no significancies among the other groups ($p>0.05$) (Figure 1-b).

Serum SOD was lower in the LPS than controls ($p<0.05$), and Mel ($p<0.01$) groups. While in the Mel, serum SOD was increased compared to LPS ($p<0.01$) and LPS+Mel ($p<0.05$) (Figure 1.c).

Table 3.1. Comparison of Fe^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	74.1989±0.76		
LPS	8	79.7941±0.13		
Mel	8	72.7111±0.19	70.47	<0.0001
LPS + Mel	8	75.7933±0.19		
Total	32	75.6241±0.29		

Fe^{++}: Iron, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.2. Comparison of Cu^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	6.1855±0.15		
LPS	8	6.6643±0.10		
Mel	8	6.0727±0.18	1,064	<0.0001
LPS + Mel	8	6.4699±0.11		
Total	32	6.3481±0.11		

Cu^{++}: Copper, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.3. Comparison of Zn^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	33.1812±0.1		
LPS	8	35.6833±0.3		
Mel	8	32.5158±1.1	65.02	<0.0001
LPS + Mel	8	34.1238±0.8		
Total	32	32.8760±0.5		

Zn^{++}: Zinc, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 3.4. Comparison of Se^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	0.7168±0.06		
LPS	8	0.7020±0.01		
Mel	8	0.7710±0.09	30.36	<0.0001
LPS + Mel	8	0.7363±0.05		
Total	32	0.7315±0.06		

Se^{++}: Selenium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.1. Comparison of Na^{+} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	649,373±18.2		
LPS	8	698,340±12.1		
Mel	8	636,352±10.1	137.5	<0.0001
LPS + Mel	8	673,147±11.8		
Total	32	664,305±10.3		

Na^{++}: Sodium, LPS: Lipopolysaccharide, Mel: Melatonin.

As an Early Biomarkers Serum YKL-40 Findings

YKL-40, an early marker of sepsis, was found decrease in the LPS compared to the results observed in other groups significantly ($p<0.05$). There is no observed significant change in the other groups ($p>0.05$) (Figure 1-d).

Electrolytes and Trace Elements Levels Analyzing

Intestinal tissue Na^{+}, K^{+}, Mg^{++}, Ca^{++}, Fe^{++}, Cu^{++}, Zn^{++}, Se^{++} levels were analyzed in all experimental groups and also Cu^{++}/Zn^{++} ratio was calculated. Except for Mg^{++} and Se^{++}, all electrolyte and trace element levels in the LPS group significantly increased compared to all experimental groups ($p<0.001$). Also, Mg^{++} and Se^{++} levels were lower in the LPS group than in other groups significant; for Se^{++}, and control and Mel groups for Mg^{++} ($p<0.001$). There were significantly changing between control and LPS + Mel group compared to control for Na^{+}, Mg^{++}, Ca^{++}, Fe^{++}, Cu^{++} ($p<0.05$) (Figures 2-3).

Histological Findings

It is observed that crypt depths and villus lengths decrease in the LPS group. It is determined that these lengths are relatively longer in the control, Mel and LPS + Mel groups compared to the LPS group. Additionally, it is observed that cell structures are disrupted in some places in the LPS (Figure 4).

DISCUSSION

The small intestine is the primary site of digestion and absorption within the gastrointestinal system. In the context of sepsis, the disruption

Table 4.2. Comparison of K^{+} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	772,917±17.6		
LPS	8	831,201±13.4		
Melatonin	8	757,419±21.5	71.07	<0.0001
LPS+Mel	8	778,321±12.9		
Total	32	784,965±17.7		

K^{++}: Potassium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.3. Comparison of Mg^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	43.7777±0.50		
LPS	8	42.1159±0.25		
Mel	8	46.2185±0.36	100.1	<0.0001
LPS + Mel	8	44.8372±0.91		
Total	32	44.2373±0.62		

Mg^{++}: Magnesium, LPS: Lipopolysaccharide, Mel: Melatonin.

Table 4.4. Comparison of Ca^{++} (mg/kg ppb) values according to groups

Groups	n	Mean	F	p
Control	8	895,880±7.6		
LPS	8	983,150±9.1		
Mel	8	914,245±3.2	757.6	<0.0001
LPS + Mel	8	954,479±5.9		
Total	32	936,938±8.1		

Ca^{++}: Calcium, LPS: Lipopolysaccharide, Mel: Melatonin.

of its microbial ecosystem, coupled with tissue injury resulting from inflammatory and oxidative damage, contributes to a marked deterioration of the intestinal barrier. This heightened significance of the small intestine in the prognosis of sepsis underscores its critical role in maintaining bodily functions.[16]

Oxidative stress is the result of an imbalance between oxidants and antioxidants, caused by an excess of ROS formation and a decrease in antioxidant levels. A multitude of studies have observed an increase in ROS levels and a decrease in antioxidant levels in septic patients. It has been proposed that oxidative stress may trigger the release of inflammatory mediators, thereby inducing inflammation through redox reactions that facilitate transcriptional activation, such as NF-κß.[17] Despite the development of numerous antioxidant therapeutic agents, treatment outcomes have been unsatisfactory. This failure has been attributed to the absence of effective antioxidant therapy at the mitochondrial level. The basis of the etiopathophysiology of organ dysfunction in septic conditions is suggested to be damage mediated by oxidative stress in mitochondria.[18]

Mel has been reported to have profound antioxidant activity by reacting with reactive species derived from both oxygen and nitrogen, and its metabolites also have antioxidant function. Mel exhibits both lipophilic and hydrophilic characteristics, with its highest concentrations being found in mitochondria. It has been demonstrated to possess both anti-inflammatory and antioxidant properties, capable of removing hydrogen peroxide, strengthening antioxidant pathways, and reducing nitric oxide formation. Mel has been documented to impede mitochondrial dysfunction, energy failure, and cell death. In animal models of oxidative damage, Mel has been shown to improve the release of inflammatory cytokines and increase GSH-Px, GR, and superoxide dismutase enzymes in the antioxidant enzyme chain.[19-21] Furthermore, Mel has been demonstrated to function as a mucosal protective agent, although its precise physiological role within the gastrointestinal tract remains to be fully elucidated. Studies have demonstrated that it reduces intestinal permeability, which increases due to damage caused by various reasons.[22, 23] The mechanism through which Mel exerts this effect remains to be fully elucidated, although studies have suggested a role for structural changes in the tight junctions of the intestinal epithelium.[24]

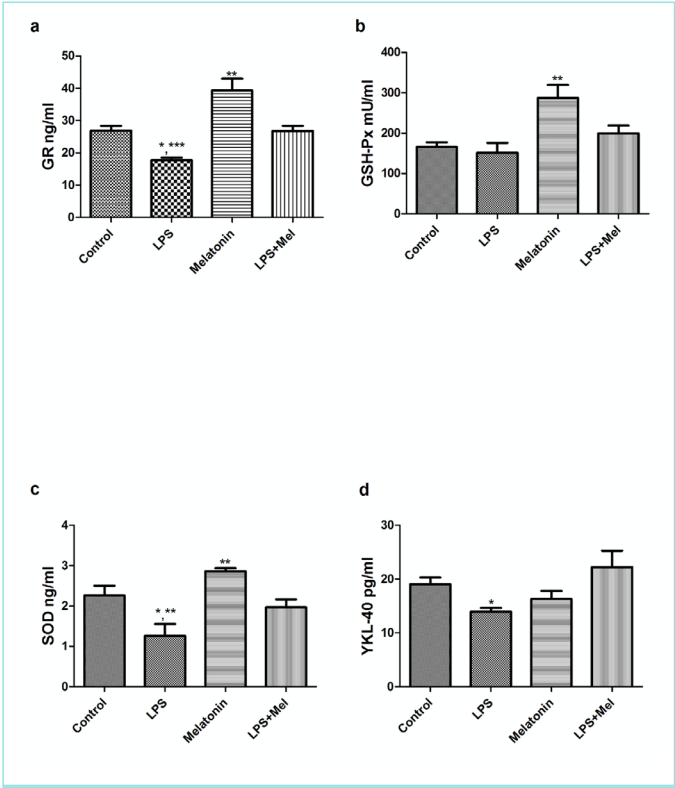


Figure 1. Serum antioxidant enzymes chain (GR, GSH-Px, SOD) and YKL-40 levels in all experimental groups; control, LPS, Mel, LPS + Mel. (a) *p<0.05 LPS vs. control and LPS + Mel; **p<0.01 Melvs. control and LPS + Mel; ***p<0.001 Melvs. LPS. (b) **p<0.01 Mel vs. control and LPS; (c) *p<0.05 LPS vs. control and Mel vs. LPS + Mel; **p<0.01 Mel compared to LPS; (d) *p<0.05 LPS vs. control.

GR: Glutathione reductase, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, LPS: Lipopolysaccharide, Mel: Melatonin.

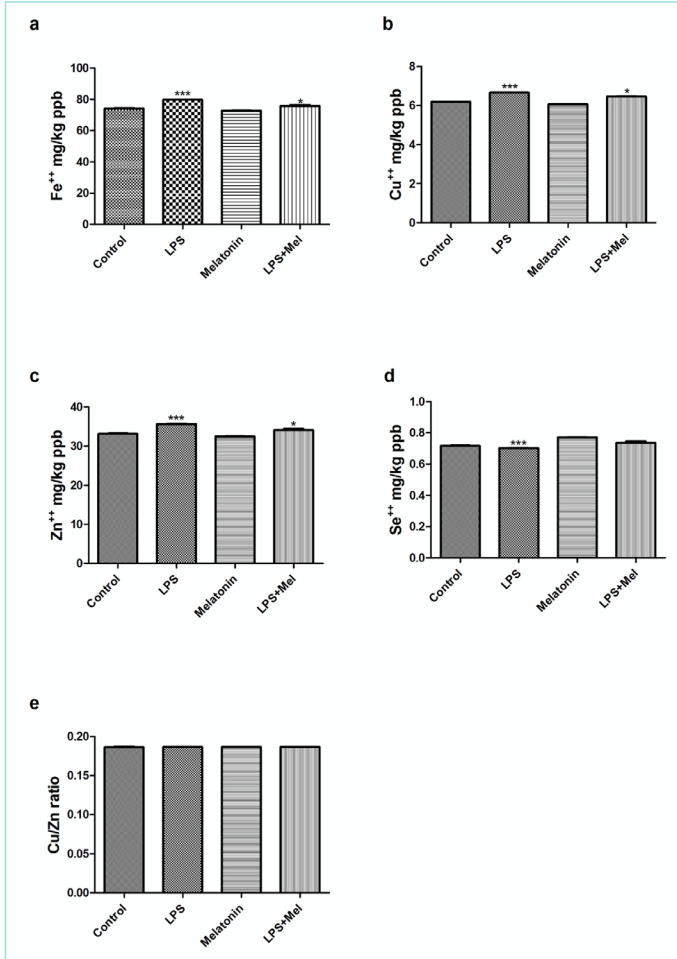


Figure 2. Trace elements levels for experimental groups (a-e). (a) Fe^{++} levels of intestinal tissue. (b) Cu^{++} levels of intestinal tissue. (c) Zn^{++} levels. (d) Se^{++} levels of intestinal tissue. (e) Cu^{++}/Zn^{++} ratio of intestinal tissue; *p<0.05 LPS + Mel vs. control and Mel; ***p<0.001 LPS vs. all experimental groups.

Fe^{++}: Iron, Cu^{++}: Copper, Zn^{++}: Zinc, Se^{++}: Selenium, LPS: Lipopolysaccharide, Mel: Melatonin.

Superoxide, an oxidant molecule, is released by mitochondria, primarily through the process of oxidative phosphorylation. It has been established that SOD represents the primary biochemical response to superoxide, the predominant harmful oxidant radical produced by mitochondria. SOD functions by dismutating superoxide radicals into hydrogen peroxide and molecular oxygen. An imbalance, whether it be an excess of superoxide anion or a deficiency in SOD, can lead to the accumulation of highly reactive superoxide radicals within the mitochondria. It has been reported that these radicals, when formed in excessive amounts, can interact with mitochondrial nitric oxide and form peroxynitrite, a reactive nitrogen species and a strong oxidant.[25]

The concentration of glutathione (GSH) is established by the catalysis process of GSH-related antioxidant enzymes called GSH-Px, which consumes reduced GSH, and GR, which regenerates reduced GSH from oxidized matter in mitochondria. Research has indicated that a decline in the activity of this enzyme may result in an accumulation of hydrogen peroxide, consequently leading to elevated oxidative stress and tissue damage.[20,26] It has been proposed that deficiencies in these enzymes may render cells more susceptible to oxidative damage.[27]

In our study, it was observed that in the group in which we induced sepsis with LPS, the levels of GSH-Px, GR, SOD, which are the main enzymes in the anti-oxidant chain, decreased significantly compared to other experimental groups, and enzyme levels increased significantly in septic rats administered Mel. In the group where Mel was administered alone, the levels of this internal enzyme were found to be higher compared to the control group. These outcomes align with existing literature, demonstrating that decreased mitochondrial antioxidant enzyme levels, which play a role in mitochondrial dysfunction due to oxidative damage in sepsis, can be mitigated by Mel. This finding further substantiates the substantial antioxidant activity of Mel.

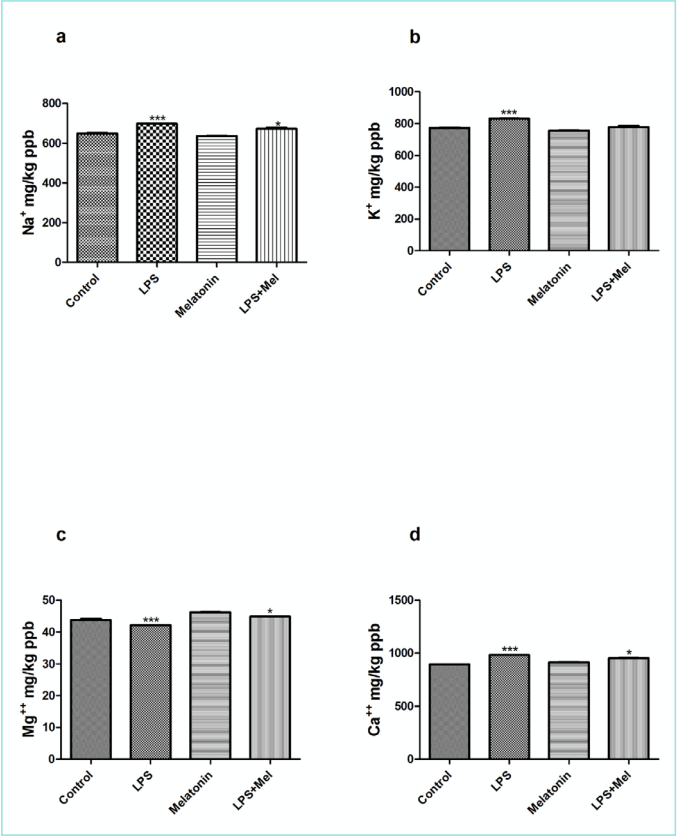


Figure 3. Electrolytes levels for all experimental groups (a-e). (a) Na^{+} levels of intestinal tissue. (b) K^{+} levels of intestinal tissue. (c) Mg^{++} levels of intestinal tissue**.** (d) Ca^{++} levels of intestinal tissue; *p<0.05 LPS + Mel vs. control and Mel; ***p<0.001 LPS vs. all experimental groups.

Na^{++}: Sodium, K^{++}: Potassium, Mg^{++}: Magnesium, Ca^{++}: Calcium, LPS: Lipopolysaccharide, Mel: Melatonin.

Disturbances in mineral and electrolyte homeostasis are common in many serious diseases, including sepsis. Moreover, the frequency of these changes in clinically critical is unclear.[28] It is reported that more than 66% of patients in intensive care have multiple electrolyte and acid-base abnormalities.[29] Since the functions of electrolytes and minerals in the protection and maintenance of cellular action, tissue perfusion and acid-base balance are vital in preventing complications and adverse outcomes. It has been reported that it is critical to keep aforementioned parameters at physiological concentrations.

It was detected that the levels of Na^{+} and K^{+} increased significantly in the LPS. In the LPS receiving Mel treatment, a significant decrease appeared compared to the LPS. It has been suggested that changes in electrolyte levels in the small intestine tissue in the sepsis group may occur due to impaired absorption levels due to sepsis, especially in studies that glucose transport decreases in sepsis and accordingly sodium accumulation may occur in the small intestine tissue.[30] It has also been stated that the effectiveness of the sodium potassium pump may change due to sepsis.[31,32]

Calcium is an ion that plays a role in triggering the apoptotic pathway, functions as a clotting factor (factor-IV), and has a role in the second messenger system. It has been reported that calcium can modify blood viscosity. Its potential involvement in coagulation, observed inflammation, and cell and tissue apoptosis in small intestinal injury has been suggested. In this study, calcium levels in the LPS group were found to be significantly lower compared to the other groups. The administration of Mel led to a reduction in this increase, approaching the level observed in the control group.

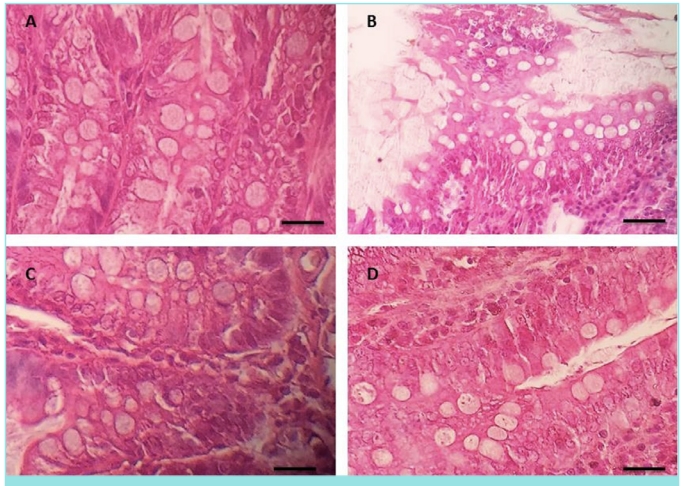


Figure 4. Image of histological section for all experimental groups (A-D). (A) control, (B) LPS, (C) Mel and (D) LPS + Mel groups.

LPS: Lipopolysaccharide, Mel: Melatonin.

Magnesium is a trace element that behaves as a cofactor of more than 300 biochemical catalyzer. In addition to its crucial for immunity, also magnesium has a function to induce of many enzymes such as phospho kinase and ATP.[33] In this study, we observed that magnesium levels in septic conditions intestinal tissue were significantly lower than the control group. We found that magnesium levels increased in the group to which we applied Mel. Low magnesium levels in sepsis are considered a sign of poor prognosis in terms of mortality.[34]

Selenium has antioxidant functions such as participating in the structure of selenoenzymes such as GSH-Px and being a component of other selenoproteins.[35] Many studies have shown that there is a insistent decrease in plasma selenium concentration in systemic inflammatory response syndrome and septic condition. It has been observed that the decrease in GSH-Px activity shows a parallel tendency with selenium levels.[12,36]

Copper is one of the trace elements required for many cellular enzymes to perform physiological function and also as a cofactor in the catalytic reactions that can lead to ROS formation when present in high intracellular concentrations.[37]

Research has demonstrated that Mel's primary function is not to serve as an effective radical-scavenging antioxidant by reacting with different pro-oxidant molecules, but rather to protect against the toxicity caused by copper by binding to copper via chelation within the cell.[38] In vitro and in vivo experiments have demonstrated that Mel, administered at concentrations of 1 mM and 50 mg/kg (i.p.), effectively mitigates the generation of hydroxyl radicals by high copper concentrations and polyphenols, thereby preventing DNA damage through copper chelation.[39] It has been reported that Mel exerts a bacteriocytic effect against pathological bacteria. However, the precise mechanism underlying this effect remains to be fully elucidated. Mel has been reported to exhibit high metal binding properties, including those of iron, copper, and zinc. It is imperative to note that bacteria require metals, particularly free iron, for their proliferation. One postulation suggests that Mel's metal-binding property may exert a bacteriocytic effect by diminishing the metal utilization of bacteria.[40]

In the present study, we observed that trace elements of Fe^{++}, Cu^{++}, and Zn^{++} exhibited a significant increase in the LPS group, while Se^{++} levels demonstrated a decrease. The administration of Mel resulted in a reversion of these trace element levels to levels more consistent with the control group.

It has been established that minerals are predominantly absorbed by the duodenum and subsequently transported into the circulation via the portal vein. It has been proposed that the assessment of villus height and crypt depth can serve as a metric for evaluating intestinal development, digestive processes, and absorptive capacity. While villus height and crypt depth are indicators of the digestive and absorptive function of the small intestine and the maturity of epithelial cells, the ratio of villus to crypt depth has been shown to be a more sensitive indicator of digestive and absorptive function.[41]

In the intestinal tissue sections of the LPS group, a decrease in crypt depths and villus lengths was observed. This phenomenon has been documented as a prevalent finding, suggesting a potential reduction in absorption. Acute excessive intestinal motility and especially glucose and amino acid absorption are impaired in sepsis. In the future, the intestinal barrier, which is disrupted as a result of tissue damage, may become excessively permeable and thus allow the invasion of bacteria.[3,40,42] It is noteworthy that the crypt depths and villus lengths in the sepsis group induced with LPS and in the group treated with Mel were greater than those observed in the LPS group.

Plasma levels of YKL-40, a promising prognostic marker for sepsis, have been found to be significantly elevated in patients with a poor prognosis. In this study, YKL-40 levels in the LPS group were found to be significantly lower compared to the other experimental groups. A previous study observed that the YKL-40 level exhibited an average increase by the 14th hour in patients with sepsis and poor prognosis. The present study is more acute than the aforementioned study. It is hypothesized that the results at the protein level may be influenced at a subsequent stage.[14,15]

Study Limitations

The limitations of our study include the inability to examine the acute model of sepsis as well as the chronic model and the lack of genetic or molecular pathway data to provide a balanced view of antioxidant mechanisms.

CONCLUSION

In this study, we evaluated the impacts of Mel on serum antioxidant enzymes, the trace element and mineral levels of small intestine tissue with the biomarker YKL-40. Our findings indicated that Mel ameliorated the injury caused by sepsis as a result of LPS stimulation. The observed activity of Mel can be attributed to its antioxidant, anti-inflammatory, and immunomodulatory.

MAIN POINTS

- Sepsis is one of the cases with the highest incidence. Small intestinal damage and impaired intestinal permeability due to sepsis are among the most frequently observed and fatal symptoms.

- Melatonin (Mel) is a powerful endogenous antioxidant. With its anti-oxidant and anti-inflammatory effects, it is very effective on the oxidative damage caused by sepsis and the resulting fetal damage.

- It is important to examine tissue trace element and electrolyte levels and the effects of Mel on these, as they are present in the structures of important enzyme systems, such as anti-oxidant enzymes, whose levels are affected in sepsis.

ETHICS

Ethics Committee Approval: Ethical approval for the research was received from İstanbul Bağcılar Training and Research Hospital Animal Experiments Local Ethics Committee (approval number: 2016/62, date: 30.05.2016).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.A., H.Y., E.Ö., Concept: G.A., H.Y., E.Ö., Ş.T., Design: G.A., H.Y., E.Ö., Ş.T., Data Collection and/or Processing: G.A., H.Y., Ş.T., İ.E.Y., V.O., Analysis and/or Interpretation: G.A., H.Y., E.Ö., İ.E.Y., V.O., Literature Search: G.A., Ş.T., Writing: G.A., E.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

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CASE REPORT

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-49
Cyprus J Med Sci 2025;10(1):79-82

Secondary Chronic Osteomyelitis of the Mandible and Concomitant Mature Florid Cemento-Osseous Dysplasia: A Case Report

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Abstract

Florid osseous dysplasia and osteomyelitis are distinct disease entities that exhibit similar clinical and radiographic manifestations. The avascular nature of florid cemento-osseous dysplasia (FCOD) may predispose individuals to osteomyelitis. This case study aimed to present a patient with mature FCOD in the mandible accompanied by secondary chronic osteomyelitis. A 72-year-old East African woman was admitted to the Oral and maxillofacial radiology clinic with chronic diffuse pain in the left mandible. A panoramic image from a previous dental center shows a sequestrum and multiple sclerotic radiopaque masses in the left mandibular molar region. Intraoral examination revealed necrotic bone exposed to the oral cavity in the same region. Subsequent surgical intervention was conducted under local anesthesia with appropriate antibiotic prophylaxis to expose necrotic, irregular, and pitted bone trabeculae on histopathological examination. Cemento-osseous dysplasias, including FCOD, can attain considerable sizes and may become exposed along the alveolar mucosa. In the present case, secondary osteomyelitis developed in association with exposed bone. The diagnosis of FCOD relies on a combination of radiological and clinical findings. Following diagnosis, regular monitoring and consistent follow-up are imperative for disease management.

Keywords: Florid cemento-osseous dysplasia, osteomyelitis, sequestration

INTRODUCTION

The term "osteomyelitis" originates from the ancient Greek words "osteon" (bone) and "muelinos" (marrow), signifying inflammation of bone marrow.[1] Today, it is recognized that the disease process extends beyond the medullary portion of the bone, encompassing the cortical and cancellous bone, as well as the periosteum.[2] According to the Zurich classification system, osteomyelitis of the jaws can be categorized into three groups based on clinical appearance, disease progression, and radiological features: (1) acute osteomyelitis, (2) secondary chronic osteomyelitis, and notably less frequently diagnosed, (3) primary chronic osteomyelitis.[3]

The introduction of a large inoculum of pyogenic organisms (bacteria, fungi, and mycobacteria) into deeper tissue planes through hematogenous dissemination from a distant source or direct inoculation of bone through trauma or surgery leads to an infectious process.[4] The establishment of this infectious process is influenced by factors such as the number and virulence of the pathogens, host immunity, and tissue perfusion. Systemic and local conditions that alter bone physiology and vascularization predispose individuals to osteomyelitis. Host factors that facilitate the development of acute and secondary chronic osteomyelitis of the jawbone due to compromised local blood supply include smoking, diabetes mellitus, Paget's disease, osteopetrosis (Albers-Schonberg disease), osteoporosis, bisphosphonate-induced

To cite this article: Efeoğlu S, Aktay E, Polat Balkan E. Secondary chronic osteomyelitis of the mandible and concomitant mature florid cemento-osseous dysplasia: a case report. Cyprus J Med Sci. 2025;10(1):79-82

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Received: 01.06.2024
Accepted: 02.12.2024
Publication Date: 14.03.2025

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osteochemonecrosis, tobacco use, radiation therapy, osteoradionecrosis, and bone malignancy. Fibro-osseous lesions represent one of the pathological conditions contributing to the increased incidence of osteomyelitis in the jaws.[1]

Benign fibro-osseous lesions (BFOL) are characterized by the replacement of normal bone with varying degrees of fibrous tissue and some bone/cementum-like tissue.[5] These lesions constitute a heterogeneous group of pathologies in the head and neck region, manifesting through reactive, dysplastic, and neoplastic mechanisms.[6] Cemento-osseous dysplasia (COD) stands out as the most prevalent BFOL in the alveolar regions of the gnathic bones. CODs are categorized into three subtypes based on their location: periapical COD, associated with the apical areas of mandibular anterior teeth; focal COD, linked to a single tooth; and florid COD [florid cemento-osseous dysplasia (FCOD)], displaying multiquadrant involvement.[7]

FCOD is often incidentally discovered through radiographic findings, and routine clinical diagnosis is challenging unless there is secondary infection.[8] Approximately half of FCOD lesions are asymptomatic radiographic findings, with symptoms such as dull pain, drainage, focal expansion, and facial deformities appearing when infection occurs.[9] It is important to note that FCOD and chronic osteomyelitis are independent conditions, and distinguishing between infected fibro-osseous lesions and true osteomyelitis poses challenges due to insufficient criteria.[10]

Although panoramic radiography is generally sufficient for initial diagnosis, advanced imaging techniques, such as computed tomography, are valuable for comprehensive diagnosis and treatment planning.[11] Nevertheless, the definitive diagnosis of fibro-osseous lesions relies on a combination of clinical, radiological, and histological findings.[6]

In this particular case, the objective was to present a mature FCOD of the mandible along with a case of secondary chronic osteomyelitis.

CASE PRESENTATION

The methods and reporting of this study adhere to the Surgical CAse REport guidelines[12], ensuring comprehensive and transparent documentation of the surgical case presented in this report.

Ethics Statement

According to our institutional guidelines, case reports or case series that do not involve experimental interventions or research on human subjects do not require formal ethics approval. Therefore, no specific ethics committee or IRB approval was obtained for this report. Informed consent was obtained from all participants.

A 72-year-old East African woman with no systemic disease presented to the oral and maxillofacial radiology clinic with a chief complaint of chronic diffuse pain in the left mandible that had been ongoing for 1 year. A sequestrum on a radiolucent background in the left mandibular molar region and multiple sclerotic radiopaque masses were observed in the panoramic image of the patient, which was taken at another dental treatment center. It was reported by the patient that the residual roots of teeth #32, #34, #43 and teeth #44, #42, #41, and #31 observed in the panoramic image were removed approximately 1.5 months ago. Intraoral examination revealed a necrotic bone exposed to oral cavity in the left mandibular molar region. A new panoramic image of the patient was obtained at our clinic. The panoramic image of the patient reveals the untreated large bone sequestrum (Figure 1). A cone beam computed tomography was prescribed for further three-dimensional examination of bone sequestrum in the left mandible and radioopaque lesions associated with the roots of teeth #17, #24, and #46 (Figures 2-4). Under local anesthesia with proper antibiotic prophylaxis, bone sequestrum was surgically inserted (Figure 5). Histopathological examination revealed necrotic, irregular bone trabeculae particles without nuclei (Figure 6). FCOD and chronic diffuse osteomyelitis of the mandible were diagnosed based on the clinical, radiographic, and histopathological features.

DISCUSSION

Fibro-osseous lesions are a group of bone disorders characterized by the replacement of normal bone with abnormal bone or fibrous connective tissue containing cementum. FCOD, also referred to as florid osseous dysplasia, is a rare non-neoplastic condition affecting the teeth and spongy part of the jaw. It presents as a cellular fibrotic structure with unencapsulated calcified formations that can be radiolucent and/or radiopaque. Contrary to the typical occurrence in middle-aged black women, the present case featured FCOD in women of advanced age.[13] However, this may be attributable to the delayed diagnosis of the case.

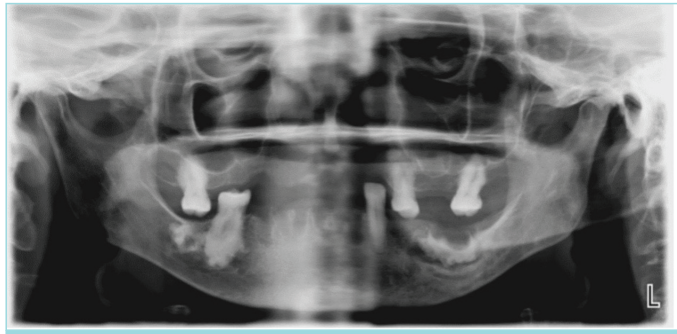


Figure 1. Panoramic view.

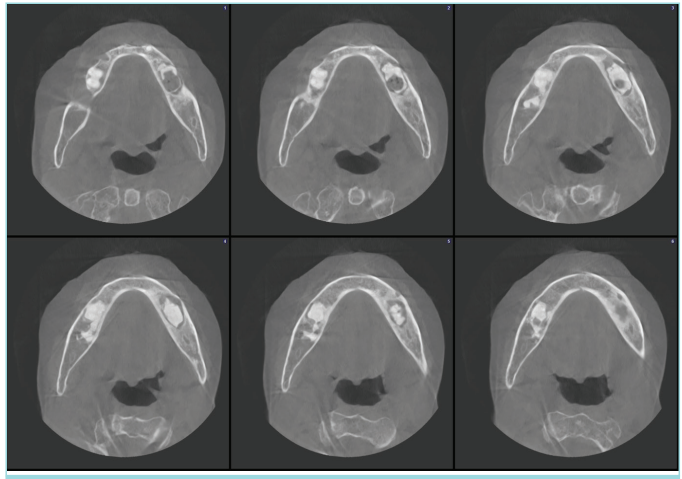


Figure 2. Axial view of the case in the CBCT: The imaging shows well-defined lesions in the right and left mandibular posterior regions, causing resorption and lytic areas in the mandibular bone, with well-defined radiopaque areas within.

CBCT: Cone beam computed tomography.

Delayed diagnosis may be attributed to the asymptomatic nature of the growth of FCOD.

COD can reach very large sizes over time and can be exposed along the alveolar mucosa. FCOD is prone to decreased vascularization; thus, exposed bone is susceptible to infection. Chronic osteomyelitis may develop from this bone base, which is susceptible to infection.[9,14] In the present case, secondary osteomyelitis with exposed bone was observed.

COD, including FCOD, can attain considerable sizes and may become exposed along the alveolar mucosa. The susceptibility to infection arises from decreased vascularization in FCOD, leading to the potential development of chronic osteomyelitis.[9,14] In the present case, secondary osteomyelitis developed in association with exposed bone.

Because of the avascular nature of FCOD lesions and the potential risk of infection or jaw fractures, biopsy is generally not recommended. Management primarily involves clinical-radiographic follow-up, with regular examinations for prophylaxis and reinforcement of good oral hygiene practices to control periodontal disease and prevent tooth loss. Antibiotics may have limited efficacy in treating FCOD lesions because of poor tissue diffusion.[15,16]

In the management of FCOD, it is imperative to differentiate it from other bone lesions to tailor the treatment approach. Tooth extraction in the presence of FCOD should be approached cautiously because it can potentially increase susceptibility to infection and the subsequent development of secondary osteomyelitis due to existing vascularization weakness.

Asymptomatic patients diagnosed with FCOD typically do not require active treatment. After diagnosis, further intervention is generally unnecessary. However, regular examinations during follow-up and recall appointments are crucial for prophylaxis, emphasizing the reinforcement of good home hygiene practices to control periodontal disease and prevent tooth loss.

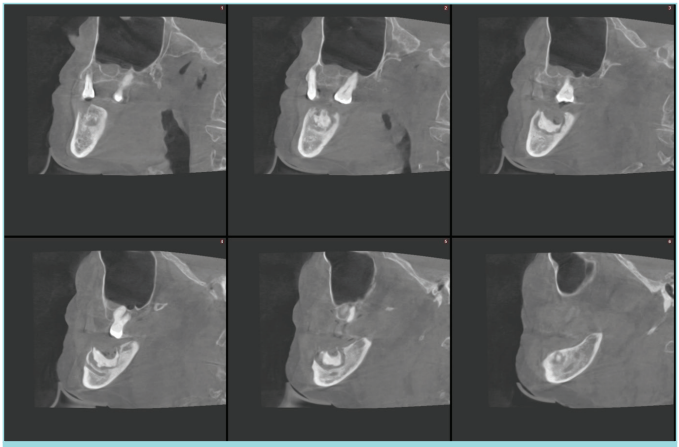


Figure 3. Sagittal view of the case in CBCT.

CBCT: Cone beam computed tomography.

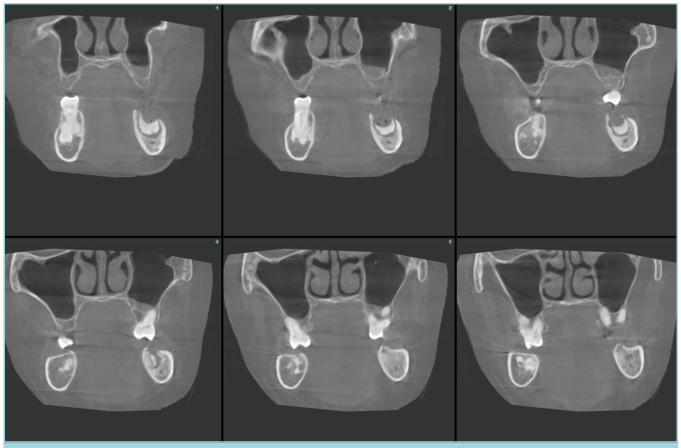


Figure 4. The coronal view of the patient in the CBCT. In this image, the relationship between the radiopaque lesions and the mandibular bone and teeth is clearly visible.

CBCT: Cone beam computed tomography.

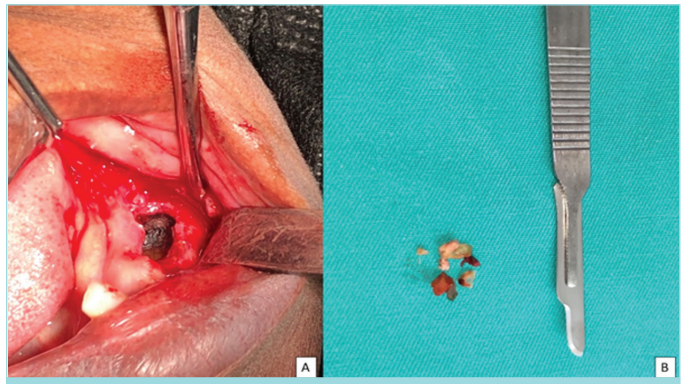


Figure 5. Intraoperative (A) intraoral view and (B) biopsy material.

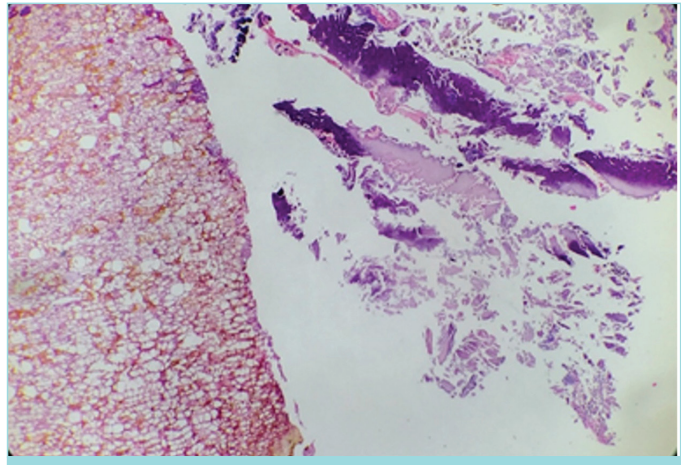


Figure 6. In the histopathology image, the left side of the sample shows an area containing remnants of vegetable matter, likely indicating foreign material or food debris. The right side reveals partially necrotic bone trabeculae that are irregularly shaped and lack distinct nuclei. These findings suggest a combination of chronic inflammation and necrosis. The bone trabeculae show signs of degradation, and no osteocyte nuclei are present within the lacunae, further supporting necrotic changes. The sample was stained with hematoxylin and eosin (H&E) at a magnification of 40x, providing clear contrast to visualize both soft tissue and bone structure abnormalities.

Antibiotics are generally ineffective in treating FCOD due to poor tissue diffusion. Despite these limitations, regular follow-up is mandatory due to the susceptibility of FCOD to infection and the potential risk of jaw fractures. To mitigate the risk of secondary infection, a biopsy was performed under antibiotic coverage, and the infected area was surgically debrided. The patient is currently under follow-up.

Re-evaluation using panoramic radiographs should be conducted asymptomatically every 2 or 3 years as part of the ongoing monitoring for individuals with FCOD. The management of symptomatic patients becomes more challenging due to the development of chronic inflammation and infection within the dense mineralized tissue. Antibiotics are indicated for symptomatic patients, and surgical interventions such as debridement and enucleation may be necessary. However, it is important to note that these interventions may not always yield a positive response to antibiotics, primarily because of the avascular nature of the lesion.[11,13-16]

Bone diseases carry significant diagnostic implications and have the potential for devastating consequences if not identified early. Therefore, accurate and early diagnosis is crucial for informed decisions regarding the most appropriate treatment. This involves a thorough analysis of the risks and benefits associated with each potential intervention. Regular re-evaluation, especially for asymptomatic patients, plays a key role in ensuring the ongoing health and well-being of patients with FCOD.

In the typical diagnosis of jaw lesions, histological assessments, clinical examinations, and radiographic analyses collectively inform the diagnostic process. However, FCOD, characterized by its avascular nature and increased vulnerability to infection, deviates from this norm. Diagnosis relies exclusively on meticulous examination of radiological manifestations and clinical presentations, eschewing biopsy procedures. Following the diagnostic stage, diligent follow-up and routine controls are imperative for effective disease management, ensuring comprehensive oversight and control of the condition.

MAIN POINTS

- Bone diseases carry significant diagnostic implications and can have devastating consequences if not identified early.
- Regular re-evaluation, especially for asymptomatic patients, plays a key role in ensuring the ongoing health and well-being of patients with florid cemento-osseous dysplasia (FCOD).
- Tooth extraction in the presence of FCOD should be approached cautiously because it can potentially increase susceptibility to infection and the subsequent development of secondary osteomyelitis due to existing vascularization weakness.

ETHICS

Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.A., E.P.B., Concept: E.A., E.P.B., Design: E.A., E.P.B., Data Collection and/or Processing: E.A., E.P.B., Analysis and/or Interpretation: E.A., E.P.B., Literature Search: E.A., E.P.B., Writing: E.A., E.P.B.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study has received no financial support.

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CASE REPORT

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-51
Cyprus J Med Sci 2025;10(1):83-85

Iatrogenic Botulism Following Botulinum Toxin Injection in Palmar Hyperhidrosis: A Case Report

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Abstract

Botulinum toxin (BT) injection is commonly used for the treatment of hyperhidrosis, with palmar hyperhidrosis being a frequent indication. Although generally safe, rare cases of botulism following BT administration have been reported. It is a potentially serious complication of inappropriate administration of high doses with unlicensed product application; therefore, prompt recognition and appropriate management are crucial for satisfying outcomes. We present the case of a 28-year-old woman who presented with progressing clinical condition mimicking myasthenia gravis (MG). Diagnosis was confirmed through clinical examination, laboratory testing, neuroimaging, and electrophysiological findings. The aim of this case report is to highlight the importance of considering iatrogenic botulism in patients presenting with neurological symptoms, particularly weakness, ptosis, dysphagia, and dysarthria, and to underscore the utility of a MG treatment protocol in managing cases of iatrogenic botulism presenting late.

Keywords: Botulism toxin, iatrogenic botulism, muscle weakness, myasthenia gravis, palmar hyperhidrosis

INTRODUCTION

Botulinum toxin type A (BT-A) has grown across various medical fields, particularly neurology and dermatology, for both therapeutic and cosmetic purposes. It is used by a wide range of healthcare providers, including estheticians and specialists. Although generally safe, BT-A can occasionally cause iatrogenic botulism, a potentially life-threatening condition resulting from improper dosage or unlicensed products.[1] This condition can mimic symptoms of myasthenia gravis (MG) and include general weakness, difficulty swallowing, speech disorders, and double vision due to extraocular muscle involvement.[2] The symptoms related to impaired neuromuscular transmission can be observed with both BT-A and antiacetylcholine receptor antibodies.[3]

Here, a case of iatrogenic botulism diagnosed in a patient initially referred to the neurology department with symptoms resembling MG is presented.

CASE PRESENTATION

A 28-year-old woman with no significant medical history presented with weakness in her arms and legs, difficulty swallowing, double vision, and drooping eyelids. Two weeks earlier, she received 200 MU of BT-A (Dysport®) injection into both palms to treat excessive sweating. Neurological examination revealed bilateral ptosis, limited outward gaze on the left side, and peri-orbital muscle weakness. Despite these symptoms, her pupils were isochoric, and her direct and consensual light reflexes were normal. The patient also exhibited slurred speech, weak tongue and masseter muscles, dysphagia, and mild proximal extremity weakness. Laboratory tests and magnetic resonance imaging of the brain and cervical cord were normal. Electroneuromyography (EMG) revealed normal nerve conduction velocities, normal amplitudes of the compound muscle action potential, and no decrement in low-frequency repetitive nerve stimulation at 3 Hz in the facial

To cite this article: Önder Ö. Iatrogenic botulism following botulinum toxin injection in palmar hyperhidrosis: a case report. Cyprus J Med Sci. 2025;10(1):83-85

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Received: 30.05.2024
Accepted: 09.12.2024
Publication Date: 14.03.2025

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nerves. Single-fiber EMG of the left orbicularis oculi showed impaired neuromuscular transmission with a mean jitter of 133 µs and 60% blocking. Acetylcholine receptor antibodies were negative. Based on these findings, the patient was diagnosed with iatrogenic botulism and treated with oral pyridostigmine (240 mg daily) and supportive therapy. Due to persistent proximal weakness, oral prednisolone was added at a dose of 1 mg/kg/day for 4 weeks. After 2 weeks, ptosis and periorbital muscle strength significantly improved. The patient still had mild nasal speech and weakness in the tongue, masseter, extremities, and neck retroflexion muscles. At the 2-month follow-up, the patient was only on pyridostigmine 120 mg daily, with normal neurological examination. Treatment was tapered and discontinued after 3 months. A written informed consent was obtained from the patient prior to the publication of this report and accompanying images.

DISCUSSION

Botulism is caused by neurotoxins, primarily serotypes A, B, E, and F, which are some of the most potent toxins known to be produced by *Clostridium botulinum*. However, this can also occur in other Clostridium species.[4] Although the lethal dose for humans is not precisely known, experimental animal studies suggest that the lethal dose of purified crystalline BT-A could be around 1 µg/kg in oral intake.[5] Although contaminated food is the most common source of botulism, botulism can also arise from wound colonization or aerosol exposure developed for biological weapon purposes.[6] In recent years, there has been an increase in iatrogenic botulism. Although the primary use of BT-A is therapeutic, treating conditions such as muscle dystonia, spasticity, hyperhidrosis, neurogenic bladder, strabismus, and various pain syndromes, the increase in iatrogenic botulism cases has been linked to the widespread use of high-dose BT-A injections, which are frequently employed for cosmetic applications.[7] Contributing factors include the utilization of unlicensed and inappropriate applications, such as those observed at events termed "botox parties". The circulation of unlicensed products with potentially dangerous contents has also increased due to increased global migration and trade.

Botulism commonly manifests as symmetric descending flaccid paralysis with notable bulbar palsies, frequently involving cranial nerve involvement.[8] Although rare, sensory involvement and even rarer, accompanying pain have been reported.[9] An important feature of iatrogenic botulism is that similar to the toxin's primary effects, its associated adverse effects are typically transient. The diagnosis of iatrogenic botulism hinges on the precise identification of recent BT-A injection history along with clinical manifestations.[10] The differential diagnoses mainly include Guillain-Barre syndrome, MG, stroke, and Eaton-Lambert syndrome.[11] It has been reported in the literature that masked MG can emerge following BT-A injections.[12] Hence, the diagnosis of MG was excluded based on the absence of characteristic clinical fluctuations, minimal abnormalities in EMG findings, and normal antibody levels.

Electrophysiological tests, including assessment of CMAP increment following exercise or high-frequency repetitive nerve stimulation, demonstrate high specificity and sensitivity in the diagnosis of botulism.[13] In contrast, a decremental response after low-frequency repetitive nerve stimulation can be observed, as in our case.[14] In botulism, compound muscle action potential values typically exhibit a decrease, while single-fiber electromyography often reveals increased jitter and blocks, as evidenced in our case.[15] It is noteworthy that exaggerated jitter and blocking were detected in asymptomatic subjects following therapeutic BT-A injections for various conditions. The sensitivity of single-fiber electromyography suggests its utility in detecting subclinical iatrogenic botulism.[16,17]

While awareness of the clinical signs and symptoms of botulism is critical for early diagnosis, there is no need to wait for confirmation of diagnosis via laboratory or electrophysiological tests to provide initial treatment. The rapid administration of antitoxin within the first 48 hours can significantly reduce respiratory complications and mortality.[8-11] Botulinum toxin (BT) is a key component of the treatment, and it neutralizes free circulating toxin by binding concomitant and irreversible.[18] Since supportive treatments have a crucial role in achieving a better response in addition to antitoxin therapy, it cannot reverse settled neurotoxicity. Pyridostigmine, which is commonly used to treat MG, has been investigated for the treatment of botulism, including iatrogenic cases. It works by inhibiting acetylcholinesterase, thus increasing the availability of acetylcholine at the neuromuscular junction. However, in some countries, limited antitoxin availability and high costs have represented critical issues in recent years. Given the critical 48-hour window for administration, emphasis is often placed on the use of pyridostigmine, despite it not being considered a first-line treatment.[19] As the symptoms persisted into the second week, antitoxin administration was not pursued, and treatment commenced with pyridostigmine alongside supportive measures.

Recovery involves axonal sprouting, new motor endplate growth, and protein regeneration, and complete recovery can take months. The recovery reaches a peak after 5-10 weeks in a mouse model.[13] Paralysis can persist for months, depending on factors such as the dosage and

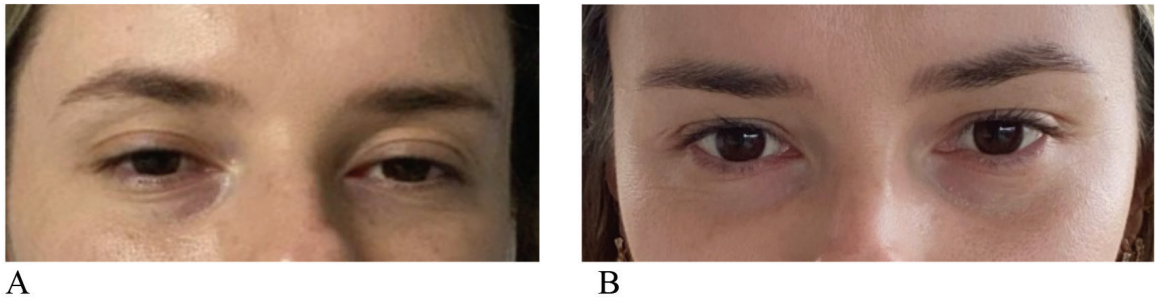


Figure 1. (A) Bilateral ptosis at presentation. (B) Improvement in ptosis at the end of eight weeks follow-up period (a written informed consent was obtained for using her images from the patient).

specific serotype of the toxin. Prolonged muscle paralysis can result in secondary effects, such as neuromuscular junction degeneration and muscle atrophy. Respiratory paralysis can persist for an extended period, sometimes for weeks or even longer. Chronic ventilation may be required in cases of persistent respiratory paralysis. However, prolonged mechanical ventilation poses significant risks of complications and comorbidities, including ventilator-associated pneumonia and other respiratory infections. Furthermore, prolonged ventilation can cause muscle weakness, atrophy, and psychological challenges for the patient.[20] Despite the absence of respiratory symptoms, full recovery was achieved in approximately 3 months.

This case underscores the importance of careful differential diagnosis in botulism, especially in distinguishing botulism from MG, and highlights the necessity of administering botulism-based antibiotherapy (BT-A) under appropriate conditions by experienced professionals.

Physicians and patients must be aware of the risks associated with the illegitimate use of unlicensed BT products. Physicians should ensure that they use only licensed products clinically to minimize potential complications and should include symptoms indicating possible complications clearly in their medical consent forms to protect themselves from medicolegal issues. Entities inappropriately marketing, selling, or using unlicensed BT products should be prosecuted and subjected to full criminal and civil penalties to protect the community from potential future issues.

MAIN POINTS

- With the increasing use of botulinum toxin, clinicians must identify the clinical manifestations of associated complications to facilitate prompt diagnosis and effective management.
- Strict adherence to approved formulations and professional guidelines is essential for safe and effective treatment.
- Iatrogenic botulism and neuromuscular disorders, such as myasthenia gravis can exhibit overlapping clinical presentations. This case underscores the importance of meticulous clinical assessment and the application of precise diagnostic methods to avoid misdiagnosis.

ETHICS

Informed Consent: A written informed consent was obtained from the patient prior to the publication of this report and accompanying images.

Footnotes

DISCLOSURES

Financial Disclosure: The author declared that this study has received no financial support.

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