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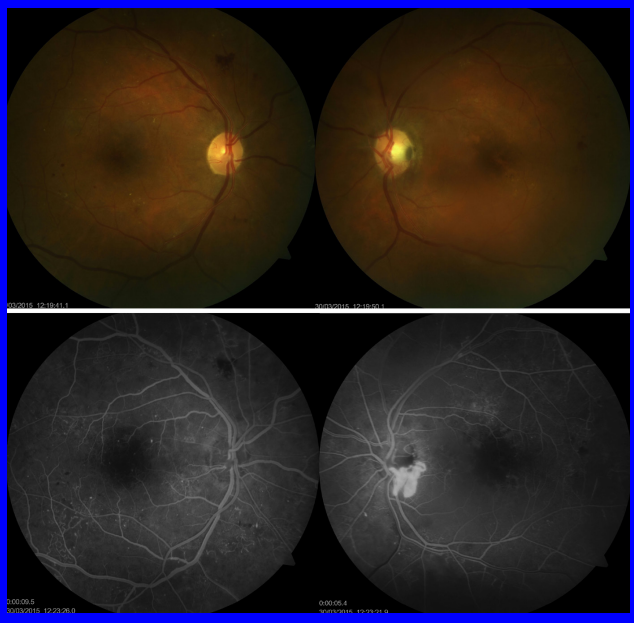

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Original Articles

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EDITORIAL

Dear Readers;

We are together again in the second issue of 2024. After a year full of scientific studies, academic activities and congresses, we are happy to see the reflections of increasing scientific activities in the many articles submitted to our journal.

In this issue, most of the articles come from the field of Dentistry. I would even like to point out that we had difficulty choosing the cover image. We could have chosen it from an area where there were the names and authors of the articles were written, but the image in the case report of Şahin et al. titled "Unilateral Optic Disc Neovascularization in a Patient with Optic Disc Pit Dependence on Proliferative Diabetic Retinopathy" was chosen as the cover image due to the majority voting. Optic pit seen in the eye is defined as an optic disc excavation that is usually asymptomatic, but sometimes causes symptoms with the development of maculopathy. In this case report, a patient with diabetic retinopathy who had a unilateral optic pit and advanced disc neovascularization in the same eye was examined, and we shared the visual with you. We thank Şahin and his team for this case report.

Other notable articles are:

1- Şahin A, Ekinci Aslanoğlu C, Özdemir H. Unilateral Optic Disc Neovascularization in a Patient with Optic Disc Pit Depending on Proliferative Diabetic Retinopathy.

2- Elter B, Paken G, Çömlekoğlu ME. The Effect of Glass Ceramic Layering on the Marginal Leakage of Zirconia Supported Crowns.

3- Hösükler E, Erkol ZZ. Forensic Geriatric Trauma Cases.

4- Maraşlı M, Keleş E, Yıldız P, Kırlangıç MM, Baki RB, Mat E, Yıldız G, Adanır İ, Dane B. Clinical Importance of the First Trimester Uterine Artery Doppler Measurements in Patients with Hyperemesis Gravidarum.

5- Kılıç B, Ünal HY, Ekinci E. Orthodontic Materials Interacting with Fifth Generation (5G) Electromagnetic Waves.

In this issue, we also present to you a selected review of the approach to fibromyalgia, which still contains uncertainties in diagnosis and treatment, under the title "Approach to Fibromyalgia and the Role of Phytotherapy in Treatment". We observe that the popularity of traditional and complementary medicine in our country is increasing day by day. In this review, the epidemiology, pathophysiology, diagnostic methods, pharmacological and non-pharmacological methods used in treatment, and the most used phytotherapeutic products of fibromyalgia syndrome are discussed.

As an advantage of being a multidisciplinary journal, I hope that you will find an article related to your field, as in every issue. I would like to thank my assistant editors, referees, publishing house, and you, our valued readers, who contributed to the preparation of this issue. Everything you get your heart desires…

Kind regards,
Prof. Dr. Adem AKÇAKAYA
Chief Editor

Original Article

DOI: 10.14235/bas.galenos.2023.26818
Bezmialem Science 2024;12(2):149-54

The Effect of Glass Ceramic Layering on the Marginal Leakage of Zirconia Supported Crowns

Cam Seramik ile Tabakalamanın Zirkonya Destekli Kronlarda Marjinal Sızıntıya Etkisi

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ABSTRACT

Objective: The purpose of this study was to evaluate the effect of veneering with lithium-disilicate glass-ceramic material on the marginal microleakage of zirconia-supported crowns.

Methods: Ten freshly extracted human third molars were embedded in an acrylic mold from the roots. Crown preparation of each tooth half was handled differently. The distal half of each tooth was prepared with a chamfer-type margin (1.2 mm), while the mesial half was finished with a mini-chamfer (0.6 mm). Zirconia frameworks were designed and manufactured with computer-aided design/computer-aided manufacturing, and the frameworks were veneered on the distal surfaces of the framework using lithium-disilicate glass-ceramic. Specimens were thermocycled and immersed in a basic fuchsine dye solution for 24 hours. Four cross-sections were made from each specimen (n=40), and dye penetrations were recorded under a stereo microscope for microleakage measurements. The microleakage values were statistically analyzed with a Mann-Whitney U test (α=0.05).

Results: Mean microleakage values were recorded for each group. The values for the ceramic veneered margin group were noted as 1.17±0.69 mm, while the values for the zirconia margin group were noted as 1.03±0.74 mm. The results did not show significant differences for the compared groups (p=0.102).

Conclusion: Lithium-disilicate glass-ceramic veneering for zirconia-supported restorations did not enhance the marginal seal capability.

Keywords: Finish-line, glass-ceramic, marginal microleakage, zirconia

ÖZ

Amaç: Bu çalışmanın amacı, lityum-disilikat cam-seramik ile tabakalamanın zirkonya destekli kronlardaki marjinal sızıntıya etkisini değerlendirmektir.

Yöntemler: Yeni çekilmiş 10 adet yirmi yaş dişi akrilik ile doldurulmuş kalıplara kök hizalarından gömüldü. Her bir dişin distal yarısı chamfer basamak (1,2 mm), mezial yarısı ise mini-chamfer (0,6 mm) olacak şekilde prepare edildi. Zirkonya altyapılar bilgisayar destekli tasarım/bilgisayar destekli üretim yöntemiyle hazırlandı. Altyapıların distal yarısı lityum-disilikat cam-seramik ile tabakalandı. Örnekler ısıl döngüye tabi tutulduktan sonra 24 saat süreyle bazik fuksin boyayıcı solüsyona daldırıldı. Her örnekten dört kesit alındı (n=40) ve mikrosızıntı ölçümleri için boya penetrasyonları stereomikroskop altında kadyedildi. Mikrosızıntı değerleri Mann-Whitney U testi ile analiz edildi (α=0,05).

Bulgular: Her grup için ortalama mikrosızıntı değerleri kaydedildi. Cam-seramik ile tabakalanmış zirkonya grubunda ortalama değerler 1,17±0,69 mm olarak kaydedilirken zirkonya kenar grubunda 1,03±0,74 mm olarak kaydedildi. Gruplar arası fark istatistiksel olarak anlamlı değildir (p=0,102).

Sonuç: Zirkonya destekli restorasyonlarda lityum-disilikat cam-seramik ile tabakalamanın marjinal sızdırmazlığa katkısı gözlenmemiştir.

Anahtar Sözcükler: Bitim hattı, cam seramik, marjinal mikrosızıntı, zirkonya

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Introduction

In recent years, the increasing demand of patients for high esthetic restorations has contributed to the development of new restorative materials that aim to ensure the longevity of the restoration by improving its mechanical and optical properties and minimizing technical problems. Zirconia has many beneficial properties, such as excellent biocompatibility, high strength, and low plaque accumulation. The material also has low translucency owing to its crystalline microstructure. High-strength zirconia is usually used as a framework and should be layered with veneering ceramic to meet patients' esthetic expectations (1-3). With computer-aided design/computer-aided manufacturing (CAD/CAM) technology, it is possible to fabricate the optimal zirconia coping/framework for crown and bridge restorations. Despite its high costs CAD/CAM ensures reproducible results by reducing the technician errors that occur in the laboratory and providing standardization (4). After manufacturing the zirconia framework, glass-ceramics can be applied through several manufacturing processes, including the layering and press techniques (5,6). In the layering technique, ceramic powder is applied directly to the zirconia core before firing. In the press technique, a ceramic ingot is heated and forced under pressure after lost wax technique application (7).

In addition to the material's esthetic properties, strength, and biocompatibility, precise marginal fit and marginal seal are also fundamental requirements for the clinical success of dental restorations (1). The marginal finish lines of full-crown restorations can be classified as feather-edged, shoulder, mini chamfer, and chamfer (8). Studies have shown that finish line type, ceramic veneering procedures, differences in the thermal expansion coefficients between the core and the veneering ceramic, and veneering ceramic thickness affect the marginal adaptation of the restorations (9-12). An inadequate marginal adaptation can result in a gap between the restoration and the prepared tooth, thus accelerating the dissolution of the cement. Subsequently, oral bacteria and food debris accumulate in this space, leading to secondary caries, pulpal lesions, postoperative sensitivity, periodontal disease, and marginal discoloration (8,13-15). Also, the cementation process affects the marginal adaptation and, subsequently, the marginal microleakage of the restoration (12). The microleakage amount of a cemented restoration depends on many other factors, like tooth preparation, restoration material, restoration fabrication method, cement type, and cementation procedures (16,17).

In the literature, the effects of different manufacturing methods (18), various cement types (19), and finish line designs (20) on marginal microleakage of zirconia frameworks have been investigated. To the authors' best knowledge, there is limited study on the effect of the finish line material on the microleakage amount of zirconia-supported crown restorations. The zirconia-supported restorations mainly have zirconia finish lines. However, due to the absence of glass phase or silica in their structure, they do not show sufficient adhesion ability to the tooth as much as glass-ceramic (1). The surface roughening process, which is applied to glass-ceramics, such as hydrofluoric acid, before cementation, is not effective for zirconia due to its high volume of crystals and absence of glass matrix. It is easy to achieve strong, predictable adhesive retentions on silica-based ceramics, but zirconia has limitations in bonding with dental tissue due to its composition (21,22).

Finishing the margin area with lithium-silicate glass-ceramic material was thought to contribute to a better marginal seal and lower marginal microleakage with the help of increased adhesion to the tooth structure. Therefore, the purpose of this study was to evaluate the influence of veneering with lithium-disilicate glass-ceramic on the marginal microleakage of zirconia-supported crowns using optical image processing software.

The null hypothesis was that there would be no difference in marginal microleakage values between the two finish lines prepared with zirconia and lithium-disilicate glass-ceramic veneered zirconia.

Methods

This study was approved by the İstanbul Okan University Clinical Research Ethics Committee (approval number: 56665618-204.01.07, date: 22.07.2020).

Ten freshly extracted and cleaned third human molars were embedded in an acrylic mold (Figure 1a). The distal half of each tooth was prepared with a chamfer margin (1.2 mm), while the mesial half was finished with a mini-chamfer (0.6 mm) (Figure 1b). The preparations were carried out by the same researcher, and a new diamond bur (Brasseler, Savannah, GA, USA) was used under water cooling. The prepared teeth were then scanned with an intra-oral digital scanner (CEREC Omnicam, Dentsply Sirona, Bensheim, Germany). The crown frameworks were designed using computer-aided design software (CEREC 4.3, Dentsply Sirona) (Figure 1c). Margin lines were drawn, leaving a homogenous margin thickness of 0.6 mm in the zirconia margin halves. On the other half side, an additional space of 0.6 mm was left for veneering ceramic material in the ceramic veneered margin group. Partially sintered zirconia blocks (inCoris Z.I., Dentsply Sirona, Bensheim, Germany) were milled (CEREC inLab MC XL, Dentsply Sirona, Bensheim, Germany) for the fabrication of the frameworks (Figure 2). The milled frameworks were then sintered with a sintering furnace (inFire HTC speed, Dentsply Sirona, Bensheim, Germany) according to the manufacturer's instructions.

Following the sinterization period, the zirconia frameworks were cooled down carefully. Each zirconia framework was fitted on the corresponding prepared tooth and checked for optimal adaptation. The frameworks were veneered on the distal surfaces of the zirconia framework using a lithium-disilicate glass-ceramic (IPS e.max Ceram, Ivoclar Vivadent, Schaan, Liechtenstein), which had a convenient coefficient of thermal expansion in terms of zirconia (Figure 2). The ceramic veneering process was completed in two firings using a ceramic furnace (Vita Vacumat 6000 M, Vita Zahnfabrik, Baden-Wurttemberg, Germany).

The prepared crowns were cemented using a dual-curing resin cement (Multilink N, Ivoclar Vivadent, Schaan, Liechtenstein).

Following cementation, ten specimens were subjected to 5.000 cycles of thermal cycling between 5 °C and 55 °C with a dwelling time of 20 seconds (23). Then, the specimens were immersed in a basic fuchsine dye solution for 24 hours. After the specimens were removed from the solution, residual surface stains were cleaned with a toothbrush under running water. Four cross-sections were taken from each restoration-tooth complex for the microleakage measurements (n=40). A total of 80 measurements of dye penetration were recorded under a stereo microscope at a magnification of 20 (Leica Microsystems, Germany). Each microleakage value was defined and recorded as the distance of the stain penetrated from the outer border of each margin using optical image processing software. The recordings were then converted to a millimetric scale. Both zirconia and ceramic veneered zirconia halves of restorations were measured to compare the results.

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, USA) for Windows.

Results

The Shapiro-Wilk test was used to examine whether the data showed normal distribution. The microleakage values were analyzed with Mann-Whitney U test since the data did not show normal distribution. P-values less than 0.05 were considered statistically significant. Mean microleakage values were evaluated and the values for ceramic veneered margin group was 1.17±0.69 mm, (Figure 3a) while the values for non-veneered zirconia margin group was 1.03±0.74 mm (Table 1, Figure 3b). There were not significant differences between the two margin groups (p=0.102).

Discussion

The null hypothesis was accepted because veneering of zirconia frameworks with lithium-disilicate glass-ceramic did not cause a difference in marginal microleakage values.

Studies have investigated the effect of veneering ceramics in zirconia framework on marginal, internal, and horizontal discrepancies (24-26). However, not enough study was found on the effect of finish line material on microleakage after cementation. Pak et al. (27) evaluated the marginal fit of two different zirconia based crown systems (Digident CAD/CAM, Lava CAD/CAM) before and after ceramic veneering. The ceramic veneering process was found to have increased the marginal gaps between the teeth and the restorations for both groups (Digident increased from 61.52 to 83.15 µm; Lava increased from 62.22 to 82.03 µm). Kohorst et al. (26) also evaluated the marginal fit of two different, four-unit zirconia (VITA In-Ceram YZ Cubes, KaVo Everest ZS Blanks) frameworks after veneering with recommended ceramic systems. While frameworks from Everest ZS Blanks veneered

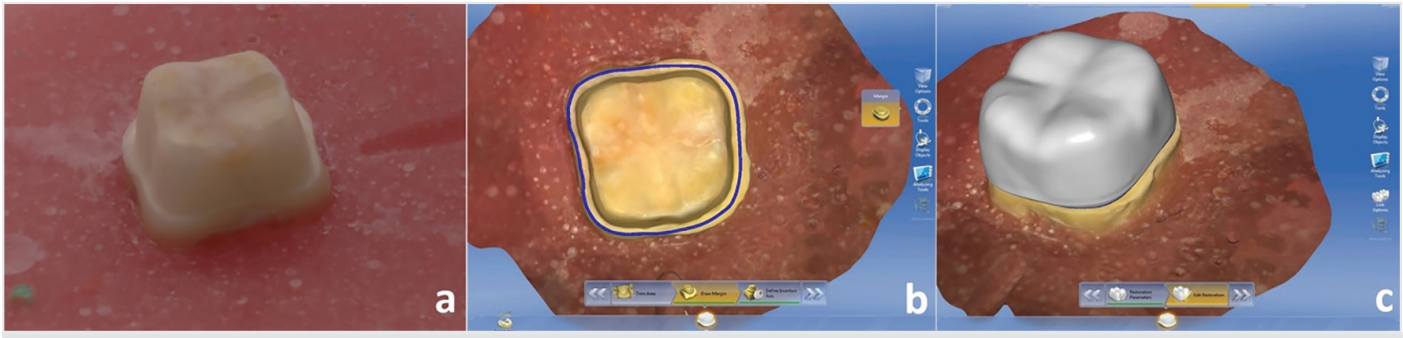

Figure 1. Representative image of a human third molar embedded in an acrylic mold (a), drawn margin lines for the zirconia framework (b), designed zirconia framework, using a computer-aided design software (Cerec 4.3, Sirona) (c)

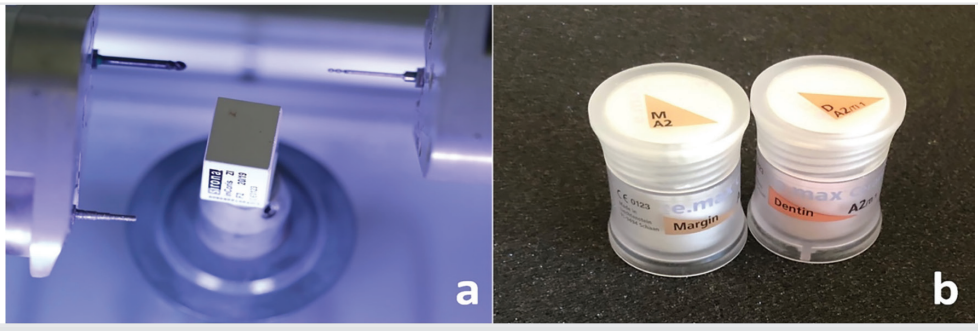

Figure 2. Representative image of a CAD/CAM zirconia block (a) and lithium-disilicate glass-ceramic layering material (b) used in the study

CAD/CAM: Computer-aided design/computer-aided manufacturing

with GC Initial Zr ceramic showed decreased marginal gap (p=0.019) and internal gap (p=0.001); frameworks from In-Ceram YZ Cubes veneered with VITA VM9 ceramic showed no differences after the veneering process. It was concluded that, extend of distortions after the veneering process depended on the type of zirconia and ceramic combination. Vigolo and Fonzi (28) evaluated the marginal fit of three different ceramic veneered zirconia systems (Everest veneered with Vita D-ceramic, Procera veneered with NobelRondo Zirconia, Lava veneered with Lava Ceram). The measurements were handled before ceramic application, after ceramic firing and after glaze firing. It was reported that ceramic firing cycles and glaze cycles did not alter the marginal fit of zirconia based ceramic restorations. The results were in accordance with the results of the present study. In the present study, the ceramic veneered margin group showed similar microleakage values to the non-veneered zirconia group. Different from the mentioned studies, in the present study, the effect of ceramic veneering on zirconia frameworks were evaluated by measuring the marginal microleakage values after cementation on natural human teeth. Single type of a veneering ceramic and a zirconia material was used; therefore, the methodology differences might contribute to the different conclusions.

In the present study, the layering technique was used as a veneering method. Both the layering technique and the press technique can be utilized to veneer zirconia frameworks (1,6). The layering technique achieves superior esthetic results far more often than the pressing technique by means of individual contouring of the ceramic veneer. This veneering technique, which comprises a firing procedure at a high temperature (750-900 °C) followed by a cooling process, is often performed two to five times of firing cycle (29). Balkaya et al. (11) investigated the effect of the firing cycles on the marginal fit of ceramic crowns, and reported that porcelain firing cycle altered the marginal adaptation of the ceramic crowns. Numerous firing processes could bring about a distortion and a decrease in the marginal adaptation of the framework (27). However, in the present study, ceramic veneering was completed after two firings. Therefore, the increased microleakage values due to distortion and poor adaptation created by repeated firings were tried to be eliminated. Furthermore, distinctions in the ceramic materials' structures and different manufacturing methods may create different results (30).

In the literature, shoulder, chamfer, and mini-chamfer finish lines of CAD/CAM-fabricated crowns have been investigated (8,30-32). Comlekoglu et al. (8) recommended rounded shoulder and chamfer preparation for the finish line designs of zirconia-supported restorations as they showed better marginal adaptation than feather-edged finish line. Komine et al. (32) also evaluated the marginal adaptation of three types of finish lines as shoulder, rounded shoulder, and chamfer. The three groups had no significant differences, and marginal adaptation values were within clinically acceptable limits. Al-Zubaidi and Al-Shamma (30) evaluated the effect of 90° shoulder and deep chamfer finish lines on the marginal adaptation of zirconia crowns and reported that the deep chamfer finish line was better than the shoulder, especially for zirconia crowns. Pan et al. (33) concluded that chamfer-type finish line with a 0.4-0.6 mm or shoulder-type finish line with a 0.5 mm thickness showed lower peak stress values than feather-edged finish lines in zirconia restorations. In the present study, 0.6 mm thickness chamfer finish line was prepared for the zirconia support.

Marginal discrepancies can be detected using various measurement techniques, such as a direct view of the crown on a die, the impression replica technique, observation with scanning electron microscopy (SEM), light microscopy, or X-ray microtomography (12,34). In the present study, the effects of different finish-line materials on the marginal microleakage

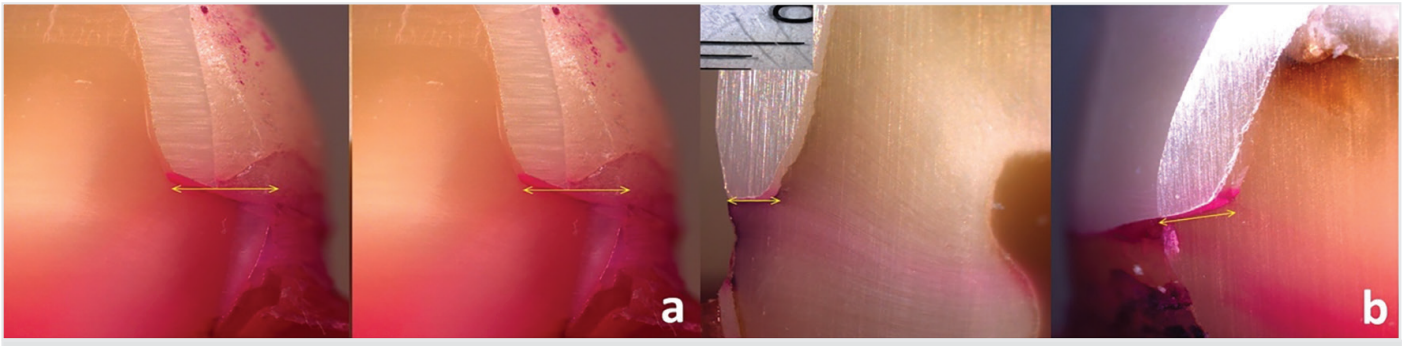

Figure 3. Optical image analysis of microleakage in ceramic veneered margin group (a), Optical image analysis of microleakage in zirconia margin group (b)

Table 1. Microleakage scores (mm) between test groups with means and standard deviations (mean ± SD)

	Mean ± SD	Min.	Max.	Median	Interquartile range
Ceramic veneered margin group	1.17±0.10	0.30	3.17	0.99	0.50
Zirconia margin group	1.03±0.11	0.26	3.88	0.87	0.42

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

of veneered zirconia crowns were evaluated using a stereo microscope. Groten et al. (35) investigated both SEM and light microscopy while evaluating marginal adaptation and concluded that there was no significant difference in validity between these imaging techniques. On the other hand, obtaining a small number of cross-sections from a specimen is a limitation of the evaluation with a microscope (12).

Study Limitations

In the present study, one type of resin cement was used while luting the crowns, different cementing procedures and cements might result in different marginal leakage values. Also, one type of zirconia and veneering ceramic was used, further studies were required to measure the marginal microleakage values of different zirconia and compatible veneering ceramic combinations. Various techniques may be combined while evaluating the marginal microleakage values to eliminate the technique-related limitations.

Conclusion

Within the limitations of this *in vitro* study, the non-veneered zirconia margin group showed similar microleakage values to the ceramic veneered margin group. It could be concluded that lithium-disilicate glass-ceramic veneering for zirconia-supported restorations did not enhance the marginal seal capability.

Ethics

Ethics Committee Approval: This study was approved by the İstanbul Okan University Clinical Research Ethics Committee (approval number: 56665618-204.01.07, date: 22.07.2020).

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: M.E.Ç., Design: M.E.Ç., Data Collection or Processing: G.P., B.E., Analysis or Interpretation: G.P., B.E., Literature Search: G.P., B.E., Writing: G.P., B.E.

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

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COVID-19 Phobia in Pregnant Women and its Effect on Vaccination Attitude

Gebelerde COVID-19 Fobisi ve Aşı Tutumuna Etkisi

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ABSTRACT

Objective: Coronavirus disease-2019 (COVID-19) vaccination rates among pregnant women are lower than the general population. This study aimed to determine the impact of COVID-19 phobia and vaccination attitudes among pregnant women.

Methods: This descriptive and cross-sectional study was conducted online with 254 pregnant women between May 2022 and December 2022; sociodemographic characteristics, COVID-19 vaccination data, Coronavirus Phobia Scale, and Anti-vaccine Scale of women with pregnancies older than 12 weeks of gestation were compared.

Results: Our study determined that 68.5% of pregnant women received COVID-19 vaccination before pregnancy and 4.7% during pregnancy. It was determined that 30% of pregnant women did not know about COVID-19 vaccines. It was determined that there was a significant positive correlation between COVID-19 phobia and anti-vaccination levels. COVID-19 phobia was higher in pregnant women with children and low economic income. It was determined that women who had never been vaccinated had higher levels of anti-vaccination.

Conclusion: Lack of information, having children, low economic income, the belief that the vaccine will have adverse side effects on the pregnant woman and her baby, and COVID-19 phobia were associated with low vaccination rates in pregnant women. These factors should be considered to raise public awareness and increase vaccination in pregnant women.

Keywords: COVID-19, COVID-19 phobia, vaccine attitude

ÖZ

Amaç: Gebe kadınlar arasında koronavirüs hastalığı-2019 (COVID-19) aşılama oranları, genel nüfusa göre daha düşüktür. Bu çalışmanın amacı gebelerde COVID-19 fobisi ve aşı tutumuna etkisini belirlemekti.

Yöntemler: Mayıs 2022-Aralık 2022 tarihleri arasında 254 gebe ile çevrimiçi olarak gerçekleştirilen, tanımlayıcı ve kesitsel tipte olan bu çalışmada 12. gebelik haftasından büyük gebeliğe sahip kadınların sosyodemografik özellikleri, COVID-19 aşısına ait verilerle Koronavirüs Fobisi Ölçeği ve Aşı Karşıtlığı Ölçeği karşılaştırıldı.

Bulgular: Çalışmamızda gebelerin %68,5'inin gebelikten önce, %4,7'sinin ise gebelikte COVID-19 aşısı yaptırdığı belirlendi. COVID-19 aşıları ile ilgili gebelerin %30'unun bilgisi olmadığı tespit edildi. COVID-19 fobisi ile aşı karşıtlığı düzeyleri arasında ileri düzeyde pozitif yönde bir ilişki olduğu tespit edildi. Çocuğu olan ve ekonomik geliri az olan gebelerde COVID-19 fobisi daha yüksek saptandı. Hiç aşı olmayan kadınların aşı karşıtlığının daha yüksek olduğu belirlendi.

Sonuç: Bilgi eksikliği, çocuk sahibi olma, ekonomik gelirin az olması ve aşının gebenin kendisi ve bebeği üzerinde olumsuz yan etkisi olacağına dair inanç ve COVID-19 fobisi gebelerde aşılanma oranının düşük olması ile ilişkilendirilmiştir. Bu faktörler toplumun bilinçlendirilmesi ve gebelerde aşılanmayı artırmaya yönelik çalışmalarda dikkate alınmalıdır.

Anahtar Sözcükler: COVID-19, COVID-19 fobisi, aşı tutumu

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Introduction

The coronavirus disease-2019 (COVID-19), which emerged in late 2019, continued to have an impact worldwide and caused at least 6.3 million people to die in mid-2022 (1). Many variants of the COVID-19 virus have emerged especially Delta and Omicron. The COVID-19 virus affects individuals in some risk groups more and causes a severe disease course. Pregnant women are among these risk groups (2-4).

There are no different measures to be taken specifically for pregnant women to prevent disease transmission. Among the methods to prevent virus transmission, vaccination has been reported to reduce pregnancy deaths caused by COVID-19 (5). The Centers for Disease Control and Prevention vaccine safety monitoring program reported no data on complications such as gestational diabetes, preeclampsia, intrauterine growth retardation, miscarriage, stillbirth, or premature birth due to mRNA vaccines administered during pregnancy (6). On September 02, 2021, the Coronavirus Scientific Committee of the Ministry of Health in Türkiye recommended that pregnant women should be vaccinated starting from the 12th week of pregnancy to prevent adverse outcomes of the COVID-19 pandemic in the mother and baby (7).

It has been reported that COVID-19 in pregnancy causes various psychological problems such as fear, panic, or phobia due to being associated with maternal and neonatal morbidity and mortality, complete control of the virus, and increasing need for intensive care (8). A high level of fear or phobia is a condition that reveals avoidance behaviors in individuals and significantly restricts life. Karkın et al. (9) found that pregnant women had a higher level of COVID-19 phobia than non-pregnant women. Fear of coronavirus causes more protection behavior (10). One of the protective behaviors against coronavirus is vaccination. However, pregnant women refuse to be vaccinated due to the desire to protect themselves and the fetus, mistrust of global vaccines, and some sociocultural factors (11,12). Therefore, evaluating the data on COVID-19 vaccine refusal in pregnant women in different countries is essential. World Health Organization reported in 2019 that vaccine refusal had become a significant threat to global health (13). Because the success of a vaccine depends not only on its effectiveness but also on its acceptance. Little is known about the relationship between COVID-19 phobia and vaccine refusal in pregnant women. To the best of our knowledge, no study in the literature evaluates phobias and vaccine refusal in pregnant women with a validated and reliable tool. Determining the relationship between this phobia and vaccine refusal in pregnant women with reliable tools may explain why or how fear can lead to increased depressive symptoms and help target interventions for vaccine refusal, which has become a global problem. This study was conducted to determine the impact of COVID-19 phobia and vaccine attitudes in pregnant women.

Methods

This descriptive and cross-sectional study was conducted between May 2022 and December 2022. The study population consisted of women with pregnancies older than 12 weeks of gestation in Türkiye. The sample size was 196, calculated with a 95% confidence level and a sensitivity level of 0.03, based on a population of 1152859 using the birth statistics (14) of Turkish Statistical Institute for 2021 and the prevalence of COVID-19 among pregnant women in the literature (4). Considering the possibility of missing data, the study was completed with 254 pregnant women. Since maximum diversity was aimed in this study, the data were collected online. Pregnant women who were literate, in their 12th or higher gestational week, and who did not have a psychiatric disorder were included in the study. Ethical approval for the study was obtained from İstanbul Arel University Ethic Committee (decision no: 07, date: 18.07.2022). Also, the study was conducted under the principles of the Declaration of Helsinki.

The Introductory Information Form, Coronavirus Phobia Scale, and Vaccine Opposition Scale (long form) prepared in line with the literature were used as data collection tools. Research data were collected through an online form (Google form). Participants were invited to the study electronically. After clicking on the link to the study, they were directed to a section providing brief information about the study and confirming their willingness to participate voluntarily. After confirming this section, they filled out the Turkish forms.

The descriptive information form consisted of 31 questions developed by the researchers to determine women's sociodemographics, general health status, pregnancy, and coronavirus history.

The coronavirus phobia scale is a 5-point Likert-type self-assessment scale developed by Arpaci et al. (15) to measure the phobia that may develop against the coronavirus. Scale items are evaluated between 1 "strongly disagree" and 5 "strongly agree"; items 1, 5, 9, 13, 17, and 20 measure the psychological sub-dimension; items 2, 6, 10, 14, and 18 measure the somatic sub-dimension; items 3, 7, 11, 15, and 19 measure the social sub-dimension; and items 4, 8, 12 and 16 measure the economic sub-dimension. Scores ranging from 20 to 100 points indicate higher scores in the sub-dimensions and general corona phobia. In a study in which the C19P-S scale used in our study was compared with another scale in Türkiye, the factorial structure of the C19P-S scale consisted of psychological, somatic, social, and economic dimensions, and the Cronbach's alpha value was found to be 0.92 (15). In our study, the Cronbach's alpha coefficient for the scale in terms of reliability was found to be 0.939.

Anti-vaccination scale (long form); The form developed by Kılınçarslan et al. (16) to measure anti-vaccination is a 5-point Likert-type scale. Scale items are evaluated between 1 "strongly disagree" and 5 "strongly agree". The 21 items are grouped into four sub-dimensions. Of these, 1, 2, 3, 5, and 8 constitute the first sub-dimension. These items are related to "vaccine benefit and protective value". Items 10, 14, 16, 17, 18, and 19 constitute the second sub-dimension of "vaccine opposition". Items 27, 28, 30, 32, and 33 constitute the third-factor solutions for not getting vaccinated. Items 6, 7, 13, 15, and 21 form the

fourth factor with the sub-dimension "legitimization of vaccine hesitancy". The higher the score, the greater the opposition/hesitancy to vaccination (section A items are reverse scored because they consist of statements in favor of vaccination). The Cronbach's alpha value of the scale is found to be 0.905 (16). In our study, the Cronbach's alpha coefficient for the scale in terms of reliability was found to be 0.711.

Findings

The mean age of the pregnant women was 29.73±5.122 years, and the mean age of the spouses was 32.73±5.633 years. It was found that 70.5% of the pregnant women were university graduates, 52% were not employed, 73.2% lived with their spouses and children, 90.9% had social security, and 61% had moderate income.

The mean gestational week of the women who participated in the study was 25.88±9.113. In addition, 51.6% of the pregnant women did not have children, and 49.1% of those who had children had a previously expected delivery. The risk status and attitudes of pregnant women regarding coronavirus were analyzed in Table 1.

Figure 1 shows the total scale scores of the pregnant women who participated in our study. The mean score on the Coronavirus Phobia Scale was 44.18, and the mean score on the Opposition to Vaccination Scale was 55.17. The comparison of pregnant women with the Coronavirus Phobia Scale and the Anti-vaccination Scale according to some variables was analyzed in Table 2.

It was determined that the scores obtained by pregnant women from the Coronavirus Phobia Scale differed statistically according to having children and income status ($p<0.05$). In addition, it was determined that the scores obtained by women from the Vaccine Opposition Scale differed statistically according to the status of receiving coronavirus vaccination before pregnancy and receiving information from health personnel ($p<0.05$). The regression model (Table 3) established to examine the effect of coronavirus phobia on vaccine opposition was statistically significant ($F=10.254$, $p<0.05$). Coronavirus phobia had a positive effect on vaccine opposition (=0.198) was determined. Coronavirus phobia explained 0.039 of the change in vaccine opposition (Table 4).

Statistical Analysis

Data were analyzed with SPSS 26.0. In the study, the scores for the coronavirus phobia scale and the anti-vaccination scale were calculated, and the kurtosis and skewness coefficients were examined to determine the suitability of the scores for normal distribution. According to this result, it was concluded that the scores were normally distributed. Since the scores showed normal distribution, parametric test techniques were used in the study. The t-test and ANOVA test were used to analyze whether the scale score differed according to demographic characteristics. Pearson correlation analysis was used to examine the direction and severity of the relationship between the coronavirus phobia scale and the anti-vaccination scale, and linear regression analysis was used to examine the effect of coronavirus phobia on anti-vaccination.

Discussion

This study determined that COVID-19 phobia and vaccine opposition in pregnant women were at moderate levels. There was a significant positive correlation between COVID-19 phobia

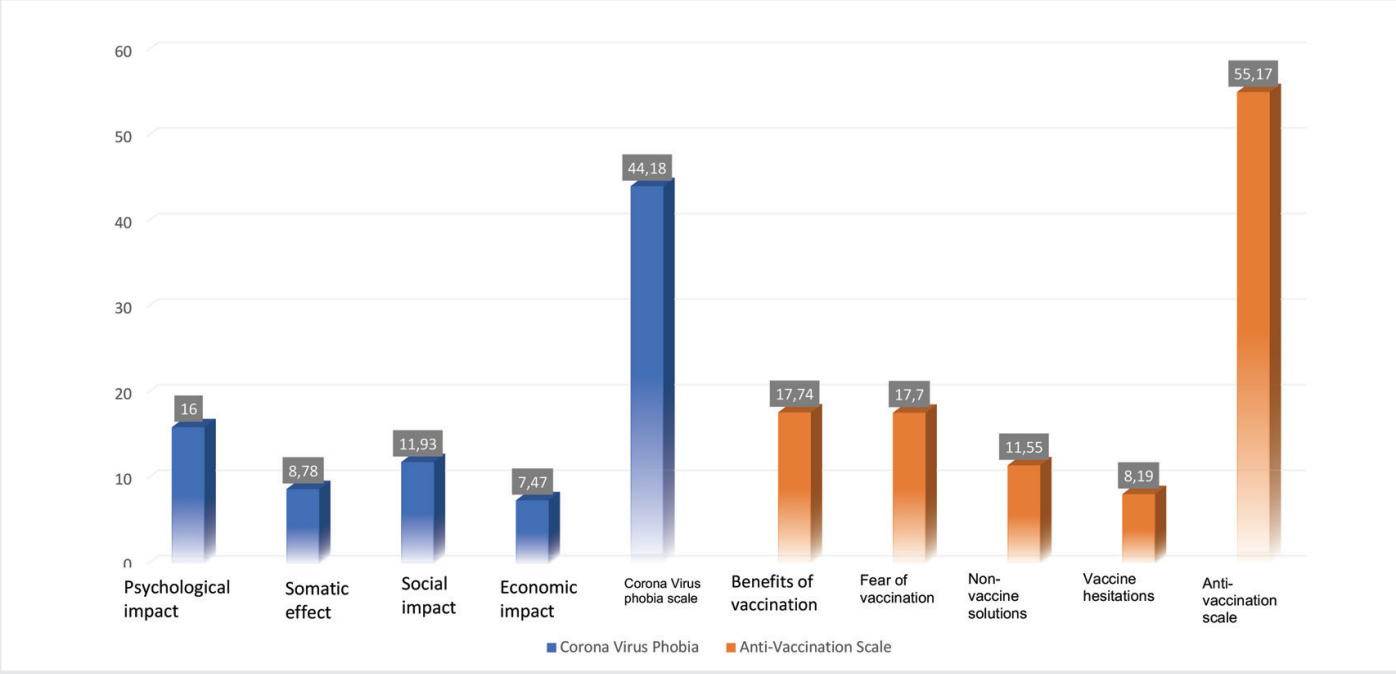

Figure 1. Pregnant women's scores on the coronavirus phobia scale and anti-vaccination scale (n=254)

Table 1. Risk status and attitudes of pregnant women regarding coronavirus

		n	%
Having a chronic disease	Yes	53	20.9
	No	201	79.1
Cigarette smoking	Yes	32	12.6
	No	222	87.4
The problem in current pregnancy*	No problems	189	74.4
	Preeclampsia	4	1.6
	Gestational diabetes	19	7.5
	Threat of miscarriage	18	7.1
	The threat of premature birth	12	4.7
	Placental problems	5	2.0
	Other	7	2.8
Having coronavirus before pregnancy	Yes	117	46.1
	No	137	53.9
Having a coronavirus disease during pregnancy	Yes	37	14.6
	No	217	85.4
Vaccinations during pregnancy*	Tetanus	135	53.1
	Hepatitis (A-B)	11	4.4
	Flu vaccine	4	1.6
	Coronavirus	12	4.7
	No vaccination	92	36.2
Coronavirus vaccination before pregnancy	Yes	174	68.5
	No	80	31.5
Persons from whom she/he received information or counseling on vaccination during pregnancy*	Family	21	8.3
	School	2	0.8
	Friends	16	6.3
	Online	41	16.1
	From TV	17	6.7
	From books/magazines/newspaper	10	3.9
	From the physician	121	47.6
	From the nurse	79	31.1
	No information	76	29.9
Reasons for not getting vaccinated against coronavirus*	I do not believe there is a coronavirus disease	3	1.2
	I do not believe the vaccine protects against coronavirus	23	9.1
	I think the vaccine will have side effects on my health	41	16.1
	I think the vaccine will have side effects on my baby	42	16.5
	My spouse/partner does not want me to get vaccinated	4	1.6
	I do not know enough about vaccination	7	2.8
	No reason	9	3.5

*Question with more than one marking

Table 2. Distribution of pregnant women's coronavirus phobia and opposition to vaccination by variables

Variables			Coronavirus phobia scale				Anti-vaccination scale		
		n	Avg	SD			Avg	SD	
Education Status	Literate/primary school	15	48.13	11.46	F=2.153	p=0.094	57.13	7.68	F= 2.493 p=0.061
	Middle school	17	50.06	16.38			59.24	7.74	
	High school	43	45.72	13.88			56.05	7.40	
	University and above	179	42.92	13.54			54.40	8.17	
Having children	Yes	123	46.37	13.23	t=2.478	p=0.014*	123	55.80	t=1.236
	No	131	42.12	14.02			131	54.56	p=0.218
Income Status	Low	56	49.21	17.04	F=7.400		56.20	8.72	F=1.125 p=0.326
	Middle	155	43.85	11.74	p=0.001*		7.56		
	High	43	38.81	13.94	54.56 56.00		8.88		
Previous birth history	Normal birth	57	44.12	11.19	t=-1.059		56.23	6.29	t= 0.517 p=0.606
	Cesarean section	52	46.81	14.82	p=0.292 55.54		7.62		
Having coronavirus before pregnancy	Yes	117	43.87	14.05	t=-0.326		55.06	7.66	t= -0.192 p=0.848
	No	137	44.44	13.59	p=0.745 55.26		8.41		
Having a coronavirus disease during pregnancy	Yes	37	45.46	15.65	t=0.612		53.46	7.92	t=-1.396 p=0.164
	No	217	43.96	13.46	p=0.541 55.46		8.07		
Coronavirus vaccination before pregnancy	Yes	174	43.22	13.62	t =1.641		53.20	7.83	t=-6.149 p=0.000*
	No	80	46.26	13.97	p=0.102 59.45		6.84		
Receiving information from health personnel	No	133	44.26	13.63	t=0.104		56.17	8.16	t= 2.087 p=0.038*
	Yes	121	44.08	13.99	p=0.917 54.07		7.84		

*p<0.05, SD: Standard deviation, Avg: Average

Table 3. The Effect of coronavirus phobia on vaccine opposition

Dependent variable	Independent variable	Unstandardized coefficients		Standardized coefficients	t	p-value	R^2
		B	Standard error	Beta			
Anti-Vaccination scale	Fixed	50.056	1.671		29.955	0.000	0.039
	Coronavirus phobia scale	0.116	0.036	0.198	3.202	0.002	

Model: F=10.254, p=0.000

Table 4. The relationship between Coronavirus Phobia Scale and Anti-vaccine Scale

		Psychological impact	Somatic impact	Social impact	Economic impact	Coronavirus phobia scale	Benefits of vaccination	Fear of vaccines	Non-vaccine Solutions	Vaccine hesitation	Anti-vaccination scale
Psychological impact	r	1									
	p										
	n	254									
Somatic impact	r	0.648**	1								
	p	0.000									
	n	254	254								
Social impact	r	0.797**	0.712**	1							
	p	0.000	0.000								
	n	254	254	254							
Economic impact	r	0.628**	0.761**	0.662**	1						
	P	0.000	0.000	0.000							
	n	254	254	254	254						
Coronavirus Phobia Scale	r	0.907**	0.860**	0.914**	0.828**	1					
	p	0.000	0.000	0.000	0.000						
	n	254	254	254	254	254					
Benefits of vaccination	r	0.220**	0.067	0.256**	0.090	0.196**	1				
	p	0.000	0.288	0.000	0.152	0.002					
	n	254	254	254	254	254	254				
Fear of vaccines	r	0.120	0.066	0.059	0.120	0.104	-0.511**	1			
	p	0.057	0.293	0.348	0.056	0.099	0.000				
	n	254	254	254	254	254	254	254			
Non-vaccine solutions	r	-0.082	0.045	-0.082	0.039	-0.037	-0.694**	0.684**	1		
	p	0.191	0.474	0.193	0.535	0.554	0.000	0.000			
	n	254	254	254	254	254	254	254	254		
Vaccine hesitation	r	-0.034	0.237**	0.030	0.229**	0.100	-0.488**	0.432**	0.612**	1	
	p	0.592	0.000	0.633	0.000	0.111	0.000	0.000	0.000		
	n	254	254	254	254	254	254	254	254	254	
Anti-Vaccination Scale	r	0.150*	0.190**	0.156*	0.231**	0.198**	-0.269**	0.839**	0.759**	0.661**	1
	p	0.016	0.002	0.013	0.000	0.002	0.000	0.000	0.000	0.000	
	n	254	254	254	254	254	254	254	254	254	254

*p<0.0, **p<0.01

and anti-vaccination levels. COVID-19 phobia was higher in pregnant women with children and low economic income. Women who had never been vaccinated had higher levels of vaccine hesitancy. However, this study shows that the level of vaccine hesitancy prevents and affects vaccination.

In Türkiye, the tetanus vaccine, one of the vaccination policies commonly administered to women, is closely followed; primarily, women receive this vaccine. The fact that the tetanus vaccine has been administered for many years and there are no reported severe side effects increases the applicability of the vaccine (17,18). In this study, it was determined that pregnant women had high rates of tetanus vaccination but were reluctant to receive new vaccines such as COVID-19 and influenza. The increasing knowledge about COVID-19, the lack of certainty about its effects on pregnancy, or the negative discourses of society about the vaccine suggests that this result is effective on this result. This result underlines that the prevalence of vertical transmission and the effects of the COVID-19 and influenza vaccine on the fetus are still unclear. This information does not reach pregnant women even if there are available data. In addition, it suggests that the pandemic has not impacted pregnant women's attitudes toward routine pregnancy vaccinations. Severe acute respiratory syndrome coronavirus-2 vaccines are reported safe in pregnant women and recommended (19,20). Current data further support the safety of COVID-19 vaccine administration during pregnancy. In a retrospective study by Kharbanda and Vazquez-Benitez (21) with more than 40,000 pregnant women, it was reported that the COVID-19 vaccine administered during pregnancy was not associated with preterm delivery or low birth weight. Despite reliable data in the literature, it is known that pregnant women are reluctant to be vaccinated. It is seen that women need more information about vaccines and their effects. Health professionals should include COVID-19 and prevention methods in preconception care, pregnancy, delivery, and postnatal counseling. There is also a need for social studies to increase trust in vaccines.

Our study determined that 4.7% of pregnant women received the coronavirus vaccine and had moderate vaccine opposition. A study conducted with pregnant and breastfeeding women in Saudi Arabia reported that 93.1% of women received two doses of the coronavirus vaccine (22). A prospective study conducted in Türkiye found that 37% of pregnant women were willing to receive the COVID-19 vaccine. Low vaccine acceptance is associated with concerns about the vaccine's safety and insufficient knowledge about its potential harm to the fetus (23). It could be said that the negative attitudes of the pregnant women who participated in our study towards the vaccine were related to the feeling that the pandemic was under control and the decrease in vaccine information.

A striking feature of this study was that more than half of the participants had been vaccinated before pregnancy. Despite the high vaccination prevalence, there was a high level of opposition to vaccination. We found that few participants thought the vaccine would adversely affect their baby and health. In the study by Hosokawa et al., (24) it was reported that the rate of COVID-19 vaccination among pregnant women was 13.4%. Low vaccination rates were associated with distrust towards those who recommended the vaccine (24). The study determined that 30% of pregnant women did not know about COVID-19 vaccines. Refusal rates against all vaccines are increasing in our country (25). This distrust of international pharmaceutical companies and health organizations result in the result. The vaccine's side effects, the idea that it contains a live virus, and the belief that COVID-19 does not exist are also effective in the prevalence of the COVID-19 vaccine. In a study on the acceptance of the COVID-19 vaccine among pregnant women in Türkiye, approximately half of the participants believed it would harm their baby's health (26). Although the consequences of the pandemic have diminished, it continues to have an impact. Therefore, reliable health organizations and professionals must update and represent information on COVID-19 vaccines in pregnancy.

During the pandemic, the risk of contracting COVID-19 caused pregnant women to fear being unable to protect their own and their baby's health. Long hours spent at home, restrictions on going out, and the inability to contact their loved ones contributed to COVID-19 phobia in pregnant women. A study conducted in Türkiye reported that pregnant women had higher COVID-19 phobia compared to other women (9). Although the official authorities state that COVID-19 vaccines are available and can be applied to pregnant women, pregnant women have remained in the background of vaccination. Studies conducted in Türkiye have also indicated that pregnant women are hesitant about COVID-19 vaccines (26,27). The phobia of contracting the COVID-19 virus may increase the vaccine response by creating avoidance behaviors in pregnant women. In studies conducted at different times, it was observed that pregnant women were reluctant to receive the COVID-19 vaccine (26,27). Therefore, it could be concluded that the phobia of pregnant women about being infected with COVID-19 was not limited to the period when the pandemic was most effective and continued.

In this study, women who were parents had more COVID-19 phobia. Mothers who are parents may feel higher anxiety and fear about the risk of transmission of the virus to family members. However, low-income women also have higher levels of coronavirus phobia. In studies conducted with women living in low-income countries such as Vietnam, China, and Indonesia, it was observed that women were most willing to be vaccinated (28-30). This result may be attributed to countries such as Vietnam, China, and Indonesia being more affected by the pandemic, and the measures are more stringent.

It was determined that the women who participated in the study had a moderate coronavirus phobia and opposition to vaccination. A similar study in Poland reported positive attitudes toward vaccines and moderate coronavirus phobia (31). During the COVID-19 pandemic, women in the perinatal period experienced mental distress such as anxiety, fear, and depression due to isolation, quarantine, and lack of social support, regardless of their psychiatric history. This led to an increase in vaccine

hesitancy. In the study by Gencer et al., (32) it was reported that the decrease in virus cases effectively decreased the vaccine hesitancy of pregnant women.

Study Limitations

We used an online questionnaire, which may lead to non-response, but the potential for bias was reduced as the questionnaires took approximately seven months to complete. Conducting the study with web-based data collection might be associated with some bias. In addition, since this study reflected a period when the impact of the pandemic was waning, and most of the population was vaccinated, most participants were vaccinated before pregnancy. The results were limited by the number of participants who participated in the study and did not represent the views of all pregnant women regarding COVID-19 phobia-vaccine opposition.

The fact that the participants had different sociodemographic characteristics and included women with previous pregnancies suggested that the study might reduce the effect of the limitation and be generalizable.

Conclusion

Identifying factors related to women's opposition to vaccination in possible future pandemics or increased infectious infections worldwide is essential. Lack of knowledge, having children, low economic income, belief that the vaccine will have adverse side effects on the pregnant woman and her baby, and COVID-19 phobia have been associated with low vaccination rates in pregnant women. These factors should be considered to raise public awareness and increase vaccination in pregnant women.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from İstanbul Arel University Ethic Committee (decision no: 07, date: 18.07.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: E.Y.A., Design: A.A., Data Collection or Processing: A.A., Ö.T., Analysis or Interpretation: A.A., E.Y.A., Ö.T., Ü.O., Literature Search: A.A., E.Y.A., Ö.T., Writing: A.A.

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

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Original Article

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Development of a Novel Plasmid-based Eukaryotic Model to Investigate Crimean-Congo Hemorrhagic Fever Virus

Kırım-Kongo Kanamalı Ateşi Virüsünü Araştırmak için Plazmit Tabanlı Yeni Bir Ökaryotik Modelin Geliştirilmesi

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ABSTRACT

Objective: Crimean-Congo Hemorrhagic Fever (CCHF) is a severe tick-borne viral disease, caused by the Crimean-Congo Hemorrhagic Fever virus (CCHFV). The global expansion of CCHF and high mortality rates underline the critical need for research and development of effective treatments and vaccines. However, the high risk of transmission and requirement for high-containment facilities hinder investigations involving live virus. In this study, we aimed to address these challenges by employing a plasmid-based virus-like particle (VLP) system and a minigenome model to investigate the biology and immunology of CCHFV.

Methods: The plasmids encoding CCHFV structural genes of CCHFV were transfected into Huh-7 cells. Viral protein expression was confirmed using fluorescence imaging, immunological and molecular methods. A minigenome system was developed, eliminating the need for T7 polymerase, T7-expressing cellular lines, or viral ribonuclear protein complexes, allowing autonomous virus replication without a helper virus or transfections using plasmids in trans.

Results: Fluorescence microscopy showed successful production of NP-EGFP and GC-EGFP proteins with various subcellular localizations. Western blot analysis demonstrated the presence of pre-Gc, Gc, pre-Gn, Gn, and Np proteins in cell lysates and supernatants. ELISA analysis suggested that transfection of Np

ÖZ

Amaç: Kırım-Kongo Kanamalı Ateşi (KKKA), Kırım Kongo Kanamalı Ateşi Virüsü'nün (KKKAV) neden olduğu, kenelerle bulaşan ciddi bir viral hastalıktır. KKKA'nın küresel yayılımı ve yüksek ölüm oranları, etkili tedavilerin ve aşıların araştırılmasına ve geliştirilmesine yönelik kritik ihtiyacın önemini vurgulamaktadır. Bununla birlikte, yüksek bulaşma riski ve yüksek biyogüvenlikli tesislere duyulan gereksinim, canlı virüsle yapılan araştırmaları engellemektedir. Bu çalışmada, KKKAV'nin biyolojisini ve immünolojisini araştırmak için plazmid tabanlı virüs benzeri partikül (VLP) sistemi ve minigenom modeli kullanarak bu zorlukların üstesinden gelmeyi amaçladık.

Yöntemler: KKKAV yapısal genlerini kodlayan plazmitler, Huh-7 hücrelerine transfekte edildi. Viral protein ekspresyonu, floresan görüntüleme, immünolojik ve moleküler yöntemler kullanılarak doğrulandı. T7 polimeraz, T7 ifade eden hücresel hatlar veya viral ribonükleer protein komplekslerine olan ihtiyacı ortadan kaldıran ve bir yardımcı virüs olmadan otonom virüs replikasyonuna izin veren bir minigenom sistemi geliştirildi.

Bulgular: Floresan mikroskopisi, NP-EGFP ve GC-EGFP proteinlerinin çeşitli hücre altı lokalizasyonlarda başarılı bir şekilde üretildiğini gösterdi. Western blot analizi ile pre-Gc, Gc, pre-Gn, Gn ve Np proteinlerinin varlığı hücre lizatlarında ve süpernatanlarında gösterildi. ELISA analizi, yalnızca Np'nin, Np

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ABSTRACT

alone, in combination with Gc, or all three proteins might cause distinct VLP formations. Huh-7 cells successfully expressed reporter genes after transfection of minigenome RNA transcripts.

Conclusion: The study advances CCHFV research by using novel tools for virus biology and immunology. The findings may provide new avenues for research that promise better public health preparation against this neglected viral disease.

Keywords: Crimean-Congo Hemorrhagic Fever virus, virus-like particle, vaccines, bunyavirus, RNA virus infections, immunology

ÖZ

ile Gc'nin birlikte veya üç proteinin aynı anda transfeksiyonunun farklı VLP oluşumlarına neden olabileceğini göstermektedir. Ayrıca, minigenom RNA transkriptlerinin transfeksiyonu sonrasında Huh-7 hücreleri raportör genleri başarıyla açıklamıştır.

Sonuç: Bu çalışma, virüs biyolojisi ve immunolojisi için yeni araçların kullanılmasını sağlayarak KKKAV araştırmalarına katkı sağlayacaktır. Elde edilen bulgular kullanılarak, bu ihmal edilmiş viral hastalığa karşı halk sağlığı alanında daha hazırlıklı olma imkanları araştırılacaktır.

Anahtar Sözcükler: Kırım-Kongo Kanamalı Ateşi virüsü, virüs benzeri partikül, aşılar, bunyavirüs, RNA virüsü enfeksiyonları, immünoloji

Introduction

The etiological agents responsible for many of the human infections causing the millions of deaths are RNA viruses (1-5). Both the increased contact of humans with domestic animals and wild populations, brought on by globalization, as well as the high virulence and evolutionary plasticity of RNA viruses cause viruses to find new hosts. As seen with the emergence of AIDS, H1N1 or H5N1 flu, Nipah, Ebola and more recently coronavirus disease-2019, RNA viruses have triggered a series of pandemics originating from wildlife reservoirs, increasing the concerns on public health (6). In recent years, the World Health Organization (WHO) has published a list of diseases that should be primarily investigated. Due to its high potential for epidemic and public health emergency of international concern and the lack of effective therapeutics or vaccines for human or animal use, Crimean-Congo Hemorrhagic Fever (CCHF) is listed among these priority diseases for research and development in emergency contexts by WHO and as well as by National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID) (7,8). The CCHF is a tick-borne viral hemorrhagic disease caused by Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and causes sporadic outbreaks with mortality rates ranging from 5% to 80% (9,10). So far, virus isolation and/or disease have been reported in more than 30 countries over a wide geographical area covering Africa, Asia, and Eurasia (11). In 2019, WHO estimated that three billion people were at risk of contracting the disease because of the expanding transmission of the virus by Hyalomma ticks (12). Also, the CCHF became a growing public health problem in Türkiye, where the highest number of cases in the world were documented (11,041 cases between 2002 and 2018) (13).

The CCHFV belongs to the genus *Orthonairovirus* within the family *Nairovirideae* of the order *Bunyavirales* (14,15). The viral genome consists of three single-stranded negative-sense RNA molecules called L, M, and S segments that encode structural proteins such as RNA-dependent viral RNA polymerase, glycoproteins, and nucleoproteins (Np), respectively. Each contains a single open reading frame (ORF) and highly conserved non-coding terminal complementary sequences. The non-covalent interaction between both ends forms panhandle-like structure that provides a functional promoter region for viral polymerase binding (16-19). Encapsulation of the viral segments by Np by homo-oligomerization as pentamers and formation a ribonucleoprotein complex together with RNA-dependent RNA polymerase are crucial for the initiation of viral replication and transcription in the host cell (16). Two glycoproteins, Gn and Gc, are embedded in the lipid bilayer of the viral envelope and are responsible for viral attachment and internalization into the host cell (20). The maturation of glycoproteins begins with cotranslational cleavage of polyprotein into the glycoprotein precursors PreGn (140 kDa) and PreGc (85 kDa), likely by signal peptidase (21,22).

The CCHFV has been spreading to new regions and causing more frequent outbreaks in recent years. It is considered as a neglected disease, and efforts are needed to improve our understanding of CCHF pathogenesis and the diagnosis, treatment, and prevention of the disease. The high risk of transmission and the necessities for BSL-4 facilities hamper the studies with live virus. So, any information on virus biology, especially that provided by individual proteins, will be a valuable contribution to the knowledge on CCHFV.

Understanding the life cycle of a virus is critical for the development of vaccines and antivirals, and the establishment of robust methods plays a critical role in investigating the underlying molecular mechanisms of the disease. Reverse genetic systems are effective tools that enable mutation analyses, which help determine the molecular basis of the viral life cycle in all its aspects (23). While viral genome replication and transcription are modeled using minigenome systems, the morphogenesis, budding, and infection of target cells are simulated using virus-like particles (VLPs), which shed light on unknown mechanisms of the virus life cycle and the pathophysiology of diseases. The absence of genomic material facilitates research on such viruses in BSL-2 laboratories, which are easily accessible to many researchers worldwide. Therefore, here it was aimed to produce viral proteins in mammalian expression systems to establish a plasmid based VLP system which could contribute to existing knowledge about CCHFV biology and immunology. With this study, it was also aimed to develop a minigenome system that could be utilized in the other studies aiming to generate VLP models which could

simulate one cycle of virus replication. Unlike studies in the literature, it was aimed to develop a unique minigenome system that eliminated the need for both T7-expressing cell lines and a viral ribonuclear protein complex (RNP) generally aided by either a helper virus or by plasmid transfections in trans. Thus, the expression studies conducted here may further facilitate studies of the virus life cycle, the discovery of new therapeutics, and the development of diagnostic tools.

Methods

Cell Culture and Virus

Human hepatocellular carcinoma cells (Huh-7) were grown at 37 °C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin.

Antibodies and Sera

Mouse monoclonal antibodies targeting CCHFV strain Ibar10200 anti-PreGn/GP38 (clones 8F10), anti-PreGc (clone 11E7), and anti-NP (clone 9D5) and were obtained from the Joel M. Dalrymple-Clarence J. Peters USAMRIID Antibody Collection through BEI Resources, NIAID, NIH. The CCHFV-immunized rabbit and mouse sera were kindly given by Prof. Aykut Özdarendeli (Erciyes University Vectors and Vector Borne Diseases Implementation and Research Center, and Department of Medical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Türkiye).

Construction of Plasmids

The nucleotide sequences of CCHFV Kelkit06 S (1673 nt) and M (5364 nt) segments retrieved from GenBank (accession numbers: GQ337053 and GQ337054, respectively) were synthesized with flanking *Sap*I recognition sites at both ends and cloned separately by *Kpn*I and *Bam*HI enzymes into pUC19 vector (Synbio Technology USA Inc., Suzhou, China). The constructed plasmids (named as pUC_S and pUC_M) were propagated in One Shot™ TOP10 Chemically Competent *E. coli* (ThermoFisher Scientific, USA) cells. The ORFs of CCHFV Kelkit06 Np, PreGn and PreGc were cloned into pcDNA 3EGFP (13031, Addgene) and pcDNA3-neo-cterminal-3HA (102643, Addgene). The constructed plasmids were transformed into XL10-Gold™ ultracompetent *E. coli* cells (Agilent, CA, USA). Three positive transformant colonies for each construct were selected and analyzed by restriction digestion analysis. The minigenome plasmid 7miniC was commercially syntesized (Synbio Technology USA Inc., Suzhou, China) and propagated in One Shot™ TOP10 Chemically Competent E. coli cells. The eGFP gene was replaced with mCardinal to generate 7miniE plasmid by using directional cloning utilizing *Kpn*I and *Xho*I enzymes.

Transfections

One day before transfection $5x10^5$ cells/well were seeded in 6-well plates and grown in DMEM (Gibco™, ThermoFisher Scientific, USA) supplemented with 10% fetal calf serum, (Gibco™, ThermoFisher Scientific, USA) and Pen-Strep (Penicillin-Streptomycin, Gibco™, ThermoFisher Scientific, USA) at 37 °C and 5% CO_2 in a humidified chamber until they reached 50-60% confluency on the day of the experiment. The next day, cell culture media was removed, and the cells were incubated with DMEM including 10% FBS with Pen-Strep. Meanwhile, polyethyleneimine (PEI) (Polysciences, Germany) complexes were prepared. Briefly, 6 µl of 1 mg/mL PEI was diluted in 100 µl DMEM (-), then mixed with 2 µg plasmids diluted in 65 µl DMEM (-) (1:2 ratio, 15 ng/µl DNA: 30 ng/µl PEI). Then PEI complexes were added, and the cells were incubated for 72 hours at 37 °C and 5% CO_2 in a humidified chamber. Three days post-transfection, cell culture supernatants and pellets were collected and stored at -20 °C for further analysis.

Cell Lysis

Transfected cells were washed two times with cold PBS and were collected by scraping followed by centrifugation at 1000 g for 3 min. The cell pellets were lysed in lysis buffer (20 mM Tris pH7.5, 1% Triton X-100, 0.05% SDS, 0.5% Sodium Deoxycholate, 150 mM NaCl, 1mM Protease inhibitor cocktail).

RT-PCR

Total RNA was isolated from cells post-transfection using TRI-Reagent (BioShop, Canada) and samples were treated with DNAse I for 15 min at 37 °C and then purified from DNAse I. RT-PCR was performed by the SensiFAST™ cDNA Synthesis Kit (Bioline, UK) using the Oligo d(T) primer to generate cDNA. Then qPCR was performed via of BlasTaq™ 2X qPCR MasterMix using different primer sets that could amplify approximately the last 130 bp of ORF sequences for each of the viral genes. The qS forward (5' CATACAGGACATGGACATTGTG 3') and qS reverse (5'TTAGATGATGTTGGCACTGGTG 3') for Np; qM forward (5'CAGGCTACAGAAGGATTATTGAAAGAC3') and qM reverse (5'TTAGCCAATGTGTGTTTTTGTGGAG 3') for Gc; Primer 8 forward (5' CTTGGTACCGCCACCATGGTCTGCAAACGC3') and Primer 3 reverse (5'GTGGATCCTTATGCAGAGGTGCTAAC 3') were used for Gn.

Western Blot

The proteins to be analyzed were separated on either by SDS-PAGE or Native-PAGE, described below.

Polyacrylamide Gel Electrophoresis

SDS-PAGE: The protein samples were diluted 1:1 in Laemmli buffer [4% (w/v) SDS, 10% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, 0,004% (w/v) bromophenol blue, 0,125 M Tris-HCI] and heated to 95 °C for 10 min unless otherwise stated. For some assays, Np and Gn samples were denatured in reducing loading buffer (5X Blue Loading Buffer, 200 mM Tris HCl pH6.8, 10% SDS, 500 mM β-mercaptoethanol and 50% glycerol) at 95 °C for 5 min. Proteins were run on an 8-12% polyacrylamide gel, first at 50 V for 20 minutes, then at 200 V for 40 minutes. After electrophoresis, the gel was stained with Coomassie brilliant blue G-250 (Sigma-Aldrich, Taufkirchen, Germany).

Native-PAGE: Samples were diluted in non-reducing loading buffer (5X Blue Loading Buffer, 200 mM Tris HCl pH6.8, 20% SDS, and 50% glycerol), incubated at 50 °C for 10 min and electrophoresed on 4-9% polyacrylamide gels.

Following electrophoresis, proteins were transferred to a polyvinylidene fluoride membrane (GVS North America, Sanford, USA) immediately after methanol induction of membrane, in transfer buffer [24 mM Tris, 192 mM glycine and 20% (v/v) methanol] either at 25 V for 7 minutes on semi-dry blotter (Trans-Blot Turbo, Bio-Rad, California, USA) or wet at 100 V for 90 minutes in Mini Trans-Blot cell (Bio-Rad, California, USA). The membrane was blocked with TBST [10 mM Tris pH 7.4, 0.9% (w/v) NaCl, and 0.05% (v/v) Tween-20] containing 5% (w/v) skim milk (Sigma-Aldrich, Taufkirchen, Germany) for two hours at room temperature and incubated with primary antibody overnight at 4 °C, followed by secondary antibody incubation for one hour at room temperature. Between each step, the membrane was washed five times of 10 min using TBST and processed for chemiluminescence detection (WesternBright Sirius Chemiluminescent Detection Kit, Advansta, California, USA), according to the manufacturer's instructions. Membranes were visualized by using Fusion FX Solo imaging system (Vilber, Collégien, France).

ELISA

The 96 well microplates (Immulon 2 HB, Invitrogen, Waltham, USA) were coated overnight at 4 °C with supernatant or cell lysate samples diluted in carbonate-bicarbonate buffer (pH 9.6). Then, the plates were washed four times with PBST [PBS + 0.05% (v/v) Tween 20] and blocked with PBST containing 5% (w/v) skim milk for two hours at room temperature. Plates were then incubated at 37 °C for 1 hour each, first with the primary antibody and then with a corresponding HRP-conjugated secondary antibody, depending on the analytes. Between each step, the wells were washed four times with PBST. Then, TMB solution (Abcam, Cambridge, UK) were added to the wells and the plates were incubated for 20-25 minutes at room temperature in the dark. The reaction was stopped by adding 2N H_2SO_4 to the wells and absorbance of samples measured in iMark microplate reader (Bio-Rad, California, USA) at 450 nm. All washing steps performed using Wellwash Versa Microplate Washer (Thermo Fisher Scientific, Waltham, USA). Data were calculated by the mean of absorbance measurements obtained from duplicated samples. The cut-off value was calculated by the given formula: the mean absorbance of each negative samples + 2 SD.

***In Vitro* Transcription**

The minigenome cassettes were excised by *Eco*RI enzyme and used as template for in vitro transcription. Then it was processed by using HiScribe® T7 High Yield RNA Synthesis Kit (New England Biolabs, MA, USA), according to the manufacturer's instructions.

Statistical Analysis

For the analysis of results obtained from RT-PCR, Ct values measured by Rotor-Gene® Q (Qiagene, USA) for each gene and were normalized to the *GAPDH* gene used as internal control. The relative expression level was analyzed by 2^{-DDCt} method using the following formula:

$$DDCt:[(Ct_{targetgene}-Ct_{housekeeping})-(Ct_{normal}-C_{thousekeeping})]$$

ELISA data were calculated by the mean of absorbance measurements obtained from duplicated samples. The cut-off value was calculated by the given formula: the mean absorbance of each negative samples + 2 SD.

Results

For the establishment of a plasmid based CCHFV VLP model in eukaryotic cells, pCDNA3.1 plasmids encoding Np, PreGn and PreGc regions were utilized to express viral proteins in Huh-7 cells. Fluorescence microscopy images at 48 hours post transfection showed that both NP-EGFP and GC-EGFP proteins were successfully produced and had various subcellular localizations (Figure 1). While Gc was uniformly distributed across the cell surface, Np was primarily found in the perinuclear region. These different expression profiles

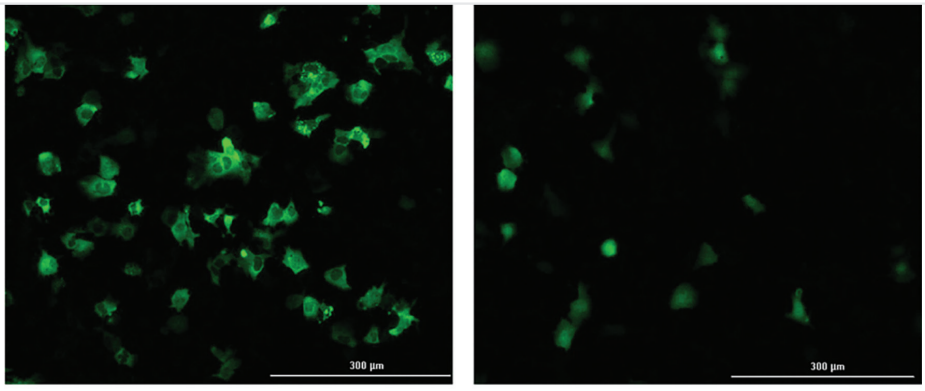

Figure 1. The visualization of differential cellular localization of NP-EGFP and GC-EGFP proteins in Huh-7 cells under fluorescent microscopy

*Scale bar 300 µm

might imply that viral proteins were expressed recombinantly and functioned properly.

As the presence of viral proteins was indirectly demonstrated by fluorescence microscopy, additional expression analyzes were performed to assess production. Proteins were first analyzed by ELISA method, in which the supernatant samples from transfected Huh-7 cells were analyzed using CCHFV-immunized mouse and rabbit sera, human convalescence sera, and CCHFV-specific mouse mAb antibody cocktail including anti-NP (9D5), anti-GN (8F10), and anti-GC (11E7) primary antibodies. It was observed that positive samples had almost similar pattern in all tests (Figure 2). All antibodies detected the viral proteins in the samples of "3in1", in which all viral plasmids were transfected simultaneously. The reproducibility of this results suggested that CCHFV VLP might be formed. Remarkably, each test consistently yielded positive results for Np + Gc cell culture samples, indicating the potential ability of Np and Gc to form a VLP structure. The Gn was also tested positive in all assays except specific antibody ELISA, suggesting that Gn might be able to be packed alone in the Golgi, due to its Golgi retention signal on their sequence. Additionally, Np was detected in all assays tested here, which might provide additional evidence to the previous study reporting that Np formed sphere-like structures in cytoplasmic vesicles after its expression using baculovirus expression system (24). This analyzes showed that viral proteins were successfully expressed and some of their combinations might contribute to form VLPs in transfected cells.

To confirm viral protein production in transfected cells, further analyzes were conducted by western blot assays (Figure 3). When the membranes were incubated with CCHFV-immunized mice sera after SDS-PAGE, pre-Gc (85 kDa) and Gc (75 kDa) were detected in the lysates while bands around 130 kDa and 245 kDa for Gn were appeared in cell culture supernatant samples (Figure 3A). Meanwhile, Np (53 kDa) proteins were detected both in lysate and supernatant samples, when they transferred to the membrane after Native-PAGE (Figure 3B). Finally, all three proteins Np, Gn, and Gc were recognized by the antibodies found in the CCHFV-immunized rabbit sera (Figure 3C). Also, post-transcriptional analysis of viral proteins revealed at least 2x104 fold increase in viral gene expression compared to the GAPDH (Figure 3D). Accordingly, these results indicated that viral proteins were successfully expressed in Huh-7 cells

To study viral replication and transcription, as well as entry, assembly, and budding of CCHFV, a transcriptionally competent VLP model is required. To achieve this goal, a minigenome plasmid is needed to be packaged into the VLP, such as the CCHFV segment. With a unique strategy, an ambisense minigenome plasmid was so designed that might transcribe vRNA in VLP forming donor cells without using any viral polymerases. The minigenome plasmid named as the 7miniC has ambisense vRNA coding cassette, a reporter gene in positive polarity was located between the antisense 3'untranslated region (UTR) and 5'UTR sequences of the CCHFV L segments and downstream of the T7 promoter region was encoded and two additional UTR sequences (NEO UTR2 and NEO UTR 3) in sense orientation were inserted upstream of the reporter gene to increase its expression. To generate synthetic RNAs with a precise 3' end, the hepatitis delta virus ribozyme sequence was added to the final nucleotide of the viral genome (Figure 4A). To demonstrate proof of concept, in vitro transcribed minigenome RNA transcripts, carrying either mCardinal (7miniC) or eGFP (7miniE) as reporter gene were transfected into Huh-7 cells using Lipofectamine or PEI. The expression of reporter gene was visualized by laser scanning confocal microscopy (Figure 4B). In this study, a unique ambisense minigenome system was developed for CCHFV. Unlike existing systems, the fact that this new minigenome system does not require additional helper virus or viral RNP complex has brought an innovative approach for CCHFV reverse studies.

The CCHFV is a highly pathogenic virus with increasing prevalence. In this sense, the development of a safe models that simulate the virus infection particularly such as replication competent VLPs has a vital role in virology. In this study, viral proteins of CCHFV Kelkit 06 strain were expressed in

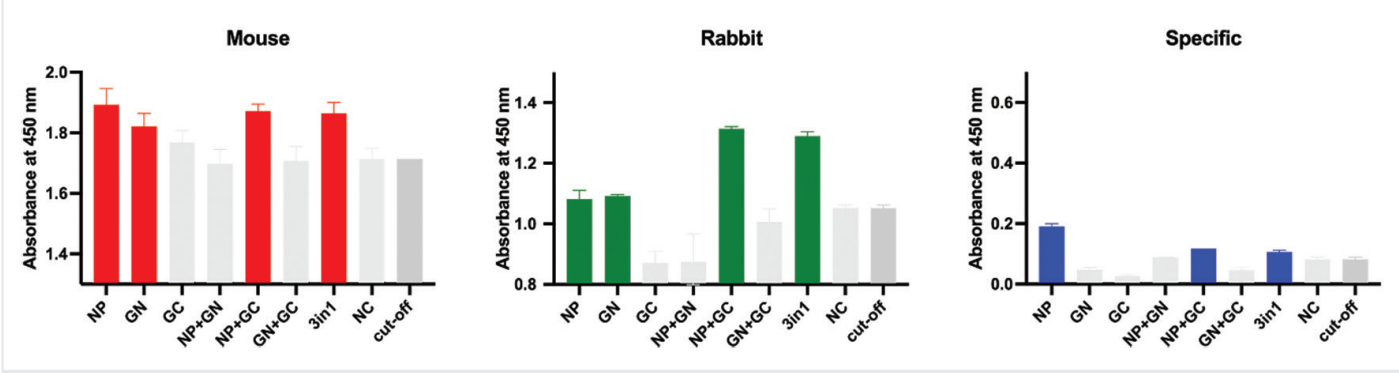

Figure 2. Analysis of supernatant samples of the Huh-7 cell transfected with different combination of viral plasmids, by in house ELISA tests

The supernatant samples were probed with CCHFV-immunized mouse (red), and rabbit sera (green), and with specific monoclonal antibody cocktail. The absorbance of each sample was measured at 450 nm using iMark microplate reader (Bio-Rad, California, USA).

CCHFV: Crimean-Congo Hemorrhagic Fever Virus

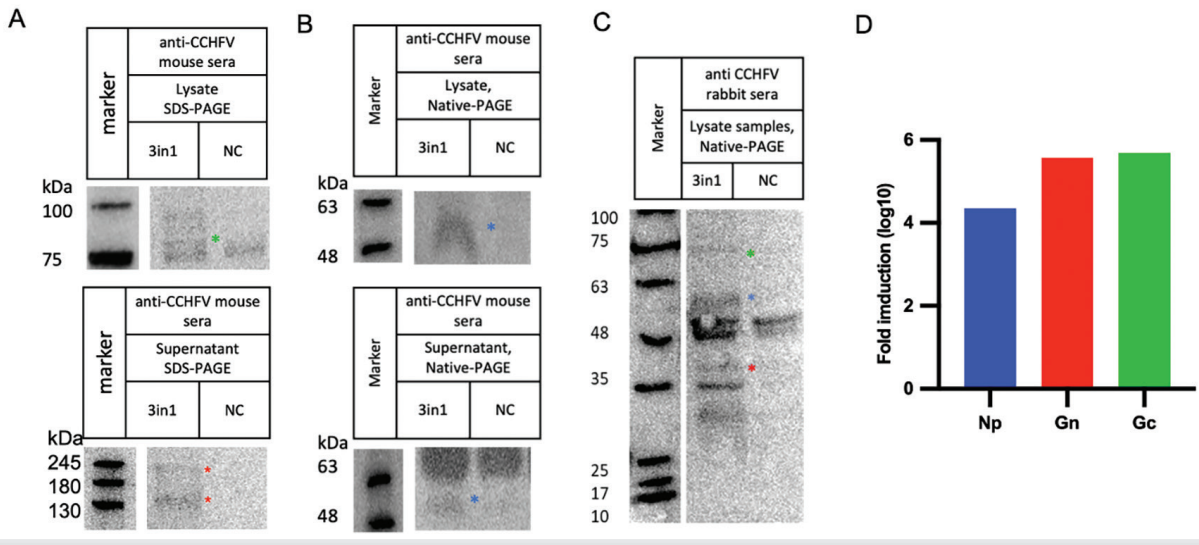

Figure 3. Analysis of viral protein expression

Western blot analysis of viral proteins in Huh-7 cells, transfected with all plasmids together. Lysate and supernatant samples analyzed using CCHFV-immunized mouse sera were either dissolved on SDS-PAGE (A) or on Native-PAGE (B), while lysate proteins dissolved on Native-PAGE were analyzed using CCHFV-immunized rabbit sera (C). (D) Post-transcriptional analysis of viral mRNAs by RT-PCR. The relative gene expression levels to GAPDH, as an internal control were measured using the $2^{-\Delta\Delta CT}$ method

CCHFV: Crimean-Congo Hemorrhagic Fever Virus

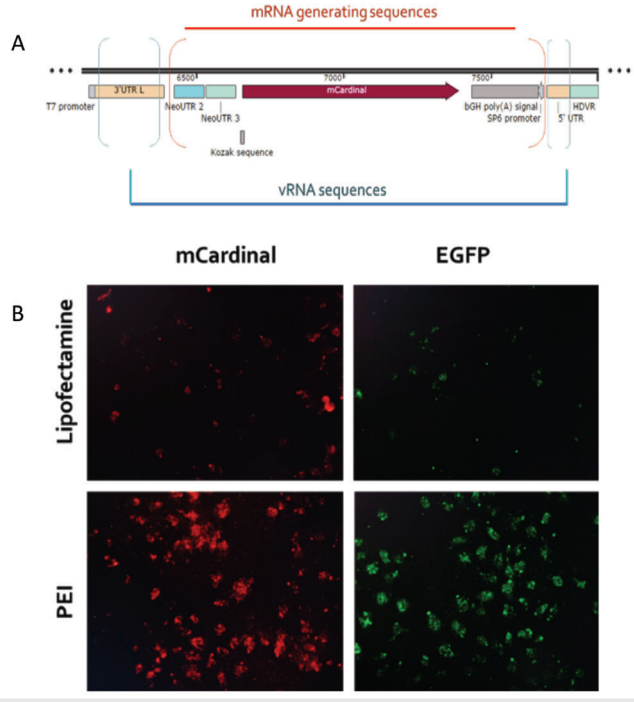

Figure 4. The design and the proof of concept of a novel CCHFV minigenome model

A) Partial vector map showing the ambisense vRNA-encoding cassette in 7miniC plasmid. The mRNA generating sequence, in which ORF of reporter gene together with the regulatory sequences, recognized by human protein translation system was inserted between negative sense viral UTR sequences, providing viral genome packaging signal. B) Confocal laser scanning microscopy images of Huh-7 cells transfected with 7miniC (red) and 7miniE (green) *in vitro* transcripts using PEI or lipofectamine at 6 hours post-transfection

CCHFV: Crimean-Congo Hemorrhagic Fever Virus, PEI: Polyethyleneimine

mammalian cells, which had the potential for development of such a VLP model. In addition, the inclusion of the newly developed minigenome system in future VLP studies will pave the way for studies on both the life cycle of CCHF and the development of antiviral treatment methods.

Discussion

Our study demonstrated that viral proteins were produced in Huh-7 cells and the expression was confirmed by immunological and molecular methods. The model developed in this study promised to be utilized in future biology, immunology, and vaccinology efforts of CCHFV. Fluorescent microscopy images revealed that eGFP displayed various intracellular signals depending on the viral protein with which it was fused. When eGFP was expressed as a fusion with Np, it fluoresced in the perinuclear region, while a diffuse fluorescent light throughout the cytoplasm was noticed when it was fused with Gc. In the literature, CCHFV Np has been reported to localize in the perinuclear region of infected cells (25-27) and Gc requires Gn for its transition to the Golgi (28). In accordance with the information in the literature, fluorescence microscopy assay provided the first data suggesting efficient production of Np and Gc proteins. Immunological analysis of viral proteins by western blot demonstrated that all viral proteins were expressed in Huh-7 cells. Furthermore, cell culture samples were examined using ELISA, and samples in which all Np, or a combination of Np and Gc, or all three proteins simultaneously were transfected were recognized by all antibodies tested in this study. According to current knowledge, the Np of Dengue virus-2 and of Hepatitis C virus can self-assemble into VLPs (16,20). Furthermore, Zhou et al. (24) reported that CCHFV Np form sphere-like structures that vary in morphology and size, ranging from 40 nm to 160 nm in diameter, within cytoplasmic vesicles of Np transfected insect cells. Our findings suggested that the model developed in this study opened the possibility to investigate the VLP formation the minimal protein composition needed for CCHFV VLP.

One aim of the study was to generate a minigenome system that could be integrated into the CCHFV VLP system. Several minigenome systems have been developed for bunyaviruses (23). The viral polymerase and Np are minimally required components for a proper genome replication and transcription in bunyaviruses (29). However, due to the difficulty of cloning 12 kb CCHFV viral polymerase gene, a unique minigenome system was designed that could simultaneously generate a single RNA that possessed the features of both vRNA of CCHFV and mRNA of a reporter gene, without the aid of any viral RNP complexes. Unlike existing systems, this system requires neither T7 expressing cells, Pol I, nor viral polymerase. This minigenome cassette has ambisense character since an mRNA cassette in sense orientation has been flanked by antisense viral UTR sequences on the same strand. Similarly to the previously reported Lassa virus minigenome system (30), the signal-to-noise ratio was significantly improved by transfecting *in vitro* synthesized minigenome RNA rather than plasmid DNA utilizing either T7 or Pol I polymerases (31). On the other hand, VLP production independent of a permanently T7 polymerase expressing cells (like BSR-T7/5) might provide versatility in CCHFV studies with various cells, as well. For this reason, the minigenome RNA used in this study was synthesized in vitro using T7 polymerase. Confocal microscopy images showing the expression of reporter genes in Huh-7 cells transfected with in vitro transcribed minigenome RNA proved that our model allowed the generation of reporter proteins without any viral RNP complexes, which were generally required for most minigenome systems. As a result, this model might enable one to scrutinize steps of the viral life cycle, such as virion assembly, genome packaging, and cell entry mechanisms, once the viral structural proteins provided in trans.

The invaluable contribution of reverse genetic studies involving VLP systems, particularly with the integration of minigenomes to the biology of CCHFV are demonstrated during the last decades (32-37). To combat CCHFV effectively, it is important to conduct studies on characterization of biological, immunological, and pathogenic features of the virus in a safe manner. The purpose of the current study was to develop such model. Here, experiments utilizing mamalian expression vectors were conducted to generate a VLP systems for CCHFV. Viral protein expressions were demonstrated in tests involving immunological and post-transcriptional expression analysis methods. Additionally, a viral RNP complex-independent ambisense minigenome system was developed for future studies, aiming to generate cell-entry competent and transcriptionally active VLPs for CCHFV Kelkit 06 strain. It is believed that the data generated in this study will pave the way for future comprehensive studies investigating each stage in the life cycle of the virus and as well as developing diagnostic, protective, and therapeutic agents against CCHFV.

Study Limitations

The results obtained from the preliminary studies required for CCHFV VLP production in this study showed that viral proteins of CCHFV Kelkit 06 strain could be produced in Huh-7 cells using pcDNA 3.1 plasmid. However, the model was not sufficiently driving the production of VLPs by which some of the virological questions could be addressed. It appears that optimization of the model is required. It appears that for generation and demonstration of sufficient quantities and commercially meaningful VLPs from plasmid based expression systems, further optimizations are needed. It should be underlined that to address the questions about the biology and immunology of these viruses through VLP and minigenome approaches, optimizations with different expression models, sequences, and transfection systems should be undertaken. Furthermore, stable expressions in susceptible cell lines could also be useful.

Conclusion

The experiments conducted in this study showed expression and detection of immunologically significant viral antigenic proteins from CCHFV. Although ELISA results imply that various viral protein combinations produce different VLP compositions, further confirmatory studies are needed to support the results

obtained in this study. As another objective of this study was to form a viral RNP complex-independent ambisense minigenome system to generate transcriptionally and entry-competent VLP for CCHFV Kelkit 06 strain. For this aim, the expression studies in the study contributed valuable information in the development of VLP systems. This information might pave the way for future studies to be conducted to comprehensively investigate each stage in the life cycle of the virus and as well as to develop vaccine and treatment agents against CCHFV.

Ethics

Ethics Committee Approval: Ethic consent is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: N.S.G.Ç., M.Z.D., Design: N.S.G.Ç., M.K.Y., M.Z.D., Data Collection or Processing: N.S.G.Ç., Ö.B., S.K., Analysis or Interpretation: N.S.G.Ç., Ö.B., M.K.Y., Literature Search: N.S.G.Ç., S.K., Writing: N.S.G.Ç., M.Z.D.

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Original Article

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How Sleep Quality Affects Postural Control?

Uyku Kalitesi Postural Kontrolü Nasıl Etkiler?

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ABSTRACT

Objective: We aimed to investigate how sleep quality affected postural control in medical and dental students.

Methods: One hundred twenty eight volunteer students with right hemispheric dominance participated in the study. Participants were divided into good and poor sleep quality groups based on their Pittsburgh Sleep Quality Index (PSQI) scores. A force platform was used for bipedal balance analysis. Participants were asked to remain upright and motionless on the platform and their body oscillations were recorded for 30 seconds in this position. Balance analysis was performed in two conditions, with eyes open and closed.

Results: There was no statistically significant difference between the demographics of the two groups. The PSQI medians of the good and poor sleep quality groups were 4 (minimum: 1, maximum: 4) and 7 (minimum: 5, maximum: 14), respectively. In the open-eye test, no significant difference was found between the two groups in any of the data. In the test performed with the eyes closed, the deviation of the center of pressure on the Y-axis and the force transferred to the anterior part of the foot on the left side were higher in the group with poor sleep quality. Parallel to this, the force transferred to the posterior part of the foot on the left side was also lower in the same group.

Conclusion: Sleep quality did not affect balance with eyes open, but negatively affected balance with eyes closed. The balance of force transferred to the non-dominant foot of the group with poor sleep quality was impaired.

Keywords: Balance analysis, sleep quality, postural control, Pittsburgh Sleep Quality Index

ÖZ

Amaç: Tıp ve diş hekimliği öğrencilerinde uyku kalitesinin postüral kontrolü nasıl etkilediğini araştırmayı amaçladık.

Yöntemler: Çalışmaya tamamı sağ el dominant olan 128 gönüllü öğrenci katıldı. Katılımcılar, Pittsburgh Uyku Kalitesi İndeksi (PUKİ) puanlarına göre iyi ve kötü uyku kalitesi olanlar olarak iki gruba ayrıldı. Bipedal denge analizi için kuvvet platformu kullanıldı. Katılımcılardan platform üstünde dik bir şekilde hareketsiz kalmaları istendi ve bu pozisyondayken 30 saniye boyunca vücut salınımları kaydedildi. Denge analizi gözler açık ve kapalı olacak şekilde iki koşulda yapıldı.

Bulgular: İki grubun demografik özellikleri arasında istatistiksel olarak anlamlı bir fark yoktu. Uyku kalitesi iyi olan ve kötü olan grupların PUKİ medyanları sırasıyla 4 (minimum: 1, maksimum: 4) ve 7 (minimum: 5, maksimum: 14) idi. Gözler açık yapılan teste, iki grup arasında verilerin hiçbirinde anlamlı fark bulunmadı. Gözler kapalı yapılan testte uyku kalitesi kötü olan grupta basınç merkezinin Y ekseni üzerindeki sapması ve sol tarafta ayağın ön bölümüne aktarılan kuvvet daha yüksekti. Buna paralel olarak sol tarafta ayağın arka bölümüne aktarılan kuvvet de aynı grupta daha düşüktü.

Sonuç: Gözler açıkken uyku kalitesi dengeyi etkilemezken, gözler kapalıyken olumsuz yönde etkiledi. Uyku kalitesi kötü olan grubun dominant olmayan ayağına aktarılan kuvvet dengesi bozuldu.

Anahtar Sözcükler: Denge analizi, uyku kalitesi, postural kontrol, Pittsburgh Uyku Kalitesi İndeksi

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Introduction

Sleep, which accounts for one-third of our lives, is an important physiological need and factor in daily performance and productivity (1). During sleep, consciousness is lost, and organic activities and voluntary muscle movements are reduced. This state is temporary and returns at regular intervals (2). During sleep, the body rests, renews, and prepares for an active life (3). Sleep quality is defined as the individual's satisfaction with sleep (4). After a good night's sleep, an individual feels fit and ready for the new day (3). Sleep quality includes quantitative aspects of sleep, such as sleep latency, duration, and the number of awakenings per night, as well as subjective aspects, such as depth of sleep and relaxation (5). Poor sleep quality, attention and memory deficits, emotional lability, hallucinations, and delusions may be observed. Accordingly, work life, social life, economic status, general health, and mental status may be affected (6). In studies that investigated the sleep quality of university students, sleep problems were common among first-year students (7) and young medical students (8). This situation negatively affects individuals' academic and physical performance (8-11). Some studies assess postural control to understand the physical effects of sleep disturbance (11-13). Postural control refers to the body's ability to maintain its position in space. It plays an important role in many activities of daily living, such as standing, moving, and reaching (11,14). The necessary information for maintaining balance in standing is obtained from the physiological system, which includes vestibular, proprioceptive, and visual elements, and the protection of balance is maintained by the coordinated work of the neuromuscular system (15,16). In their study on patients with Parkinson's disease, Gallea et al. (17) found that the pedunculopontine nucleus network was associated with postural control and sleep disturbances. They found that functional connectivity of the pedunculopontine nucleus and anterior cingulate cortex decreased in patients with sleep disturbances (17). How sleep quality affects these systems is not fully understood. Some studies stated a strong relationship between sleep disturbance and postural control (18). This relationship was linear, and it was suggested that poor sleep was related with poor postural control.

We need our ability to control posture in almost all activities of daily living (11). However, in the modern world, sleep problems, whether young or old, affect all groups (7,8,19). In particular, most young people, whom we consider healthy, suffer from sleep problems (20). In our study, we aimed to investigate the effect of sleep quality on postural control in medical and dental students by measuring balance with objective measurement tools. In addition, unlike the studies in the literature, we evaluated how the dominant side was affected by sleep quality in balance analysis.

Methods

The Ethics Committee approved this cross-sectional study for Scientific Research of the Faculty of Medicine of Trakya University under the Declaration of Helsinki (decision no: 06/04, date: 16.03.2020). The study was conducted in the Laboratory of Motion Analysis of the Faculty of Medicine of Trakya University University, Department of Anatomy. Subjects were divided into two groups: those with good sleep quality and those with poor sleep quality.

Participants

The number of volunteers to participate in the study was set at 64 for each group using the G*Power program (version: 3.1.9.7) (effect size: 0.5, alpha: 0.05, power: 0.8). Healthy volunteers (55 men, and 73 women) from medical and dental schools participated in our study. Those who had a medical condition that could affect balance or were taking medications that could affect sleep status and those who had left hemispheric dominance were excluded from the study. All participants were right-sided dominant. The study was explained in detail to the participants, and written informed consent was obtained from the volunteers under the Declaration of Helsinki.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a scale developed by Buysse et al. (21) to assess sleep quality over one month. While 19 of the 24 questions on the scale are answered by the individual, 5 are answered by the person with whom they share the room, if any. The questions assess seven components: subjective sleep quality, latency, duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and time-of-day disturbance (12). If the participants' calculated score is ≥5, sleep quality is labeled poor, and if the score is <5, sleep quality is labeled good.

Study Protocol

Participant demographics were collected, and participants were asked to complete the PSQI form. If the participants' calculated score is ≥5, sleep quality is labeled poor, and if the score is <5, sleep quality is labeled good. Based on the PSQI score, two groups were formed: those with good sleep quality (35 women, 29 men) and those with poor sleep quality (38 women, 26 men). Postural control data were measured every morning between 08:00 and 10:00 for three weeks.

Balance Analysis

A force platform (Zebris©, FDM system type FDM 3.5) was used for the measurements. The force platform was 158 cm long, 60.5 cm wide, and 2.5 cm high. The sensor area of the platform was 149 cm long and 54.2 cm wide. The number of sensors on the platform was 11264, and the measurement width was between 1-120 N/cm^2. The computer program WinFDM (Zebris Medical GmbH, Isny, Germany) was used to convert the information obtained from this platform into digital data and transmit it to the computer environment. Each participant was shown individually what the subjects would do during the measurement. Participants assumed an upright position on the platform with both feet on the floor and arms at their sides. The distance between the feet was not interfered; it was left to the participant's free will. An image was placed about two meters away from the participants and at eye level. The measurements

were performed in two ways. First, participants were kept still on the platform by looking at the image two meters away. The recordings were taken for 30 seconds. Second, participants had the same position, looked at the same image, and then closed their eyes. Recordings were made for 30 seconds after the eyes were closed. The data we obtained from the measurements were related to the movement of the center of pressure (COP), which expressed the point where the ground reaction force acted. The data we obtained with the force measurement system were as follows.

- Confidence ellipse: An ellipse containing 95% of the points through which the COP passes during the measurement (Figure 1). The data for this ellipse is the length of the ellipse's minor axis, the length of the major axis, the angle between the long axis and the Y-axis, and the ellipse area (Figure 2).

- The data relating to the COP are the path length of the COP, the standard deviation (SD) on the X-axis, and the SD on the Y-axis (Figure 1).

- We have obtained the average load distribution of the forefoot and heel on the left and right sides and the data on the total load distribution of the left and right contact area in percent (Figure 1).

- Left/right side fore (%): The ratio of the total load on the left/right foot transferred to the forefoot (Figure 1)

- Left/right side back (%): The rate of load transferred to the heel of the left/right foot (Figure 1).

- Left/right side total (%): Percentage of total load transferred to the left/right foot (Figure 1).

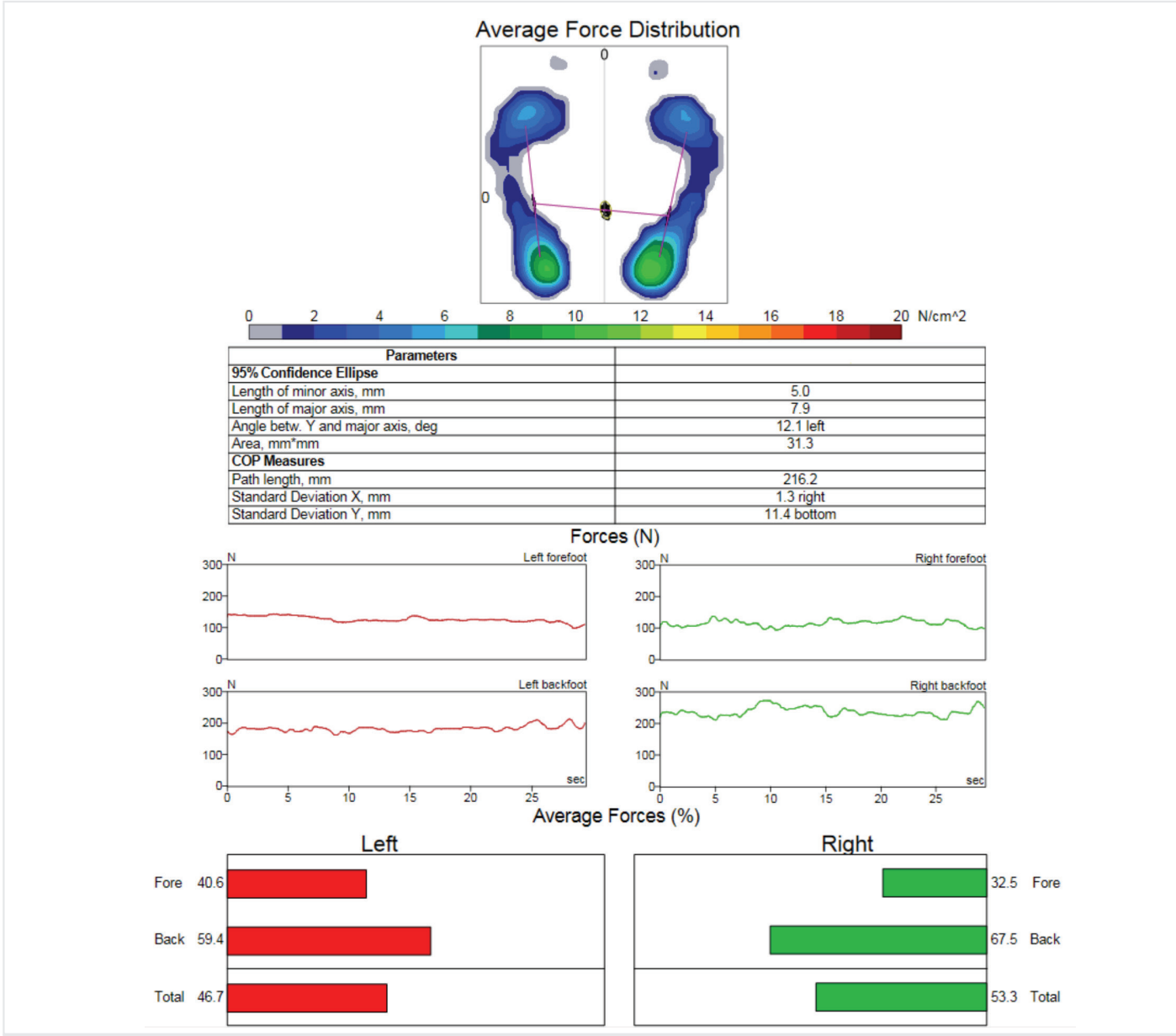

Figure 1. Data from balance analysis

Statistical Analysis

The SPSS 20.0 program (IBM SPSS software, USA) was used for statistical analysis. Results were expressed as mean ± SD, median, minimum, and maximum. The "Single Sample Kolmogorov-Smirnov Test" was used to check the conformity of the variables to the normal distribution. Comparison of weight distribution data on age, height, weight, and foot regions was performed using Student's t-test. As the data from COP did not conform to the normal distribution, the Mann-Whitney U test was used for comparisons between groups. P<0.05 was accepted as the threshold for statistical significance.

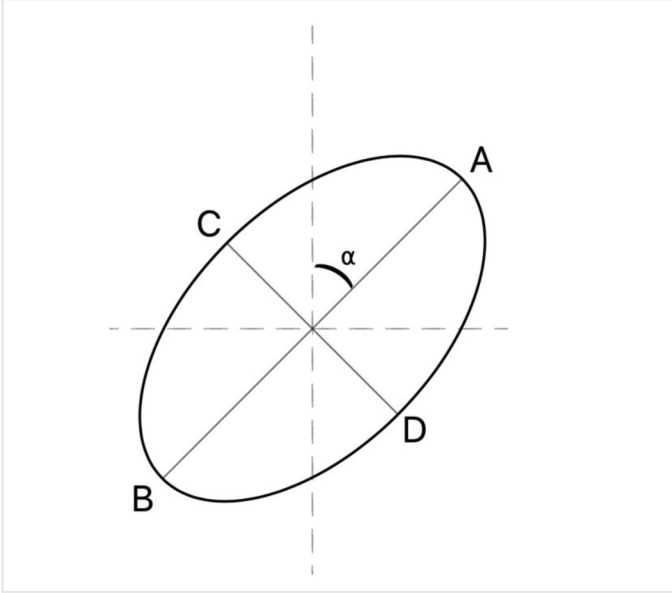

Figure 2. Confidence ellipse
A,B: Length of major axis
C,D: Length of minor axis
α: Angle between Y and major axis

Table 1. Demographic data of the participants

	Good	Poor	p-value
Age (year)	20.75±1.24	20.71±0.75	0.864
Weight (kg)	65.4±13.2	66.98±15.1	0.542
Length (cm)	169.12±8.22	171.9±8.96	0.070

Independent samples t-test was used

Results

There was no statistically significant difference between the demographics of the two groups (p>0.05) (Table 1). The PSQI medians of the good and poor sleep quality groups were 4 (minimum: 1, maximum: 4) and 7 (minimum: 5, maximum: 14), respectively.

The open-eye balance analysis found no difference between the two groups in the confidence ellipse and COP shift data. In the analysis with eyes closed, the deviation of the COP Y-axis was higher in the group with poor sleep quality (Table 2).

In the test with eyes open, no difference was found between the two groups in the pressure distribution on the foot regions. In contrast, a significant difference was found in the data for the left anterior and left posterior sides in the test performed with eyes closed. The data for the left anterior side was higher in the group with poor sleep quality than in the group with good sleep quality, and the data for the left posterior side were lower (Table 3).

Discussion

The PSQI is a commonly used and easy-to-use assessment method for evaluating sleep quality. We assessed participants' sleep quality using this index and examined its effects on postural balance. We hypothesized that poor sleep quality might negatively affect postural control. As a result of the study, we found that sleep quality did not affect balance when the eyes were open. However, when the eyes were closed, the oscillation of the COP in the anteroposterior direction was negatively affected. Poor sleep quality increased the oscillation of the COP on the Y-axis. Data on the distribution of plantar strain were also similar. There was no difference between the two groups when

Table 2. Confidence ellipse data generated by the displacement of the COP

	Eyes open			Eyes closed		
	Good sleep quality (n=64)	Poor sleep quality (n=64)		Good sleep quality (n=64)	Poor sleep quality (n=64)	
	Median (min-max)	Median (min-max)	p-value	Median (min-max)	Median (min-max)	p-value
Length of minor axis, mm	5.7 (2.8-17.7)	5.5 (1.9-11)	0.395	5 (2-13.9)	5 (1.5-12.4)	0.592
Length of major axis, mm	11.8 (4.3-46.9)	11.05 (5.9-48.5)	0.327	11.6 (5.8-25.7)	9.7 (5-53.4)	0.196
Angle between Y and major axis, deg	16.35 (0.1-89.5)	11.9 (0.1-86.3)	0.145	9.3 (0-85.3)	8.55 (0.1-75.1)	0.422
Area mm²	52.4 (10.5-375.2)	46.8 (8.9-262.8)	0.356	45 (11.9-22.5)	37.15 (8.4-520.20)	0.318
Path length, mm	224.5 (145.2-425.6)	220.25 (125-411.9)	0.286	244.35 (139-454.8)	237.95 (146.7-392.7)	0.311
Standard Deviation X, mm	6.75 (0.3-29.8)	8.75 (0.1-32.2)	0.259	7.75 (0-33.2)	8.85 (0.5-32.7)	0.378
Standard Deviation Y, mm	7.1 (0.1-40.3)	6.6 (40.1-0.2)	0.556	5.15 (0.2-23.3)	7.65 (0-29.8)	**0.042***

Mann-Whitney U test was used. *Signifcant diferences (p<0.05) between the two groups, min: Minimum, max: Maximum

Table 3. Load distribution data on right and left side

	Eyes open			Eyes closed		
	Good sleep quality (n=64)	Poor sleep quality (n=64)	p-value	Good sleep quality (n=64)	Poor sleep quality (n=64)	p-value
Left side fore %	41.74±11.4	44.86±10.55	0.112	42.33±11.09	46.29±9.64	0.033*
Left side back %	58.26±11.4	55.14±10.55	0.112	57.67±11.09	53.71±9.64	0.033*
Left side total %	51.24±4.4	50.33±5.52	0.314	51.17±5.39	50.24±5.27	0.327
Right side fore %	43.32±12.1	44.88±9.88	0.428	45.46±10.68	45.45±10.01	0.995
Right side back %	56.68±12.1	55.12±9.88	0.406	54.54±10.6	54.55±10.19	0.995
Right side total %	48.76±4.4	49.67±5.52	0.314	48.83±5.39	49.76±5.27	0.325

Independent samples t-test was used. *Signifcant diferences (p<0.05) between two groups

the eyes were open. However, there were differences in the load ratios distributed on the anterior and posterior part of the foot on the left side when the eyes were closed. We observed that the load on the anterior part of the foot increased in the group with poor sleep quality. In ideal equilibrium, the total load distribution on the right and left sides is 50% (22). When the same side is in the load distribution of the foot, 2/3 of the load is transferred to the dorsum of the foot and 1/3 to the forefoot (22). We hypothesize that worsening this distribution ratio also increases the anterior-posterior displacement of the COP. Therefore these ratios are higher in the group with poor sleep quality. In addition, because all participants had right hemisphericdominance, we found that the non-dominant side was more affected.

Some studies investigate sleep deprivation's effects on the central nervous system (23,24). It has been observed that deactivation of the cortico-thalamic network after 24 hours of sleep deprivation alters the attentional system and functions of the prefrontal cortex (23). Sleep deprivation has been found to reduce glucose metabolism in the temporal lobes, basal ganglia, white matter, and cerebellum (24). Thomas et al. (25,26) examined the rate of glucose metabolism in brain regions during sleep deprivation. They examined participants for 72 hours at 24-hour intervals and found that the metabolic rate in the thalamus, prefrontal cortex, and posterior parietal cortex decreased at the end of the 24 hours. As the duration of sleep deprivation increased, different regions were added. At the end of 72 hours, they observed that the metabolic rate decreased in the thalamus, prefrontal cortex, posterior parietal cortex, dorsal thalamus, and medial visual cortex regions. In contrast, the metabolic rate increased in the lateral superior occipital cortex, lingual and fusiform gyrus, anterior cerebellum, and primary and supplementary motor cortex. They stated that the decrease in metabolic rate in the prefrontal-thalamic network was the reason for the decrease in alertness and cognitive performance and that the brain was involuntarily driven to the onset of sleep. They explained that the increase in metabolism in the visual and motor areas indicated that the brain struggled to stay awake. In addition, the postural control system cannot use visual input with maximum efficiency during sleep deprivation (27). Postural control is more impaired during poor sleep quality when visual input is removed (11). These changes in the central nervous system associated with sleep disruption also negatively affect postural control (27).

Postural control affects not only sleep deprivation but also sleep quality (11). Furtado et al. (11) observed that poor sleep quality negatively affected postural control, similar to sleep deprivation, which was exacerbated when the eyes were closed. They used the activity average of the least active 5-hour period daily to identify groups. The reason for this was that they argued that increased sleep movements shortened the duration of the adequate rapid eye movement sleep phase, which in turn affected postural control. They showed that at this stage, adequate regulation of muscle tone was warranted as the reason (11). The grouping method in this study differed from ours because we determined sleep quality using only PSQI scores. Nevertheless, our results were similar. We found that postural control was negatively affected in those with poor sleep quality when the eyes were closed.

There is a relationship between sleep quality and the maintenance of postural balance (12). Lack of effective sleep negatively affects postural control (28). In a study that examined postural control during a dual-task cognitive task, a positive correlation was found between sleep quality and postural control (29). Another study also found a low correlation between the two parameters and a moderate correlation when the test was performed with eyes open and closed. For this reason, the researchers recommended that the severity of the correlation should be considered before accepting the hypothesis in such studies (30). However, Saraiva et al. (31) found in their study that postural control was independent of sleep quality and that sleep quality did not affect balance.

Ensuring postural control is multifaceted, and mechanisms that integrate neural inputs are used for this purpose (12). These physiological systems include vestibular, proprioceptive, and visual elements (15,16). Studies showed that sleep deprivation caused changes in some parts of the central nervous system (23-26). However, the mechanism by which these changes affected postural control was not fully elucidated. However, these studies were related to sleep deprivation, and we did not find studies on whether sleep quality caused similar changes. Nevertheless, some studies showed poor sleep quality negatively affected postural control (12,28-30). In addition, the assessment of balance in the current studies usually evaluated the movements of the COP and did not examine the differences between the dominant and non-dominant sides in weight shifting. Our study observed that the load distribution on the non-dominant side was impaired when the eyes were closed.

Study Limitations

There are some limitations in our study. First, we assessed sleep quality using only one subjective method, the PSQI. We did not track participants' sleep habits using an objective method such as the ActiGraph. Second, we assessed the proportion of load distributed to the dominant and non-dominant sides of the participants. However, we did not separately assess the postural control of each limb by performing a balanced analysis on one leg.

Conclusion

We performed a two-leg static balance analysis with eyes open. We closed on participants divided into two groups for good and poor sleep quality based on PSQI scores. We found no effects of sleep quality on postural control for the open-eye test. In the closed-eye test, we found that the shift of COP on the Y-axis was higher in the participants with poor sleep quality. Since all participants had right hemispheric dominance, we could compare the percentage of force distribution on the dominant and non-dominant sides. The group with poor sleep quality had higher values for the left front, and correspondingly lower values for the left back. Normally, 2/3 of the load is transferred to the dorsum of the foot and 1/3 to the front of the foot. We observed that this harmony was impaired on the left side (non-dominant side) of the group with poor sleep quality. We think this deteriorated rate affects the SD of the Y data of the group with poor sleep quality and therefore is higher.

Ethics

Ethics Committee Approval: The Ethics Committee approved this cross-sectional study for Scientific Research of the Faculty of Medicine of Trakya University under the Declaration of Helsinki (decision no: 06/04, date: 16.03.2020).

Informed Consent: The study was explained in detail to the participants, and written informed consent was obtained from the volunteers under the Declaration of Helsinki.

Authorship Contributions

Surgical and Medical Practices: M.K., Concept: M.K., M.P., Design: M.K., M.P., D.D.A., Data Collection or Processing: M.K., M.P., E.U., M.S., D.D.A., Analysis or Interpretation: M.K., E.U., Literature Search: M.K., M.P., E.U., M.S., Writing: M.K., M.P., E.U., M.S., D.D.A.

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

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Original Article

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Determination of Types and Frequency of Urinary Incontinence in Women

Kadınlarda Üriner Inkontinans Çeşitleri ve Sıklığının Belirlenmesi

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ABSTRACT

Objective: Finding out the forms, frequency, and contributing variables of female urine incontinence was the goal of this study.

Methods: The research is cross-sectional and descriptive. In this study, 408 women who were at least 18 years old participated. The International Consultation on Incontinence Questionnaire Short Form and the Women's Personal Data Identification Form were used to gather study data.

Results: It was discovered in this study that 37.3% of women had urine incontinence. The quality of life was found to be minimally impacted by urinary incontinence (2.81±4.02). Urinary incontinence frequency was found to be significantly correlated with age, body mass index, number of births, chronic conditions, normal delivery, menopause status, and these factors ($p<0.05$).

Conclusion: Women who experience urinary incontinence, particularly as they age, suffer from a disorder that impairs their quality of life. To help women live better lives, it is critical that medical professionals-especially nurses-screen for urine incontinence in high-risk populations, refer patients to a facility for treatment when needed, and offer education in tandem.

Keywords: Prevalence, risk factors, urinary incontinence

ÖZ

Amaç: Bu çalışmada kadınlarda üriner inkontinans çeşitleri, sıklığı ve ilişkili faktörlerin belirlenmesi amaçlanmıştır.

Yöntemler: Bu çalışma tanımlayıcı ve kesitsel bir çalışmadır. Bu çalışma 18 yaş ve üzeri 408 kadın ile yapılmıştır. Çalışmanın verileri kadınların kişisel verilerini tanılama formu ve Uluslararası İnkontinans Sorgulama Anketi Kısa Formu ile toplanmıştır.

Bulgular: Bu çalışmada kadınlarda üriner inkontinans sıklığı %37,3 bulunmuştur. Üriner inkontinansın yaşam kalitesini düşük düzeyde (2,81±4,02) etkilediği görülmüştür. İdrar kaçırma sıklığının yaş, vücut kitle indeksi, doğum sayısı, kronik durumlar, normal doğum, menopoz durumu ve bu faktörlerle anlamlı düzeyde ilişkili olduğu belirlendi ($p<0,05$).

Sonuç: Özellikle yaşlandıkça idrar kaçırma sorunu yaşayan kadınlar, yaşam kalitelerini bozan bu sorundan muzdariptirler. Sağlık profesyonellerinin özellikle de hemşirelerin riskli gruplara yönelik üriner inkontinans taramasının yapılması ve gerektiğinde sağlık kuruluşuna tedavisi için yönlendirmesi, eş zamanlı olarak eğitimlerinin verilmesi kadınların yaşam kalitesini yükseltmesi açısından önemlidir.

Anahtar Sözcükler: Prevalans, risk faktörleri, üriner inkontinans

Introduction

One prevalent and significant issue that has an impact on women's health is urinary incontinence (UI). The inability to regulate urinate unintentionally is often referred to as UI. UI can have negative physical, psychological and social effects, significantly reducing quality of life. This problem, which is especially common among women, can occur at different stages of life and may be associated with factors such as lifestyle, hormonal changes, and childbirth (1,2). According to studies, the prevalence of UI ranges from 9% to 75%, with women experiencing the highest rates.

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It is stated that this prevalence increases especially with age, obesity, menopause, and number of births (3-5).

Nursing is a multifaceted health field that aims to support the physical and psychosocial health of the individual. Women's health is an important issue that nurses are particularly interested in and work on. In this context, a common problem such as UI constitutes an important part of women's health (6-8). The goal of internal medicine nursing is to maximize patients' medical care by addressing a broad variety of health issues. Internal medicine nurses should be aware of and responsive to conditions like obesity and advanced age, which are major contributors to UI and are also risk factors for the condition. Therefore, UI is an issue that internal medicine nurses encounter and play an active role in its management. By supporting patients and their families in managing their incontinence, nurses can enhance the quality of life for individuals. Because they view this illness, which has a detrimental impact on quality of life, as a natural process that begins with birth and aging, women do not apply to medical facilities. Research on this topic has been done, according to an analysis of the literature. To increase awareness, additional research is necessary. The purpose of this study was to identify the different forms, frequency, and associated factors of female UI. The study's findings will add to the body of knowledge by directing medical professionals in the diagnosis, treatment, and education of patients with risk factors for UI.

Methods

Study design: The research was cross-sectional and descriptive.

Sample of the research: Turkish women who were over the age of eighteen made up the study's sample. Informed about the research and their rights, 408 willing participants in the study gave their informed consent prior to the study's commencement. Participants' rights were all upheld, and the concepts of voluntariness and confidentiality were taken into consideration.

Data collection method: Online surveys were used to gather data between July 18, 2023, and September 1, 2023.

Data collection: A questionnaire outlining each person's personal traits and the International Consultation on Incontinence Questionnaire Short questionnaire (ICIQ-SF) were used to gather the data.

Personal characteristics identification form of individuals

A 13-item form was used to gather the data, which were gathered through a review of the literature. The questions covered the following topics: age, height, weight, height and age of marriage, education level, presence of chronic diseases (diabetes, hypertension, coronary artery disease), smoking, number of births, type of birth, whether the birth was an intervention, status of miscarriages, whether the baby weighed more than 4 kg, and menopausal status.

International Consultation on Incontinence Questionnaire Short Form

Avery et al. (9) created the ICIQ-SF to evaluate user interface and its effects on quality of life. The number of the dimensions of the scale are four. Three dimensions are scored in the first place. Based on the individual's complaints, the type of UI is determined by the answers to the fourth dimension, which is not assessed. The assessment allows for the individual evaluation of each sub-dimension's score, and the influence of UI on quality of life is determined by adding the scores of the three dimensions. Instead of adding the dimension scores one at a time, scoring generally tends to produce a single score. The measure offers scores ranging from 0 to 21, where a low score denotes minimal impact of UI on quality of life and a high score denotes significant impact. Çetinel et al. (10) translated the ICIQ-SF into Turkish in 2004 and carried out a validity and reliability analysis. The scale's Cronbach's alpha coefficient was determined to be 0.71. The Cronbach's alpha coefficient in this investigation was found to be 0.75.

Statistical Analysis

The statistical analysis in the study was conducted using the IBM SPSS statistics 26.0 application. Along with descriptive statistical techniques, the study data were evaluated (mean, standard deviation, frequency, percent). The Mann-Whitney U test was used to examine data that did not exhibit a normal distribution, and the Student's t-test was used to evaluate data that did. The correlation between the variables was assessed using Pearson and Spearman correlation analyses. The associations between the variables were assessed using the chi-squared test. An analysis of binary logistic regression was done to look at the impact of confounding variables. The significance level of $p<0.05$ and the 95% confidence range were used to analyze the results.

Ethical aspect of the study: Permission to begin the study was acquired from Bezmialem Vakıf University Ethics Committee, with decision number 2023/133 and date of June 7, 2023. Those who consented to take part in the study provided informed consent.

Results

Table 1 displays the individual's personal characteristics as well as the types and frequency of UI. The women's average age was 41.12±12.34 years, with 69.9% having graduated from college and 70.1% being married. UI was found in 37.3% of the female participants. UI was shown to have a negligible impact on quality of life (2.81±4.02) (Table 1).

Table 2 illustrates the association between individual traits and incontinence. Age, number of births, BMI, marital status, educational attainment, status of chronic diseases (diabetes, hypertension, coronary artery disease), menopausal status, miscarriage, normal delivery, status of intervention delivery, and

Table 1. Personal characteristics of individuals, frequency and types of urinary incontinence (n=408)

	n	%
Age (mean)	41.12±12.34	
Body mass index (mean)	25.85±5.25	
Number of births (mean)	1.53±1.32	
Educational status		
Primary school graduate	39	9.6
Secondary school graduate	24	5.9
High school graduate	60	14.7
Graduate student	285	69.9
Marital status		
Married	286	70.1
Single	122	29.9
Those with diabetes	34	8.3
Those with coronary artery disease	6	1.5
Those with hypertension	54	13.2
Smokers	129	31.6
Those with menopause	106	26
Normal births	150	36.8
Those who gave birth by caesarean section	168	41.2
Miscarriage	68	16.7
Those who had an intervention birth	78	19.1
Those who gave birth to a baby weighing over 4 kilos	39	9.6
How often do you leak urine?		
Never ever	256	62.7
Like once a week or less	83	20.3
Two or three times a week	21	5.1
Like once a day	9	2.2
Like several times a day	31	7.6
Always	8	2.0
How much urine do you usually leak?		
Nothing	256	62.7
Small amount	131	32.1
Moderate quantity	21	5.1
Overall, how much does urinary incontinence affect your everyday life? (mean)	1.14±2.24	
When do you leak urine?		
Urine never leaks	256	62.7
It'll be gone before you can make it to the bathroom	103	25.2
Leaks when you cough or sneeze	120	29.4
It leaks while you sleep	9	2.2
Leaks when you are physically active or exercising	25	6.1
It leaks when you finish peeing and get dressed	11	2.7
Leaks for no apparent reason	13	3.2
It always leaks	8	2.0
Mean score for the impact of UI on quality of life	2.81±4.02	

Descriptive statistical methods (mean, standard deviation, frequency, percent), UI: Urinary incontinence

urine incontinence were found to be significantly correlated ($p<0.05$).

Table 3 shows a significant positive connection ($p<0.005$) between the persons' UI status and age, BMI, number of births, and intervention delivery. The study employed binary logistic regression analysis to examine the impact of age, BMI, number of births, and intervention delivery on the prevalence of urine incontinence in the participants. Age, BMI, and the number of births were revealed to be significant ($p<0.05$) in the binary logistic regression analysis, even in the absence of additional confounding variables. Binary logistic regression analysis revealed that while there was a positive association between the delivery of interventions and UI, it was not statistically significant ($p>0.05$).

Discussion

The study found that the prevalence of UI among women was 37.3%, and its influence on quality of life was minimal (2.81±4.02) (Table 1). Our finding is comparable with the literature. According to reports in the literature, women are more likely to have UI at ages 25 to 45, with the prevalence rising with age (3). The individuals' mean age was 41.12±12.34, and a significant correlation ($p<0.05$) was found between age and UI. The frequency of UI increased with increasing age. We could say that the reason why UI affected the quality of life at a low level was that the participants were middle-aged women.

The mean BMI of the participants was 25.85±5.25 and the mean number of births was 1.53±1.32. The frequency of UI and BMI was shown to be significantly correlated with the number of births ($p<0.05$). Furthermore, age, BMI, and the number of births-risk factors for UI-were revealed to be significant ($p<0.05$) in the binary logistic regression analysis, separate from other confounding variables. In a systematic review by Abufaraj et al., (11) age, obesity, comorbidities, smoking, postmenopausal hormone therapy were found to increase UI. Wikander et al. (12) found that the number of births was associated with UI. The study's findings are consistent with those reported in the literature. But this study revealed no evidence of a significant link between smoking and UI ($p>0.05$). Given that the literature revealed a different conclusion, it was believed that more thorough research should be done on this topic.

In addition to the risk variables discovered in earlier studies, the menopause was found to increase the incidence of UI in the study by Xue et al. (13). In this study, it was also found that menopause increased UI. We can explain this significance in two ways. First, menopause is a process seen in women at advanced ages. It is found that the frequency of UI increased with age. Although menopause does not directly affect the frequency of UI, UI can increase in an age-dependent manner. Secondly, Abufaraj et al. (11) found that menopausal hormone therapy increased UI. In this study, it can be said that menopausal hormone treatment may increase UI.

In this study, it was found that intervention, normal delivery and miscarriage increased UI. Studies in the literature also indicate that normal delivery increases UI (12). Binary logistic

Table 2. The relationship between personal characteristics of individuals and urinary incontinence (n=408)

	Patients with urinary incontinence (n)	Patients without urinary incontinence (n)	p-value
Age (mean)	45.64±12.41	38.43±11.50	0.001
BMI (mean)	27.47±5.77	24.89±4.66	0.001
Number of births (mean)	2.05±1.37	1.22±1.18	0.001
Marital status			
Married	130	156	0.001
Single	22	100	
Educational status			
Primary school graduate	22	17	0.02
Secondary school graduate	12	12	
High school graduate	21	39	
Graduate student	97	188	
People with diabetes	22	12	0.001
People without diabetes	130	244	
Those with hypertension	32	22	0.001
Those without hypertension	120	234	
Those with coronary artery disease	5	1	0.02
Those without coronary artery disease	147	255	
Those with menopause	55	51	0.001
Those without menopause	97	205	
Smokers	52	77	0.22
Non-smokers	100	179	
Miscarriage	39	29	0.001
No miscarriage	113	227	
Normal births	85	65	0.001
Those who do not give birth normally	67	191	
Those who gave birth by caesarean section	63	105	0.5
Those who did not give birth by caesarean section	89	151	
Those who had an intervention birth	38	40	0.01
Those who do not have an intervention birth	114	216	
Those who gave birth to a baby weighing over 4 kilos	20	19	0.08
Those who did not give birth to a baby weighing over 4 kilos	132	237	

Student's t-test, Mann-Whitney U test, The chi-squared test, BMI: Body mass index

Table 3. Correlation between urinary incontinence status and age, BMI, number of births and intervention births

		How often do you leak urine?	How much urine do you usually leak?	Overall, how much does urinary incontinence affect your everyday life?	Mean score for the impact of UI on quality of life
Age	r	0.198	0.216	0.274	0.270
	p	0.001	0.001	0.001	0.001
BMI	r	0.264	0.204	0.283	0.301
	p	0.001	0.001	0.001	0.001
Number of births	r	0.213	0.235	0.288	0.296
	p	0.001	0.001	0.001	0.001
Interventive delivery	r	0.050	0.094	0.110	0.107
	p	0.310	0.057	0.026	0.031

Pearson and Spearman correlation analysis. BMI: Body mass index

regression analysis revealed that while there was a positive association between the delivery of the intervention and UI, it was not statistically significant (p>0.05). Deliveries performed at older ages in women may be more risky and interventional. Interventive delivery may have increased the frequency of UI in an age-dependent manner.

In this study, people with chronic illnesses had a greater prevalence of UI. UI was found to occur often in patients with chronic illnesses, according to literature reviews (14,15). The study's findings are consistent with those found in the literature. Chronic diseases are a condition that usually occurs in individuals at an advanced age and continues to be treated throughout life, negatively affecting the systems. It may be a reason that chronic diseases are more common with age and therefore the frequency of UI is higher in those with chronic diseases. At the same time, the fact that chronic diseases cause systemic deterioration in individuals may increase the frequency of UI.

Study Limitation

This study is a descriptive cross-sectional study. The results of education or treatment that can be given to women for urinary incontinence can be supported by experimental clinical studies.

Conclusion

According to this study, 37.3% of women reported having UI, and their quality of life was not significantly affected by them. The number of births, menopause, age, BMI, chronic illnesses, and normal delivery have all been linked to an increased risk of UI in women. Women who experience UI, particularly as they age, suffer from a disorder that impairs their quality of life. According to the results of this study, women typically regarded UI, which could also occur in middle age, as a normal ailment and did not seek medical attention for it. It is crucial that medical personnel, particularly nurses, screen for UI in high-risk populations, refer them to a facility when treatment is required. In order to enhance women's quality of life, it is critical that health professionals especially nurses screen for UI in high-risk populations, refer them to a medical facility for treatment when appropriate, and offer concurrent training.

Ethics

Ethics Committee Approval: Permission to begin the study was acquired from Bezmialem Vakıf University Ethics Committee, with decision number 2023/133 and date of June 7, 2023.

Informed Consent: Those who consented to take part in the study provided informed consent.

Authorship Contributions

Concept: N.K., M.K., Design: N.K., Data Collection or Processing: N.K., Analysis or Interpretation: M.K., Literature Search: N.K., Writing: N.K.

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

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Original Article

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The Effect of Manual Pressure Applied on Infants Before Vaccine Injection on Pain Level and Crying Time

Aşı Enjeksiyonu Öncesinde Bebeklere Uygulanan Manuel Basıncın Ağrı Düzeyi ve Ağlama Süresine Etkisi

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ABSTRACT

Objective: The aim of this study was to determine the effect of manual pressure applied to the injection site before vaccine injection on the level of pain and crying time of 4-month-old infants.

Methods: This study was a randomized controlled trial. The sample of the study included 70 infants (35 infants in the intervention and 35 in the control groups). Research data were collected using an "Information Form", the FLACC Pain Assessment Scale, and a stopwatch. Before the procedure, manual pressure was applied to the injection site with the thumb for 10 seconds to the infants in the intervention group. No non-pharmacological method was applied to the infants in the control group before vaccination. Pain scores of the infants were evaluated during and after the vaccine injection, and total crying times were recorded.

Results: It was found that the pain score of the control group (6.37±1.92) was higher than that of the intervention group (4.40±2.32) ($p<0.05$) during the procedure. The pain score of the control group after the procedure (6.86±1.97) was significantly higher than the intervention group (3.00±2.00) ($p<0.05$). The mean crying time of the infants in the intervention group (5.68±5.54 seconds) was significantly shorter than the infants in the control group (81.67±31.31 seconds) ($p<0.05$).

Conclusion: In this study, manual pressure applied before injection was found to be effective in reducing the pain of infants. Manual pressure is an easy-to-apply, non-time-consuming, and cost-effective method.

Keywords: Infant, injection, pain, manual pressure, vaccine

ÖZ

Amaç: Bu araştırmanın amacı 4 aylık bebeklerde aşı enjeksiyonu öncesinde enjeksiyon bölgesine uygulanan manuel basıncın bebeklerin ağrı düzeyine ve ağlama süresine etkisini belirlemektir.

Yöntemler: Bu çalışma randomize kontrollü deneysel desende gerçekleştirildi. Araştırmanın örneklemini 70 bebek (müdahale grubu: 35 bebek; kontrol grubu: 35 bebek) oluşturdu. Araştırma verileri "Bilgi Formu", FLACC Ağrı Değerlendirme Skalası ve kronometre aracılığı ile toplandı. Müdahale grubundaki bebeklere işlemden önce enjeksiyon bölgesine 10 saniye süre ile baş parmak ile manuel basınç uygulandı. Kontrol grubundaki bebeklere aşı uygulaması öncesinde herhangi bir non-farmakolojik yöntem uygulanmadı. Bebeklerin aşı enjeksiyonu sırası ve sonrasında ağrı puanları değerlendirildi ve toplam ağlama süreleri kayıt edildi.

Bulgular: İşlem sırasında kontrol grubunun (6,37±1,92) ağrı puanının müdahale grubundan (4,40±2,32) daha yüksek olduğu bulundu ($p<0,05$). Kontrol grubunun işlem sonrasındaki ağrı puanının (6,86±1,97), müdahale grubuna göre (3,00±2,00) anlamlı olarak yüksek olduğu belirlendi ($p<0,05$). Müdahale grubundaki bebeklerin ortalama ağlama süresinin (5,68±5,54 saniye), kontrol grubundaki bebeklere göre (81,67±31,31 saniye) anlamlı düzeyde kısa olduğu saptandı ($p<0,05$).

Sonuç: Bu araştırmada enjeksiyon öncesi uygulanan manuel basıncın bebeklerin ağrısını azaltmada etkili olduğu belirlendi. Manuel basınç uygulaması kolay, zaman almayan ve maliyet-etkin bir yöntemdir.

Anahtar Sözcükler: Bebek, enjeksiyon, ağrı, manuel basınç, aşı

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Introduction

Vaccination is a routine public health practice recognized worldwide to protect against infectious diseases (1). Many diseases and deaths have been prevented after the advent of vaccines (2). An average of 21 vaccine injections given are made during infancy, although the number of injections is now less as a result of the combined vaccines (3). It is estimated that an average of 12 billion injections are made annually, of which approximately 5% are for infant vaccination (4). The implementation of vaccines and vaccination programs has been significant progress in public health practices. On the other hand, vaccine injection is one of the most common causes of pain in healthy infants and children (5). Parents may avoid or delay vaccination to avoid the pain their child experiences repeatedly with vaccination. Avoiding or delaying vaccination by parents may lead to disruption of vaccination programs, and thus to an increase in communicable diseases preventable by vaccination. Management of pain from vaccine injection in children is therefore very important (6,7).

Non-pharmacological methods are frequently preferred all over the world because they are effective in reducing the pain of children when used alone or in combination with pharmacological methods (8). There are studies examining the effects of non-pharmacological methods such as breastfeeding, oral sucrose, appropriate positioning, distraction, massage, manual pressure application, and the use of appropriate injection techniques in the control of pain during vaccination in children (1,2,5,7,9,10). One of these methods, manual pressure is applied to the injection area with the thumb for 10 seconds before the injection (11). Only limited studies examining the effect of manual pressure on the pain caused by vaccine injection in infants could be identified (9). Considering this important deficiency in the literature, the study was carried out to determine the effect of manual pressure applied to the injection site on the pain level and crying time of infants before the injection of the 5-in-1 vaccine [diphtheria-tetanus-acellular pertussis inactivated poliomyelitis and Haemophilus influenzae type B (DTaP-IPV-Hib)] in 4-month-old infants.

Research hypotheses:

Hypothesis 1 (H1): The pain scores of the infant's given manual pressure before the vaccine injection are lower than the infants who are not.

Hypothesis 2 (H2): Infants who are given manual pressure before vaccine injection have shorter crying times than infants who are not.

Methods

Study Design and Participants

This randomized controlled study was conducted between December 2021 and April 2022. The study followed the CONSORT (Consolidated Standards of Reporting Trials) checklist. The population of the study included 4-month-old infants who were brought to a family health center to have the 5-in-1 vaccine (DTaP-IPV-Hib). The sample size of this study was calculated by power analysis in G*Power 3. 1.9.4 program based on a study (5), which reported that the aspiration-free and rapid injection technique applied during vaccine injection was effective in reducing pain scores in infants aged 6 weeks to 6 months. Taking the effect size as 0.67 for the FLACC pain score, the sample size was calculated as 66 infants in the sample size analysis performed by taking the alpha error probability of 0.05 and the power value of 0.85. Considering that there may be drop-outs from the sample, it was decided to carry out the study with a total of 70 infants, 35 in both groups. As the inclusion criteria, the infants had to be healthy and born at term, had to be 4 months old, had to be receiving the 5-in-1 vaccine (DTaP-IPV-Hib), should not have any chronic conditions or a neurological disorder which might affect the infant's response to pain and should not have received an analgesic within 24 hours before vaccination.

Randomization of the study was done through an online program with the URL https://www.random.org/. Lots were drawn before the sample number was entered into the software, and the 1st set was assigned to the control group and the 2nd to the intervention group. To determine the assignment of the infants included in the study to the groups, numbers from 1 to 70 were written into the software without repeating the numbers. The infants forming the sample group were randomly distributed to the two groups by the software.

Data Collection Instruments

Information form, FLACC Pain Assessment Scale, and stopwatch were used to collect the research data.

Information form: It consisted of 11 questions on the socio-demographic descriptive characteristics (age, gender, etc.) of the infants included in the study and their parents. The form also included spaces to record FLACC pain scores and total crying times.

FLACC Pain Assessment Scale: The pain of infants in the intervention and control groups was evaluated using the "FLACC Pain Assessment Scale" in the study. It was developed by Merkel et al. (12) in 1997 to assess the pain of children aged 2 months to 7 years. The child's facial expression, leg movements, activity, crying, and consolability behaviors are evaluated in relation to pain. These five behaviours are rated with 0, 1, or 2 points. The total score of the scale ranges from 0 to 10, with high scores indicating that the child has pain. A score of "0" from the scale indicates no pain, "1-3" points indicate mild pain, "4-6" points indicate moderate pain, and "7-10" points indicate severe pain (13,14). The FLACC scale was translated into Turkish in the study by Şenaylı et al. (15) evaluating the pain of children aged 1 month to 9 years.

Stopwatch: Voit 8073 stopwatch was used to determine the crying times of the infants included in the study.

Procedures

Parents of infants who met the inclusion criteria were informed by the researcher about the study, and their verbal and written

consent was obtained. Information about the infants included in the study and their parents were recorded in the "Information Form" 5 minutes before the procedure by face-to-face interview method by the researcher. The same nurse researcher did all the vaccinations. The infant was laid back, and the vaccination area was marked. The 5-in-1 vaccine (DTaP-IPV-Hib) was intramuscularly (IM) injected. The injection site was at the right or left laterofemoral section of the vastus lateralis muscle, which was in the middle 1/3 part of the thigh. The vaccination area was disinfected with alcohol, and the vaccine was injected into the vastus lateralis muscle at a 90-degree angle.

All infants were awake and had clean diapers at the time of injection, and their parents were present in the procedure room. No non-pharmacological method was applied to the infants in the control group before vaccination, and routine vaccination was performed. Before the procedure, the infants in the intervention group were applied manual pressure to the injection site with the thumb for 10 seconds before the procedure. Parents of all infants both in the intervention and control groups were present during the procedure. During vaccination, parents were allowed to calm the infants in both groups by touching and talking to them. They were not allowed to feed and do anything that would distract the infant's attention, including giving them toys, showing them a dummy, or clapping. Once the vaccine was started to be injected, the infants' pain was evaluated by the nurse researcher using the FLACC scale. Immediately after the vaccine injection was ended and the needle was removed, the infants' pain was re-evaluated by the nurse researcher using the FLACC scale. If the infant started to cry, another nurse working at the family health center started a stopwatch and stopped when the infant stopped crying to measure the total crying time of the infant.

Ethical Considerations

This study was registered in the clinical trial registry (ClinicalTrials.gov number: NCT05143450). Ethical approval was obtained for the study from Biruni University Clinical Research Ethics Committee (decision no: 2015-KAEK-53-21-02, date: 29.09.2021). Before starting the study, the parents of the infants included in the study were informed about the purpose, duration, plan of the study, and where and how the obtained data would be used. In line with the principles of voluntariness and willingness, verbal and written consent were obtained from the parents.

Statistical Analysis

Data were evaluated with the SPSS package program for Windows (version 21, IBM Corporation, Armonk, NY). In evaluating the data, number, percentage, mean and standard deviation were given in descriptive statistics. Paired t-test was used to evaluate repeated measures of normally distributed variables and independent t-test to compare paired groups. For variables not showing normal distribution, Wilcoxon test was used to evaluate repetitive measurements, Mann-Whitney U test to compare paired groups. A p-value of $p<0.05$ was considered statistically significant.

Results

In this study, 78 infants were assessed for eligibility. Before randomization, 8 infants were excluded because their parents declined to participate. A total of 70 infants were assigned into groups (35 in the intervention and 35 in the control group) (Figure 1). Of the infants, 60% in the control group and 54.3% in the intervention group were boys, and the majority of the infants in both groups (65.7% for both groups) were born into a nuclear family. The mean age of the mothers of the infants in the intervention group was 27.20±6.63 years, and the mean age of the fathers was 31.97±5.89 years. The same values for the control group were 26.97±5.14 and 30.57±5.94 years, respectively. The two groups were well-matched in their descriptive characteristics ($p>0.05$).

The pain score of the control group (6.37±1.92) during the procedure was found to be significantly higher than the intervention group (4.40±2.32) ($p<0.05$). The pain score of the control group (6.86±1.97) after the procedure was found to be significantly higher than the intervention group (3.00±2.00) ($p<0.05$) (Table 1, Figure 2). The mean crying time of the infants

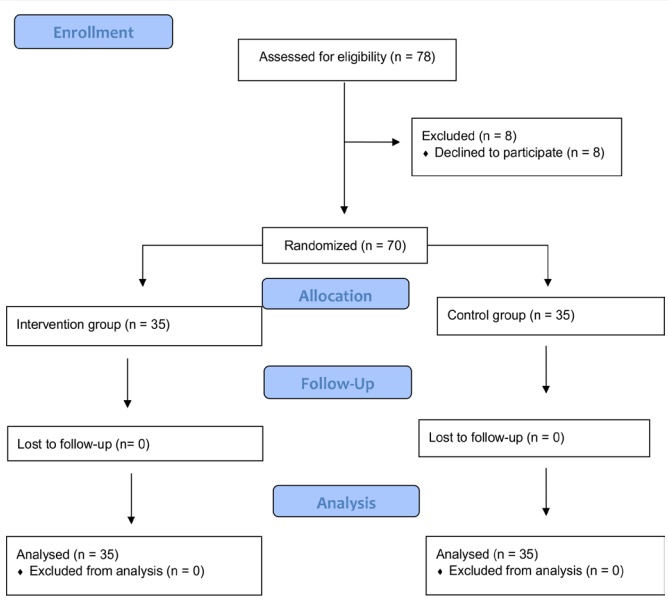

Figure 1. CONSORT participant flow diagram

Table 1. Comparison of mean FLACC scores during and after injections between the intervention and the control groups (n=70)

Time	Intervention group (n=35) M ± SD (Median)	Control group (n=35) M ± SD (Median)	U*	p-value
During injection	4.40±2.32 (4)	6.37±1.92 (5)	321.00	0.00
After injection	3.00±2.00 (4)	6.86±1.97 (7)	115.00	0.00

M: Mean score, SD: Standard deviation, *Mann-Whitney U test

in the intervention group was 5.68±5.54 seconds, compared to 35.57±39.57 seconds in the control group. The crying time of the infants in the intervention group was significantly shorter than the control group ($p<0.05$) (Table 2). The intervention and control groups did not differ statistically significantly by gender for the mean FLACC score and the mean crying time during and after the procedure ($p>0.05$) (Table 3).

Discussion

Although early childhood injectible vaccines are vital for preventive health, they are painful and often lead to fear of needles. Recent research has recommended updating evidence-based strategies for managing pain associated with vaccine injections using non-pharmacological interventions (6). Using such interventions could reduce pain and could easily become a routine aspect of the delivery of vaccine injections (16). The results of our study revealed that manual pressure was effective in pain relief in the infants administered DTaP/IPV/Hib.

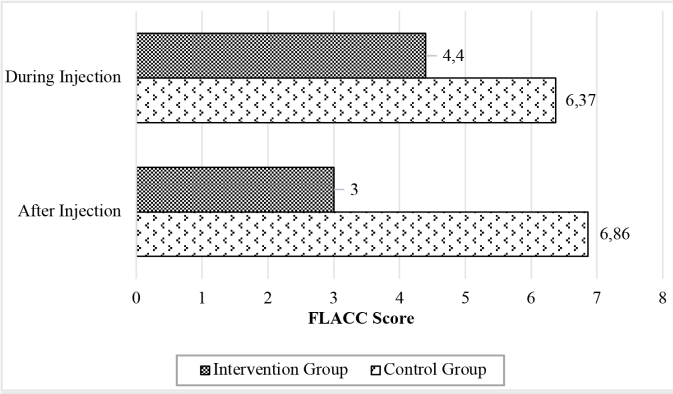

Figure 2. FLACC scores of the groups during and after the injection

Table 2. Comparison of mean crying time between the intervention and the control groups (n=70)

Crying time	Intervention group (n=35) M ± SD (Median)	Control group (n=35) M ± SD (Median)	U*	p-value
Crying time (second)	5.68±5.54 (5)	35.57±39.57 (18)	190.500	0.00

M: Mean score, SD: Standard deviation, *Mann-Whitney U test

One of the most obvious and observable behavioral responses of infants to painful stimuli is crying (17). Both the intensity (e.g., gentle or whimpering) and duration of crying are valid measurements (18). Crying time in infants is used to determine the time, severity, and duration of pain (16). In our study, the infants in the intervention group had a shorter crying time. In a study examining the effect of rapid injection without aspiration and applying 10 seconds of manual pressure in infants aged 4-6 months who were administered the 5-in-1 vaccine (DTaP-IPV-Hib), the average pain score of the infants in the manual pressure group was lower than the infants in the control group. In the same study, the mean crying time of infants in the manual pressure group (10.8±21.8) was shorter than that of the infants in the control group (27.3±27.9) (9).

The pain control effect of manual pressure is explained by the gate control theory (19). According to this theory, the presence and severity of pain depend on the transmission of neurological stimuli. Briefly, if the gate is open, the stimulus reaches the level of consciousness and pain is felt, and if it is closed, the stimulus does not reach consciousness and pain is not felt (20). Applications such as rubbing the pain area, pressing with fingers, and massage provide closing the gate to painful stimuli (11). Studies examining the effect of manual pressure on pain associated with intramuscular injection in different age groups found this method to be effective in reducing pain (11,19). In a study examining the effect of manual pressure to the IM injection area before penicillin injection on the pain level of children aged 7-19 years, the pain scores of children who were applied manual pressure were significantly lower than the children in the control group (11). Chung et al. (19) conducted a study with 74 students between the ages of 18-21 to investigate the effect of manual pressure application on pain in Hepatitis A and Hepatitis B vaccine injections and reported significantly lower pain scores of individuals who were subjected to pressure. These data indicate that manual pressure is an effective, easy to apply and cost-effective method not requiring preparation to reduce the pain of intramuscular injection.

Table 3. Comparison of the mean FLACC scores and crying time of the groups according to gender (n=70)

Variables	Intervention group (n=35)		Control group (n=35)	
	Girl (n=16) M ± SD	Boy (n=19) M ± SD	Girl (n=14) M ± SD	Boy (n=21) M ± SD
Pain/during injection	3.56±2.5	5.10±1.9	6.79±2.1	6.09±1.8
U*; p-value	96.00; 0.066		117.50; 0.325	
Pain/after injection	2.62±2.3	3.32±1.7	6.86±2.1	6.81±1.9
U*; p-value	128.00; 0.441		141.50; 0.855	
Crying time	4.44±5.3	6.74±5.6	41.71±46.8	31.48±34.5
U*; p-value	116.500; 0.243		136.50; 0.727	

M: Mean score, SD: Standard deviation, *Mann-Whitney U test

Features such as age, gender, previous painful experiences, and cultural background have been suggested as factors that increase or alleviate the effect of painful experiences in children (2). Gender differences also have complex effects on pain perception. Pain perception can also be affected by biological, psychosocial and other factors such as the intensity, frequency, and duration of pain (21). Gender differences were reported to be effective on pain only during adolescence, not affecting pain in younger children (infants, toddlers, school children) (22). Similarly, in our study, the pain scores and crying times of the infants in both groups were not affected by gender. In the study conducted by Göl and Altuğ Özsoy (9), the average pain score and crying time of infants were similar in infants of both genders, and gender was not a factor affecting pain.

Study Limitations

There were some limitations of the study. One of the limitations was that the study was limited to intramuscular vaccine injection only and was not necessarily generalizable to manual pressure prior to other intramuscular injections such as medication administration. The second limitation was that the observer, who was one of the researchers, conducted pain assessment on both groups. This might pose a natural bias in the observer's outcome. The last limitation of this study was that the previous painful stimuli of the infants were not recorded.

Conclusion

The results of our study indicated that the pain intensity of the infants who were applied manual pressure to the injection area for 10 seconds before vaccination was reduced. Thus, manual pressure before vaccination can be effective in reducing pain and shorten the duration of infant crying during vaccination. Finally, 10-second manual pressure before vaccination can be easy, quick, safe, and inexpensive method to implement in addition to other techniques in the management of vaccine pain.

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Ethics

Ethics Committee Approval: Ethical approval was obtained for the study from Biruni University Clinical Research Ethics Committee (decision no: 2015-KAEK-53-21-02, date: 29.09.2021).

Informed Consent: Verbal and written consent were obtained from the parents.

Authorship Contributions

Surgical and Medical Practices: Z.E., S.K., F.D., Concept: Z.E., S.K., F.D., Design: Z.E., S.K., F.D., Data Collection or Processing: F.D., Analysis or Interpretation: S.K., Literature Search: Z.E., Writing: Z.E., S.K.

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Original Article

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Evaluation of The Effect of Different Polyetheretherketone Materials on Biofilm Formation: An *in vitro* Study

Farklı PEEK Materyallerinin Biyofilm Formasyonuna Etkisinin Değerlendirilmesi: Bir *İn Vitro* Çalışma

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ABSTRACT

Objective: The aim of this *in vitro* study was to investigate microorganism adhesion and biofilm formation between pure and ceramic-reinforced polyetheretherketone (PEEK) materials.

Methods: A total of 72 rectangular (8 x 8 x 4 mm) samples were prepared from pure-PEEK without filler and PEEK (Ceramic-reinforced PEEK - bio high-performance polymer) containing 20% nano-ceramic filler. A profilometer contact surface measurement device was used to assess the surface roughness of the samples. PEEK groups (36 pure PEEK, 36 Ceramic-reinforced PEEK) were divided into 4 sub-groups of 9 according to the microorganism strains. *Staphylococcus aureus* [American Type Culture Collection (ATCC 29213)], *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCC 10231) standard strains were used for microbiological analysis. Blocks were added to 24-well microplates containing suspensions of microorganisms and were incubated at 37 °C for 72 hours. Microplates were read at a wavelength of 490 nm using crystal violet.

Results: No significant difference was determined between the PEEK groups in terms of surface roughness. No significant

ÖZ

Amaç: Bu *in vitro* çalışmanın amacı iki farklı tip polietereterketon (PEEK) materyali (saf ve seramik ile güçlendirilmiş) üzerinde mikroorganizma tutulumu ve biyofilm formasyonunun değerlendirilmesidir.

Yöntemler: Çalışmada doldurucu içermeyen saf PEEK (Juvora) ve %20 nano-seramik doldurucu içeren PEEK (Seramik PEEK - yüksek performanslı polimer) materyallerinden 8 x 8 x 4 mm boyutlarında dikdörtgen şeklinde toplam 72 örnek hazırlandı. Numunelerin yüzey pürüzlülüğünü değerlendirmek için profilometre temas yüzeyi ölçüm cihazı kullanıldı. Her iki PEEK grubu mikroorganizma suşları dikkate alınarak 4 alt gruba ayrıldı (n=9). Mikrobiyolojik analizde *Staphylococcus aureus* [American Type Culture Collection (ATCC 29213)], *Acinetobacter baumannii* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) standart suşları kullanıldı. Her mikroorganizma için eşit sayıda blok kullanıldı (9 saf PEEK ve 9 Seramik PEEK). Çalışma blokları mikroorganizma süspansiyonlarını içeren 24 kuyucuklu mikroplaklara eklendi ve 37 °C'de 72 saat inkübe edildi. Mikroplaklar kristal viyole kullanılarak 490 nm dalga boyunda okundu.

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ABSTRACT

differences in biofilm formation of *S. aureus*, *A. baummanii*, *E. faecalis*, and *C. albicans* strains were found between the PEEK groups (p>0.05). In the pure-PEEK, the highest adhesion was recorded in *S. aureus* (p<0.001), and the lowest adhesion in *C. albicans* (p<0.001). In the ceramic-reinforced PEEK group, *S. aureus* and *A. baummanii* adhesions were observed more than *E. faecalis* and *C. Albicans* (p<0.001).

Conclusion: The results of this investigation demonstrated no significant differences in the biofilm formation of different strains between PEEK materials. This was a preliminary study to define the biological characteristics of ceramic-reinforced PEEK. There is a need for further comparative and clinical studies on this subject.

Keywords: PEEK, ceramic-reinforced PEEK, BioHPP, biofilm formation

ÖZ

Bulgular: Yüzey pürüzlülüğü açısından PEEK grupları arasında anlamlı fark saptanmadı. Her iki PEEK materyali arasında *S. aureus, A. baummanii, E. faecalis* ve *C. albicans* suşları adezyonu açısından anlamlı farklılık bulunmadı (p>0,05). Saf PEEK bloklarında en yüksek tutulum *S. aureus* mikroorganizmasında görülürken (p<0,001), en düşük tutulum *C. albicans*'ta saptandı (p<0,001). Seramik PEEK grubunda ise *S. aureus* ve *A. baummanii* adezyonları *E. faecalis* ve *C. albicans*'tan fazla bulundu (p<0,001).
Sonuç: Bu araştırmanın sonuçları, PEEK malzemeleri arasında farklı suşların biyofilm oluşumunda önemli bir fark olmadığını gösterdi. Bu, seramikle güçlendirilmiş PEEK'in biyolojik özelliklerini tanımlamaya yönelik bir ön çalışmaydı. Bu konuda daha fazla karşılaştırmalı ve klinik çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: PEEK, seramikle güçlendirilmiş PEEK, BioHPP, biyofilm formasyonu

Introduction

Microbial dental plaque is defined as a type of highly structurally and functionally organized biofilm consisting of bacterial and non-bacterial microbes aggregations surrounded by an extracellular matrix that includes substance from serum, saliva, and blood (1). Periodontitis/peri-implantitis are pathological conditions associated with biofilm in the tissues surrounding teeth/dental implants. Although certain risk factors such as smoking, inadequate oral hygiene, and poorly controlled diabetes mellitus have been identified in the deterioration of periodontal and peri-implant health, prosthetic factors such as improper prosthetic design, unstable occlusion, the violation of supracrestal adherent tissue, uncleansable bridges, over-contoured crowns, and surface properties of prosthetic materials also play an important role in the development of periodontal and peri-implant diseases (2-7). In addition, the amount and composition of biofilm formation may be influenced by the implant and prosthetic material's chemical and physical properties such as surface roughness and surface energy (8,9).

Polyetheretherketone (PEEK) has a partially crystalline polymer structure and exhibits high-temperature resistance (over 300 °C), high mechanical strength, and good chemical resistance (10). In addition, the elastic modulus of PEEK material is close to the elastic modulus of human bone and thus, it has been reported that the longevity of PEEK material in the human body is excellent (11). PEEK has been preferred for use as an alternative to metallic implants in the field of orthopedics and traumatology because of its favorable biomechanic properties and high performance (12). It has also been reported that PEEK is resistant to chemicals in aging environments (13).

Many applicable methods such as direct surface modification, nano-level surface modification, and/or various filler additives can be produced to increase the bioactivity and osteoconductive properties of PEEK (14,15). Recently, modified PEEK materials have been used as alternative materials to titanium, zirconium, and metal alloys in various fields of dentistry, such as infrastructure material in fixed partial dentures, components in removable prostheses, temporary abutments, healing caps, and even implant materials (15,16). A modified PEEK, bio high-performance polymer (BioHPP), has been introduced as a novel material with higher biocompatibility. This modified and ceramic-reinforced PEEK is obtained by adding 20% ceramic fillers (aluminum oxide and zirconium oxide) to the PEEK material. The ceramic microparticles are approximately 0.3-0.5 microns in size, (17,18) and the addition of ceramic microparticles has been shown to improve the polishability and stability of PEEK material. The ceramic-reinforced PEEK material can be used to produce higher-quality prosthetic restorations (13,17). Moreover, the addition of filler has been reported to improve hydrophilic properties compared to pure PEEK material (19).

Prosthetic implant dentistry is an area that is constantly open to new modifications and materials such as ceramics, polymers, and modified PEEK/PEEK. At the same time, it would be beneficial for clinicians to know about improved dental materials that inhibit bacterial adherence and delay biofilm formation. Although there has been a limited number of studies to date, the research has focused on comparing PEEK and titanium surfaces with biofilm formation (20-22). This *in vitro* study was designed to compare microorganism adherence and biofilm formation of *Staphylococcus aureus, Acinetobacter baumannii, Enterococcus faecalis*, and *Candida albicans* on pure PEEK and ceramic-reinforced PEEK.

Methods

The sample size was calculated with a software program (G*Power 3.0.1). The minimum total number of specimens was determined as 72 with 0.403 effect size (f), 0.80 power (1-β err probe), and 0.05 significance level (α err probe). Considering the result of the power analysis, a total of 72 specimens were prepared in the current study, as 9 in each group.

The surfaces of pure PEEK (Juvora; Juvora Ltd. Thornton Cleveleys, Lancashire, England) and ceramic-reinforced PEEK

(BioHPP; Bredent GmbH, Senden, Germany) materials were prepared using the same processes. Nine pieces of each pathogen and a total of 72 (36 pure PEEK; 36 ceramic-reinforced PEEK) 8 x 8 x 4 mm rectangular samples were obtained from the manufacturer's prefabricated blocks with computer-aided design and computer-aided manufacturing and a precision cutting tool (Micracut 151; Metkon Instruments Inc. Bursa, Türkiye) at 400 rpm/min under water cooling. The samples were polished with 180,400,600,800,1200,1800 silicon carbide papers (ScanDia Hans P. Tempelmann) respectively under water spray at 25 N pressure in an automatic polishing device (Tegranim-20, Struers, Ballerup, Denmark) for 15 sec. All the samples were then rinsed in an ultrasonic machine (UT-206; Sharp, Osaka, Japan) with distilled water for 5 min, and the residue was cleaned from them. The samples were sterilized by autoclaving at 134 °C and 3 bar with a 60-minute program.

Surface Roughness

The surface roughness of the samples was determined with a profilometer contact surface measurement device (Sutronic S-series, Taylor/Hobson, Lester, England) using a standard diamond tip (tip angle: 90°, tip radius 2 μm) and a cut-off level of 0.25. A total of 5 measurements (3 vertical and 2 horizontal) were made from all samples. The mean roughness value (R_a) was calculated by averaging the values obtained from each sample.

Microbial Cultures

All the test microorganisms used in this study were the American Type Culture Collection (ATCC) strains including; *S. aureus* (ATCC 29213), *A. baummanii* (ATCC 19606), *E. faecalis* (ATCC 29212), and *C. albicans* (ATCC 10231).

The microorganisms were first inoculated into 5 mL nutrient broth and were incubated at 37 °C for 24 h. Microorganisms from these cultures were transferred onto a solid medium and incubated overnight. Tryptone Soy Agar (Oxoid, UK) was used for *S. aureus, E. faecalis,* and *A. baummanii*, and Sabouraud Dextrose Agar (Biolife, Italia) was used for *C. albicans*. After growth, selected colonies were transferred into a liquid medium and incubated for 4-6 h to achieve log phase growth. Tryptone Soy Broth (TSB) (Oxoid, UK) for bacterial strains and Sabouraud Dextrose Broth (BD, Difco, USA) for *C. albicans*, were the selected media for this purpose. The optical density of each culture at 490 nm (OD 490) was adjusted to 1-3x10^8 colony forming units (CFU)/mL.

Biofilm Formation Assay

Biofilm formation was measured with a modification of the method used by Peng et al. (23). One mL of a microbial suspension of *S. aureus, E. faecalis, A. baummanii,* and *C. albicans* (10^6 CFU/mL) was inoculated in all the wells of a 24-well plate. The testing samples (pure PEEK and ceramic-reinforced PEEK) were added to the wells and incubated for 72 hours at 37 °C. At the end of the incubation, the broth medium was removed and the wells were washed twice with phosphate-buffered saline (PBS) and air-dried for 1 h. After adding crystal violet (0.1% w/v) to each well, the plate was allowed to remain at room temperature for 15 min. After 15 minutes, the stain was aspirated and the plate was rinsed four times with PBS. In the last step, 1 mL of 33% acetic acid was added to the wells. Absorbance was determined at 490 nm wavelength (OD 490) on a microplate reader (Biotek, ELx800 Absorbance Microplate Reader, USA). TSB and SDB without microbial suspension were used as negative controls.

Statistical Analysis

Data were analyzed using IBM SPSS V21 software (SPSS IBM, Chicago, IL, USA). According to the Kolmogorov-Smirnov test, the data showed normal distribution (p>0.05). The comparison of the two groups was analyzed with the Independent Samples t-test. Intra-group analyses were performed with a two-way analysis of variance (ANOVA) followed by Post hoc Bonferroni adjustment. The level of statistical significance was determined as 0.05. The correlation between surface roughness and biofilm formations were analyzed with pearson correlations.

Results

The surface roughness was mean 0.56±0.88 R_a for pure PEEK, and 0.52±0.83 R_a for ceramic-reinforced PEEK, with no significant difference determined between the groups (p=0.054) (Table 1). There were no significant correlations between surface roughness of peek materials and biofilm formation (Juvora; r=-0.190, p=0.266, BioHPP r=-0.018, p=0.916) (Table 2, Figure 1). The results obtained from the microbial analysis of *S. aureus, E. faecalis, A. baummanii,* and *C. albicans* are shown in Table 2. No significant difference was found in terms of the biofilm formation of the PEEK groups for each pathogen (p>0.05). In each group evaluation, the highest biofilm formation was found in *S. aureus*, followed by *A. baummanii*, *E. faecalis,* and *C. albicans* in the pure PEEK group (p<0.001). The highest biofilm accumulation in ceramic-reinforced PEEK was recorded in *S. aureus* and *A. baummanii*. The biofilm formations of *E. faecalis* and *C. albicans* were significantly lower than those of *S. aureus* and *A. baummanii* (p<0.001) (Table 3).

Discussion

Microbial dental plaque is considered a primary etiological factor for the development of periodontal disease and should be controlled as the first step in periodontal treatment. The attachment of microorganisms to surfaces occurs through complex chemical and physical mechanisms. The surface and chemical properties such as roughness, hydrophilicity, nano topological structure, and modifications with antibacterial coatings of a prosthodontic or implant material can be effective in inhibiting biofilm covering (24,25). Therefore, it is important to know the properties of prosthodontic biomaterials that

Table 1. Comparison of PEEK materials according to surface roughness

Juvora (mean R_a ± SD)	BioHPP (mean R_a ± SD)	p-value
0.56±0.88	0.52±0.83	0.054

Independent samples t-test (Significant level <0.05). SD: Standard deviation, PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

Table 2. Correlation analyses between surface roughness of PEEK materials and biofilm formations

Juvora		Biofilm formation	Surface roughness
Biofilm formation	**Pearson correlation**	1	-0.190
	Sig. (2-tailed)		0.266
	n	36	36
Surface roughness	**Pearson correlation**	-0.190	1
	Sig. (2-tailed)	0.266	
	n	36	36
BioHPP		**Biofilm formation**	**Surface roughness**
Biofilm formation	**Pearson correlation**	1	-0.018
	Sig. (2-tailed)		0.916
	n	36	36
Surface roughness	**Pearson correlation**	-0.018	1
	Sig. (2-tailed)	0.916	
	n	36	36

Juvora; r=-0.190, p=0.266, BioHPP r=-0.018, p=0.916. PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

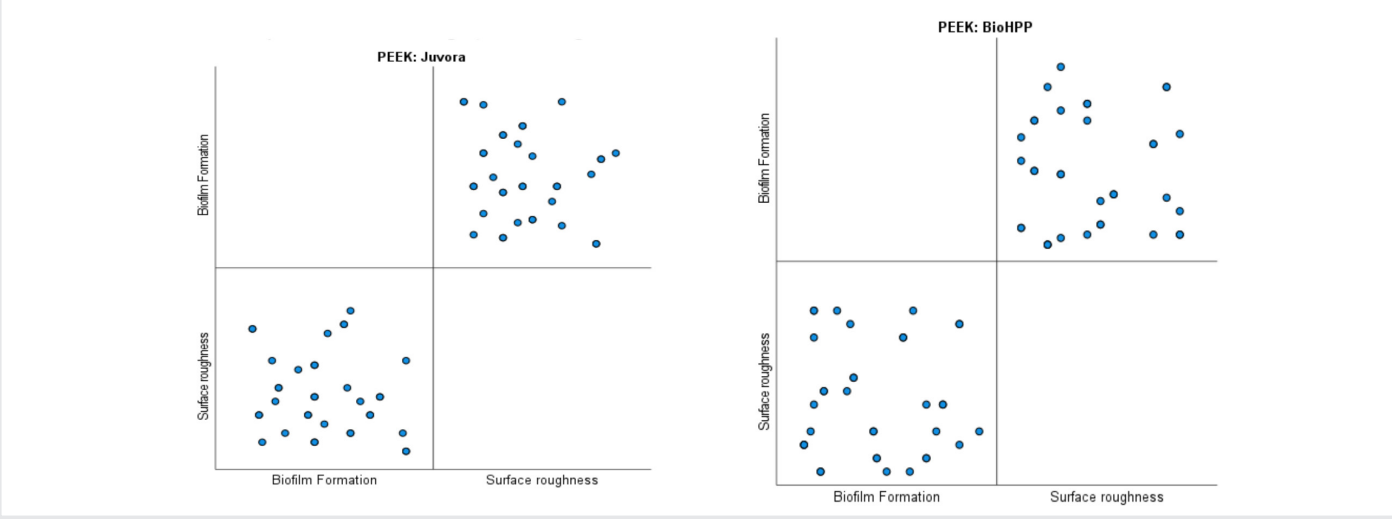

Figure 1. Scatterplot Matrix Graphics for correlations between surface roughness of PEEK materials and biofilm formation
PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer

Table 3. Comparison of PEEK materials according to biofilm formation

Pathogen	PEEK		P[a] values
	Juvora	BioHPP	
S. aureus	0.888±0.0062[aA]	0.776±0.0105[aA]	0.14
A. baumannii	0.736±0.0065[aB]	0.822±0.0117[aA]	0.071
E. faecalis	0.660±0.0045[aC]	0.576±0.0079[aB]	0.14
C. albicans	0.523±0.0037[aD]	0.480±0.0040[aB]	0.30
P[b] values	p<0.001	p<0.001	

[a]Independent samples t-test; bold p-values indicate statistical significance (p<0.05) values. SD: Standard deviation, PEEK: Polyetheretherketone, BioHPP: Bio high-performance polymer
[b]ANOVA test results (p<0.05).
The same lowercase letters (a, b) indicate that there is no significant difference in horizontal comparisons of independent peek groups (p<0.05)
The same capital letters (A, B, C, D) indicate that there is no significant difference in vertical comparisons based on Holm-Bonferonni adjustment for multiple testing (Bonferroni adjustment value α=0.05/6=0.008)).

enhance biofilm formation. A search of the literature revealed a few studies that analyzed biofilm formation on PEEK surfaces. This investigation aimed to evaluate the biofilm formation of different strains on pure PEEK and ceramic-reinforced BioHHP materials. The second aim of this study was to contribute to the literature by defining the properties of BioHPP. The hypothesis of the study was that there was a difference in biofilm formation between PEEK and ceramic-reinforced PEEK materials.

Previous studies have noted the importance of good mechanical properties of BioHPP such as good marginal quality, fracture resistance, retention, high polishing, low absorption properties, wear resistance, and aesthetics (26-29). Jin et al. (26) suggested that BioHPP, compared to titanium, could be used as an alternative material to be veneered with composite resin due to higher shear bond strength compared to composite resin. Porojan et al. (17) found that BioHPP was less affected by aging than pure PEEK due to the nanosurface topography and nanoroughness. BioHPP is reinforced with ceramic molecules; aluminum oxide, and zirconium oxide (18). Zirconia is known to attract low plaque accumulation (30,31). Therefore, assessing biofilm formation on ceramic-reinforced and pure PEEK materials would be of interest. In the current study, the differences in biofilm formation between pure PEEK and BioHPP were not significant. Wiessner et al. (32) conducted an *in vivo* study to examine the formation of biofilm on various materials, including titanium, zirconia, PEEK, and PEEK-BioHPP. They employed fluorescence microscopy and image analysis software to quantify the biofilm formation. Their findings indicated that zirconia exhibited the least biofilm formation, followed by titanium, PEEK, and PEEK-BioHPP. Their observations align with the outcomes of our study, as no difference in biofilm formation was observed between PEEK and modified PEEK materials.

Higher surface roughness can influence bacterial attachment, increasing the surface area, and causing unsmooth and uncleanable surfaces (9). A key strength of the present study was that no difference was determined in the surface roughness between the PEEK groups. In a recent study, Barkarmo et al. (22) found that while there were no significant differences in biofilm adhesion of streptococci strains between smooth PEEK and titanium surfaces, blasted PEEK material had significantly more biofilm formation. Hahnel et al. (33) reported in a laboratory study that biofilm formation of *Streptococcus gordonii*, *Streptococcus mutans, Actinomyces naeslundii*, and *C. albicans* on the surface of PEEK from zirconia and titanium abutment materials was equal or less. In addition, the surface roughness of PEEK surfaces was also found to be lower than that of zirconium and titanium abutment surfaces. In addition, one study found biofilm formation on ceramic-reinforced PEEK, BioHPP. Similarly, in this study, the adhesion of *S. aureus* and *C. albicans* on dental polymers was mainly affected by surface roughness (34). These results might be explained by the fact that the surface roughnesses were different between blast, rough, polished, and smooth surfaces and increased surface roughness had an impact on the biofilm adhesion.

In the current study, tests were made of four different biofilm-producing strains of microorganisms, namely S. *aureus, E. faecalis, A. baummanii,* and *C. albicans,* on pure PEEK and ceramic-reinforced PEEK material obtained by adding 20% ceramic (aluminum oxide and zirconium oxide). *S. aureus* is an important pathogen of implant-associated osteomyelitis infections (35,36). *Candida spp*. have been associated with denture stomatitis (37). *E. faecalis* acts as an important agent in oral infections and *A. baummanii* is a multidrug-resistant bacteria of clinical importance (38,39). The present study showed considerably high *S. aureus* accumulation and biofilm formation on both biomaterials when compared with the other pathogens. In contrast to *S. aureus, E. faecalis* and *C. albicans* strains formed fewer accumulations. Although not statistically significant, *A. baummanii* formed more biofilm on the ceramic-reinforced BioHHP group than on pure PEEK surfaces. These results led to a review of the previous studies that investigated the antimicrobial properties and biofilm-forming characteristics of aluminum oxide and zirconium oxide, which were added to PEEK material.

Compared to the nanoparticles of oxides of metals such as silver, iron, zinc, and copper, there have been few investigations of the antimicrobial efficacy of aluminum oxide nanoparticles. According to the results of a recent review, studies about the inhibitory effect of aluminum oxide nanoparticles have reported that aluminum oxide nanoparticles cause a decrease in the growth rate of *S. aureus* and *C. albicans*, as well as show an inhibitory effect at moderate concentrations and a bactericidal effect at high concentrations for *A. baumanii.* Compared to other metal oxide nanoparticles, aluminum oxide nanoparticles have the lowest microbial inhibitory effect (40).

Zirconia has also been used introduced to the field of dentistry due to mechanical stability and it can be used as pure zirconia or alumina-toughened zirconia (41). To date, limited scientific data regarding bacterial accumulation around zirconia dental implants and abutments are available and the results are conflicting. Studies investigating zirconia implants have shown less oral biofilm in vivo and conversely, more biofilm *in vitro* when compared with titanium implants. As these data can not allow a clear preference for the use of zirconia, more studies have been carried out to provide further information (42). Zeller et al. (43) investigated biofilm formation on discs of metal alloys, zirconia, and PEEK in vivo and detected higher biofilm mass formation, and the zirconia and PEEK levels were similar. Roehling et al. (44) compared biofilm formation on zirconia and titanium implant surfaces and detected a statistically significant reduction in biofilm formation. Abualsaud et al. (45) evaluated the antimicrobial effects of zirconium dioxide nanoparticles reinforcement of poly(methyl) methacrylate on surface roughness and *C. albicans* biofilm and an insignificant reduction of *C. albicans* biofilm formation was observed.

Study Limitations

The results of the current study, indicating no difference between the two tested biomaterials by means of microbial

attachment, can be interpreted through comparisons with the reports of some former studies concerning low antimicrobial efficiencies and the biofilm formation inhibitory effects of zirconia and aluminum oxide. However, as zirconia and aluminum oxides are mixed with PEEK to obtain ceramic-reinforced BioHHP material, interpretation is not straightforward. These conflicting results can be associated with the nature of the machined or modified PEEK surfaces. Moreover, biofilm involvement can be affected by many clinical factors such as the plaque removal efficiency of the patient the location of restorations, and improper finishing/polishing of restorations. Therefore, conducting our study under *in vitro* conditions is one of the limitations of our study. The certain limitation of this study was the surfaces of PEEK were standardized with silicon carbide papers and the effect of different surface modifications on biofilm formation on the surface of the PEEK materials was not evaluated. The effect of different surface modifications on biofilm formation on PEEK surfaces should be evaluated in further investigations. Another limitation was that the study evaluated only two specific PEEK materials, whereas there were various PEEK materials in the dentistry field. This study was a preliminary study to define the biological characteristics of ceramic-reinforced PEEK. Biofilm retention of PEEK materials could be evaluated with further clinical studies, and comparative evaluation of the effects of various PEEK materials on biofilm formation would be effective in obtaining comprehensive findings.

Conclusion

The study results showed no difference in terms of biofilm formation between pure and ceramic-reinforced PEEK materials. It can be suggested that the association of biofilm formation on modified PEEK materials be investigated in future clinical studies.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Surgical and Medical Practices: S.K.Y., K.A., B.K., S.S., A.D.K., Concept: S.K.Y., K.A., B.K., S.S., A.D.K., Design: S.K.Y., K.A., S.S., Data Collection or Processing: S.K.Y., K.A., S.S., Analysis or Interpretation: S.K.Y., K.A., K.D., Literature Search: S.K.Y., K.A., A.D.K., K.D., Writing: S.K.Y., K.A., A.D.K., K.D.

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Original Article

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Forensic Geriatric Trauma Cases

Adli Geriatrik Travma Olguları

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ABSTRACT

Objective: This study aimed to evaluate the general characteristics of geriatric cases aged ≥65 years, who presented at the Forensic Medicine Clinic due to trauma, and the severity of injury using trauma scoring systems.

Methods: The study included all trauma cases over 65 years with a forensic report prepared in the Forensic Medicine Clinic between 2015 and 2021. Evaluations were made using the chi-square, Mann-Whitney U, and Kruskal-Wallis (post-hoc: Dunn-Bonferroni) tests. A value of $p<0.05$ was considered statistically significant.

Results: Two-thirds of the cases were male (67.6%) and mean age was 73.23±6.25 years. More than half of the cases were exposed to battery-related trauma (54%). There was an injury in more than one region in 37.4% (n=52) of the cases. The median injury severity score (ISS) of the cases was 2 (1.5) and the median new-injury severity score (NISS) was 3 (1.8). The scores of ISS and NISS in cases aged >75 years were higher than in those aged ≤75 years ($p<0.05$). The score severity of ISS and NISS of the cases injured in traffic accidents and other accidents was higher than in those injured due to battery ($p<0.001$). Almost half (48.1%) of the traffic accident-related cases were pedestrians, and it was seen that pedestrians suffered more severe trauma.

Conclusion: Increasing the necessary safety measures in traffic, especially pedestrian safety, and taking measures to make daily life easier for the elderly may help protect this vulnerable population from the effects of severe trauma.

Keywords: Elderly, trauma, forensic medicine, traffic accident

ÖZ

Amaç: Bu çalışmada, Adli Tıp Kliniği'ne travma nedeniyle başvuran ≥65 yaş üstü geriatrik olguların genel karakteristik özellikleri ve travma skorlama sistemleri kullanılarak yaralanma şiddetlerinin değerlendirilmesi amaçlanmıştır.

Yöntemler: 2015-2021 yılları arasında adli tıp kliniğinde travma nedeniyle adli rapor düzenlenen 65 yaş üstü tüm olgular çalışmaya dahil edildi. Gruplar ki-kare testi, Mann-Whitney U testi ve Kruskal-Wallis testi (post-hoc: Dunn-Bonferroni testi) ile karşılaştırıldı. P değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: Olguların üçte ikisi erkekti (%67,6). Yaş ortalaması 73,23±6,25'ti. Olguların yarısından fazlası darp cebir nedeniyle (%54) travmaya maruz kalmıştı. Olguların %37,4'ünde (n=52) birden fazla bölgede yaralanma vardı. Olguların median yaralanma ciddiyeti skoru (ISS) 2 (1,5) ve median yeni-yaralanma ciddiyeti skoru (NISS) 3 (1,8) idi. Yetmiş beş yaş üzerindeki olgularda ISS ve NISS puanı, 75 yaş ve altı olgulara göre yüksekti ($p<0,05$). Trafik kazasına bağlı yaralanan ve diğer kazalar sonucu yaralanan olguların ISS ve NISS puanı darp sonucu yaralananlara göre daha yüksekti ($p<0,001$). Trafik kazası geçiren olguların yaklaşık yarısı (%48,1) yayaydı. Yayalar daha şiddetli travmaya maruz kalmıştı.

Sonuç: Özellikle yaya güvenliği başta olmak üzere trafikte gerekli güvenlik önlemlerinin artırılması, günlük yaşamı yaşlılar için kolaylaştıracak önlemlerin alınması ile yaşlı bireylerin şiddetli travmanın etkilerinden korunmasına yardımcı olabilir.

Anahtar Sözcükler: Yaşlı, travma, adli tıp, trafik kazası

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Introduction

As people get older, body resistance decreases, and thus less energy is required to cause serious injury (1). Although the elderly have a lower risk of trauma than the younger population as they spend less time outside, the morbidity and mortality rates following trauma are higher than for the younger (2). In a study by Burstow et al., (3) mortality resulting from both minor and major traumas was more than two-fold higher in cases aged ≥65 years compared to those aged <65 years. Trauma in the elderly causes longer hospital stays, higher hospital costs, long and grueling rehabilitation, and a higher risk of complications (4). The Turkish Statistics Institute has estimated that the elderly population, which was 8.6 million (10.2%) in 2023, will increase to 19.5 million (20.8%) in 2050 and 24.7 (27.7%) million in 2075 (5). Therefore, it is inevitable that elderly trauma cases will increase in parallel with the increasing proportion of the elderly in the population every year.

This study aimed to evaluate the general characteristics of geriatric cases aged ≥65 years, who presented at the Forensic Medicine Clinic due to trauma, and the severity of injury using trauma scoring systems.

Methods

This retrospective cohort study included a total of 139 cases for whom a forensic report was requested as a result of trauma between January 01, 2015 and December 31, 2021. Cases without traumatic injury or with incomplete data were excluded from the study. All information was collected retrospectively from the hospital automation system, forensic records, and patient files of the cases included in the study. Although the study was desinged as a retrospective study and, thus, it was out of the scope of the informed consent doctrine, all procedures in the study were performed after obtaining ethical and scientific approval of Bolu Abant İzzet Baysal University Clinical Researches Committee Approval dated 26.04.2022, no: 2022/104, and compatible with the 1964 Helsinki Declaration including its later amendments.

The cases included in the study were evaluated in terms of the following parameters: "age, gender, injuries, injury site, location of traffic accident victims, safety belt, helmet and protective equipment, degree of forensic injury, and trauma scores [injury severity score (ISS) - new-injury severity score (NISS)]". The ISS and NISS were calculated using the abbreviated injury scale (AIS) 2008 update.

Trauma scoring systems are handy tools in assessing the severity of injury (6). It is an anatomical-based coding system. It was first created by the AIS Association for the Advancement of Automotive Medicine in 1976 to classify the severity of injury, classifying each damage on a six-point scale according to body region. The ISS is basically a trauma system in which AIS is used and 6 body regions (head or neck, face, chest, abdomen and pelvis contents, extremity and pelvic girdle, external and other trauma) are evaluated. The ISS is calculated by adding the square of the AIS score of the body's three most severely injured areas. [ISS= (AIS body resgion 1)^2 + (AIS body resgion 2)^2 + (AIS body resgion 3)^2] (7). NISS is the sum of the squares of the three most severe injuries, regardless of the injured body area. Therefore NISS can be equal to or higher than ISS (8). ISS and NISS are very helpful trauma scoring systems in showing trauma severity and predicting mortality (6).

Statistical Analysis

Statistical Package For Social Science (SPSS), version 21.0 (IBM Corpn., SPSS Statistics for Window, Armonk, NY, USA) statistics program was used for data analysis of the study. The conformity of variables to normal distribution was investigated using visual (histograms plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk test). Descriptive statistics were presented as mean, standard deviation, or median (interquartile range values for quantitative data, and as number (n) and percentage (%) for categorical variables.

Categorical variables were compared with the chi-square test. Non-parametric tests were conducted to compare data with non-normal distribution. Paired groups were evaluated with the Mann-Whitney U test, and more than two groups with the Kruskall-Wallis Test (post-hoc: Dunn-Bonferroni test). A value of $p<0.05$ was considered to show a statistically significant result.

Results

The 139 cases included in the study comprised 94 (67.6%) males and 45 (32.4%) females with a mean age of 73.23±6.25 years, of the majority (n=99, 71.2%) of cases in the 65-75 year age group. More than half of the cases were exposed to battery-related trauma (54%) (Table 1). Injuries were observed in more

Table 1. Characteristics of geriatric cases

		n	%
Age (years)	65-75 years	99	71.20
	>75 years	40	28.80
Forensic event	Battery	75	54.00
	Traffic accidents	52	37.40
	Other accidents*	12	8.60
Injury site	Head-neck	36	25.90
	Extremity	32	23.00
	Chest-abdomen	19	13.70
	Multiple	52	37.40
Accident cause	Falling	7	58.34
	Dog attack	2	16.67
	Sharp object injury	1	8.33
	Gunshot injury	1	8.33
	Injection neuropathy	1	8.33
Degree of forensic injuries	Cured by simple medical intervention	61	43.90
	Not cured by simple medical intervention	55	39.60
	Life-threatening	23	16.50

*Fall from height, work accident…etc.

than one body region in 37.4% (n=52) of the cases (Table 1). Soft tissue lesions were detected in 66 (47.5%), a bone fracture in 61 (43.9%), pulmonary contusion in 10 (7.2%), cerebral hemorrhage (subarachnoidal, subdural) in 10 (7.2%), a tendon-muscle laceration in 5 (3.6%), cerebral contusion in 4 (2.9%), liver laceration in 2 (1.4%), renal laceration in 2 (1.4%), nerve laceration in 1 (0.7%), larynx laceration in 1 (0.7%), and finger amputation in 1 (0.7%) of the cases.

The median ISS of the cases was 2 (1.5) and the median NISS was 3 (1.8). There was no significant difference in ISS and NISS severity between the genders (p>0.05) (Table 2). The severity score of ISS and NISS in cases aged >75 years was significantly higher than in those aged ≤75 years (p<0.05) (Table 2). The ISS and NISS severity scores of the cases injured due to traffic accidents and other accidents were statistically significantly higher than those injured due to battery (Kruskal-Wallis: p<0.001, post-hoc: p<0.001, p<0.05, respectively) (Table 2). The severity of ISS and NISS increased with the degree of forensic injury (p<0.001) (Table 2).

Of the cases involved in traffic accidents (n=52), 25 (48.1%) were pedestrians, 15 (28.8%) were drivers, and 12 (23.1%) were passengers. The seat belt was worn in 74.1% of cases (n=27). The ISS and NISS severity score was statistically significantly higher in out-of-vehicle traffic accidents (pedestrians) than in-vehicle traffic accidents (p<0.01) (Table 2).

Discussion

Gender

The vast majority (77.63%) of the elderly who experienced trauma in Japan between 2004 and 2017 were male (2). Javali et al. (8) reported similar results (male, 74%). In contrast, Gioffrè-Florio et al. (9) reported that more than two-thirds of 4554 trauma survivors were female. Most previous studies in Türkiye stated that males were exposed to trauma more than females in the elderly population (10-15). Consistent with the literature data, more than two-thirds of the cases in the current study were male. This situation may be due to the fact that male individuals in our society have a more social lifestyle and spend more time in traffic and outside.

Table 2. Distribution of ISS and NISS according to gender, age group, injury site, traffic accidents, degree of forensic injuries

		ISS			p-value
		Median	25th per	75th per	
Gender	Male	2.00	1.00	6.75	0.909[1]
	Female	2.00	1.00	4.50	
Age group	65-75 years	2.00	1.00	5.00	0.019[1]
	>75 years	4.00	2.00	9.00	
Traumatic forensic event	Battery	2.00	1.00	4.00	<0.001[2]
	Traffic accidents	5.00	1.00	13.75	
	Other accidents	4.00	4.00	5.00	
Traffic accidents	In-vehicle	1.00	1.00	9.00	<0.01[1]
	Off-vehicle (pedestrian)	9.00	4.00	17.50	
Degree of forensic injuries	Cured by simple medical intervention	1.00	1.00	2.00	<0.001[2]
	Not cured by simple medical intervention	4.00	2.00	5.00	
	Life-threatening	18.00	13.00	22.00	
Median		**NISS**			**p-value**
		25th per	75th per		
Gender	Male	3.00	1.75	8.25	0.886[1]
	Female	3.00	1.00	7.50	
Age group	65-75 years	3.00	1.00	6.00	0.032[1]
	>75 years	4.00	3.00	10.50	
Traumatic forensic event	Battery	3.00	1.0 0	4.00	<0.001[2]
	Traffic accidents	5.50	2.25	21.00	
	Other accidents	4.00	4.00	6.00	
Traffic accidents	In-vehicle	3.00	1.00	9.00	<0.01[2]
	Off-vehicle (pedestrian)	12.00	4.00	24.50	
Degree of forensic injuries	Cured by simple medical intervention	2.00	1.00	3.00	<0.001[2]
	Not cured by simple medical intervention	4.00	3.00	6.00	
	Life-threatening	22.00	14.00	27.00	

[1]Mann-Whitney U test, [2]Kruskal-Wallis test, ISS: Injury severity score, NISS: New-injury severity score

Age

In a study by Yousefzadeh-Chabok et al., (4) the mean age of the cases was 71.55 years. In a few studies conducted in Türkiye, the average age of geriatric trauma victims was reported to be between 71.9 and 77.16 years (11-14,16), with the majority (60.4-70.3%) in the 65-75 years age group (13,16,17). In the current study, the mean age of the cases was 73.23±6.25 years, and the majority (n=99, 71.2%) were in the 65-75 years age group. The reason for this may be related to the fact that individuals >75 years are less exposed to trauma as they are less involved in social life with advancing age.

Cause of Trauma

Falls (71.49%) and traffic accidents (31.40%) have been reported to be the most common causes of injury in the elderly Japanese population (2). In a study from Türkiye, Söz and Karakaya (16) reported that elderly patients admitted to the emergency department with trauma injuries were most frequently injured due to falls (86%) and traffic accidents (7.3%). Yildiz et al. (11) stated that almost two-thirds of elderly trauma patients presented at the emergency department due to falls. In another study of 224 elderly patients admitted to the emergency department with trauma, the most common causes of trauma were reported to be traffic accidents (46.4%) and assault (43.7%) (10). In another study of 101 elderly forensic cases, the majority of the cases (82.1%) were exposed to trauma due to traffic accidents (13). However, in a study conducted in the Department of Forensic Medicine, more than half (51.6%) of elderly patients were injured as a result of physical trauma (14). Similarly, Kaya et al. (15) reported that 57% of the elder cases for which a forensic report was prepared, occurred due to assault. In this study, more than half of the cases in this study were exposed to trauma due to battery (54%). The reason for this difference between the studies from the emergency department and the forensic medicine departments might be that battered elderly individuals were not usually admitted to the hospital for superficial injuries such as soft tissue lesions, but they were sent to us for the preparation of a forensic report after complaining to the law-enforcement officers.

Injury Site

In a study conducted in an emergency room in Iran, the most common site of injury in the elderly population was the lower and upper extremities (93.5%) (4). A study in Japan reported the most common injury site to be the lower extremities (46.40%) and the head (36.91%) (2). In the study of Gioffrè-Florio et al., (9) 4554 elderly trauma victims were most frequently injured in the head region (31.4%). Söz and Karakaya (16) reported that the most common injury sites in elderly trauma patients were the extremities (58%) and head and neck (26.2%). Yildiz et al. (11) reported extremity injuries at a higher rate (56.3%), and Güler et al. (13) reported the most common injuries in the lower and upper extremities (45.5%). Kaya et al. (15) reported that the head and neck (37.2%) were the most frequently injured areas in geriatric cases for which a forensic report was prepared. In the current study, 32.7% of the cases (n=52) were injured in more than one region, and the most frequent isolated injury site was the head and neck. This could be because more than half of the cases were injured due to battery.

Injury Type

A previous study stated that soft tissue lesions (40.4%) were detected most often in elderly trauma patients who presented at an emergency department (16). In a study by Kandiş et al., (10) elderly patients presented at the emergency department most frequently with soft tissue lesions (49.1%). Şener and Baydemir Kılınç (14) reported that more than half (53.1%) of forensic geriatric cases had soft tissue lesions. In the current study, a soft tissue lesion was detected in almost half (47.5%) of the cases.

ISS/NISS

Male patients >65 years are more likely to be seriously injured and generally have a higher median ISS (3). Burstow et al. (3) calculated a median ISS score of 4 in 22,454 cases ≥65 years. In a study by Cevik et al., (18) the median ISS of elderly traffic accident victims was reported to be 4. In the current study, the median ISS of the cases was 2 (1.5) and the median NISS was 3 (1.8).

Miyoshi et al. (2) reported median ISS of 13 in elderly patients in the 65-79 years age group and the median ISS was 9 in individuals aged ≥80 years in a series of 131,088 geriatric trauma cases. In the current study, the ISS and NISS scores of the cases over 75 years old were higher than those of the cases aged ≤75 years ($p<0.05$). This showed that although individuals aged ≥75 years were exposed to less trauma, their trauma-related injuries were more severe. This may be due to the older age group being more vulnerable to trauma.

The mean ISS score of 371 elderly trauma survivors presenting at an emergency department in Bursa, Türkiye, was 9.3, and traffic accidents were significantly more fatal in the traumatized elderly population (12). Burstow et al. (3) reported that traffic accidents (motor vehicle accidents, pedestrian accidents, motorcycle accidents) had a significantly higher median ISS than other types of injury. In a study in Japan, traffic accidents and burns were the causes of the highest death rate among traumatized elderly patients (2). Şener and Baydemir Kılınç (14) demonstrated that injuries resulting from accidents (traffic accident, fall from height, work accident, etc.) were associated with higher ISS scores. The result of the current study showed that the ISS and NISS severity scores of the cases injured due to traffic accidents and other accidents (fall from height, work accident…etc.) were higher than those injured due to battery (Kruskal-Wallis: $p<0.001$, post-hoc: $p<0.001$, $p<0.05$, respectively). Remarkably, the elderly population is exposed to more severe trauma due to traffic accidents and other accidents, which can be prevented by measures to be taken. Therefore, it is necessary to increase and develop precautions and security measures for this population to prevent these accidents.

In a previous study, ISS and NISS were recommended as the best trauma-scoring systems that could be used to detect life-threatening injuries (19). The current study determined

that the degree of forensic injury and the severity of ISS and NISS increased in parallel (p<0.001). Therefore, in the elderly population, this can be an useful method to determine the life-threatening and simple medical and intervention concepts specified in Turkish Penal Code.

Traffic Accidents

Pedestrians and very old adults (≥75 years) have a higher death rate in traffic accidents (20). In a study by Etehad et al., (21) 40.5% of 1306 elderly patients injured as a result of traffic accidents were pedestrians, 22.1% were passengers, 4.6% were drivers, 7.7% were cyclists, and 19.1% were motorcyclists. In the current study, 25 (48.1%) of the cases (n=52) were pedestrians, 15 (28.8%) were drivers, and 12 (23.1%) were passengers.

Generally, elderly pedestrians are exposed to more severe trauma due to traffic accidents, and most deaths are seen in pedestrians (22). In the current study, the ISS and NISS severity scores were higher in out-of-vehicle traffic accidents (pedestrians) than in in-vehicle traffic accidents (p<0.01).

In a study conducted in Athens, 73.7% of drivers and 85.9% of passengers injured in traffic accidents were not wearing a seatbelt at the time of the accident (23). Similarly, in a study conducted in an emergency room, 93.1% of the cases injured in traffic accidents were not wearing a seatbelt (24). However, in the current study, 74.1% of the cases injured in in-vehicle traffic accidents were wearing a seatbelt, which was a higher rate than in the literature. This may be related older individuals avoiding high risk actions and protecting themselves more.

Study Limitations

This study had some limitations, primarily the retrospective design. Moreover, it did not represent the entire elderly trauma population, as it was conducted in a single clinic and only included forensic cases. There were also elderly trauma cases that were not recorded as forensic cases.

Conclusion

The result of this study showed that forensic geriatric cases were frequently injured due to battery. Severe trauma in the geriatric age group was seen to be the result of traffic accidents and other accidents, especially involving pedestrians. Cases >75 years were exposed to less but more severe trauma. Injuries to elderly individuals can be prevented by simple and small measures such as making arrangements to facilitate road-crossing in traffic, drivers being more attentive to elderly individuals, not leaving elderly individuals with insufficient mental-motor functions alone, taking measures to make daily life easier for the elderly and increasing the penalties for assault crimes committed against the elderly who are incapable of defending themselves compared to a young adult.

Ethics

Ethics Committee Approval: This study was approved by Bolu Abant İzzet Baysal University Clinical Research Committee with the approval dated 26.04.2022 and numbered 2022/104.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: E.H., Z.Z.E., Concept: E.H., Z.Z.E., Design: E.H., Z.Z.E., Data Collection or Processing: E.H., Z.Z.E., Analysis or Interpretation: E.H., Z.Z.E., Literature Search: E.H., Z.Z.E., Writing: E.H., Z.Z.E.

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Original Article

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Retrospective Investigation of The Relationship Between Coronal Restoration and Quality of Root Canal Fillings and Apical Periodontitis in a Specific Turkish Population

Belirli Bir Türk Popülasyonunda Koronal Restorasyon ve Kök Kanal Dolgularının Kalitesi ile Apikal Periodontitis İlişkisinin Retrospektif Olarak İncelenmesi

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ABSTRACT

Objective: The purpose of this study was to retrospectively assess the prevalence and efficacy of endodontic treatments in Turkish subpopulations, as well as the relationship between the type of coronal restorations, procedural errors, and their association with the periapical status of treated teeth.

Methods: Cone beam computed tomography (CBCT) images of 500 patients were analyzed. A total of 10500 teeth were evaluated and 1185 of those had received root canal therapy. Periapical condition, coronal restoration, and root canal quality of these teeth were evaluated. Periapical status was evaluated by two observers regarding to CBCT periapical index scoring system. The chi-square test was used to evaluate all data for statistical analysis.

Results: Healthy periapical status was seen in 44.9% of endodontically treated teeth. Teeth with procedural errors (broken instrument, untreated canal, perforation, ledge formation, short or overfilled canals, inadequate filling) showed more periapical pathosis than teeth with good endodontic treatment ($p<0.05$). Additionally, it was found that teeth with sufficient coronal restoration had lower prevalence of apical periodontitis. However, existence of post did not effect periapical status ($p<0.05$).

Conclusion: The effectiveness of the root canal treatment and the periapical status were considerably influenced by the quality of coronal restoration.

Keywords: Apical periodontitis, cone beam computed tomography, coronal restoration, epidemiology, root canal treatment

ÖZ

Amaç: Bu çalışmanın amacı, belirli bir Türk popülasyonunda yapılmış endodontik tedavilerin kalitesinin yanı sıra, yapılmış işlemsel hatalar ve dişlerin periapikal durumu ile koronal restorasyonların başarısının retrospektif olarak değerlendirilmesidir.

Yöntemler: Bu çalışma için, 500 hastanın konik ışınlı bilgisayarlı tomografi (KIBT) görüntüleri incelenmiştir. Bu hastalara ait 10500 diş radyografik görüntü incelenmiş ve toplam 1185 dişte kanal tedavisi yapılmış olduğu görülmüştür. Kök kanal tedavilerinin ve koronal restorasyonların kalitesi ile bu dişlerin periapikal durumu tek tek incelenmiştir. Periapikal durum KIBT uyarlanmış peiapikal indeks skorlama sistemine göre iki gözlemci tarafından değerlendirilmiş ve elde edilen tüm veriler, istatistiksel olarak ki-kare testi kullanılarak analiz edilmiştir.

Bulgular: İncelenen dişlerin %44,9'unda periapikal bölgede lezyon olmadığı görülmüştür. İşlemsel hata (kırık alet, bulunamamış kanal varlığı, perforasyon oluşumu, basamak oluşumu, kısa veya taşkın kanal dolumu) yapılmış dişlerde, daha yüksek oranda periapikal lezyon varlığı tespit edilmiştir ($p<0,05$). Ayrıca, yeterli koronal restorasyona sahip dişlerde apikal periodontitis insidansının daha düşük olduğu bulunmuştur ($p<0,05$). Bunun yanı sıra post varlığının periapikal durumu etkilemediği görülmüştür.

Sonuç: Koronal restorasyonun kalitesinin, kök kanal tedavisinin başarısını ve dişin periapikal durumunu önemli ölçüde etkilediği görülmüştür.

Anahtar Sözcükler: Apikal periodontitis, konik ışınlı bilgisayarlı tomografi, koronal restorasyon, epidemiyoloji, kök kanal tedavisi

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Introduction

Inflammation of the periapical periodontium results in a condition known as AP, and its toxins have an impact on the root canal system (1). Apical periodontitis (AP) in root canal of treated teeth is an important public health problem (2).

Epidemiologic studies are usefull to give information about incidence and prevalence of disease and related factors. Since root canal treatment (RCT) is the most frequent treatment done by endodontist, the prevalance and quality of the treatment inform dentist not only the incidence but also risk factors and reasons for failure of endodontic treatments.

Numerious epidemiological studies investigated the prevalance of the AP in all over the world. Tibúrcio-Machado et al. (3) found high prevalence (52%) of AP in their systemic review. However, Al-Omari et al. (4) (83.7%) and Marotta et al. (5) (87%) found much higher prevalance of AP. The rate of prevalence varies according to age (6), level of education, access to dental care (7) and radiographic techniques used during diagnosis (8).

RCT is indicated in patients with irreversible pulpitis and/or AP to keep the tooth functional in the patient's mouth in long term (9). Since high percentage of AP prevalence has been detected in studies (3), prevalence of RCT is expected to be high. However, the frequency of prevalence RCT was found in a very wide range of 0.7-97.3 % in different countries (10).

The difference could be because of needs and availabilty of RCT depending on the countries. Also, preventive dentistry management, using new technology or equipment and materials, or treatment procedure of deep cavity or pulp exposure may affect the results. Meantime, León-López et al. (10) indicated that global prevalence of people with at least one RCT was % 55.7 in their systemic review.

Conventional radiographs are the most common used method for detecting AP and quality of root canal filling. While radiographs show 2 dimensional images, recently introduced cone beam tomography, shows 3 dimensional images. So cone beam computed tomography (CBCT) images can be used for diagnosis of AP, detecting root canal morphology, quality of the fillings, procedural errors and healing outcomes.

Limited studies have been reported on the prevalence of AP and quality of RCT in selected population of Turkish people mostly done by detecting conventional radiographs (11-14). Also, recently introduced new technology in endodontics might improve the outcome of endodontic therapy.

The aim of the present study was to examine quality of endodontic treatments with coronal restorations and analyse the relationship of various factors with AP in a Turkish subpopulation based on radiographic examination. The null hypothesis to be tested was that there was a difference in the prevalence of periapical lesions in each specific root with regard to procedural errors.

Methods

Marmara University Clinical Research Ethical Committe was approved this study by no: 2021/07 and dated 07.10.2021.

In the present study, all CBCT images which were taken from patients refferred to University of Marmara Department of Radiology for different reasons between 2017 and 2021 were investigated. A total of 14623 teeth were examined; 500 patients with 1185 teeth who had undergone RCT were included in the study.

The CBCT images were included according to the following criteria; patients between 18-70 years old with full arch scans and also had at least one endodontically treated teeth (except 3rd molar) and signed informed consent form. Additionally, deciduous teeth and unrestorable root fragments were excluded. Images that could not be examined due to artifacts were excluded.

Evaluation Criteria and Radiographic Analysis

The CBCT images were taken using Planmeca ProMax (Helsinki, Finland, 96 kVp, 10 mA) device in accordance with the manufacturer's instructions and with a voltage of 96 kVp, a tube current of 10 mA. Voxel size for the scans was 0.200, 0.200, and 0.200 mm, and 15 bits. Images were evaluated by two experienced endodontists. Images were examined with a Dell brand monitor (China). The monitor with a screen size of 22 inches had a resolution of 1920*1080.

Teeth were evaluated with the multiplanar reconstruction view of the software Planmeca Romexis viewer (Helsinki, Finland).

All images were evaluated 40-50 cm away from the image and using the same monitor, in a reduced light room. Observers were able to adjust the contrast, density and magnification, settings as they wished. Reviewers assessed all images to get a consensus for the interpretation of the radiographic data. Both examiners reached consensus on each image examination.

The CBCT- periapical index (PAI) scores, created by Estrela et al. (15), were used to determine the score based on the location, size of the lesion and relationship with the roots of the tooth (Table 1).

Total number of RCT, root canal obturation quality, type and coronal restoration quality, periapical status, and total number of teeth were recorded. All spatial planes (sagittal, axial, coronal and cross-sectional) were used to evaluate each tooth.

Table 1. Cone beam computed tomography periapical index scores

Score	Quantitative Bone Alterations in Mineral Structures
0	Intact periapical bone structures
1	Diameter of periapical radiolucency > 0.5-1 mm
2	Diameter of periapical radiolucency > 1-2 mm
3	Diameter of periapical radiolucency > 2-4 mm
4	Diameter of periapical radiolucency > 4-8 mm
5	Diameter of periapical radiolucency > 8 mm
Score (n) + E* Expansion of periapical cortical bone Score (n) + D* Destruction of periapical cortical bone	

Assessment of Endodontic Tretament

When all root canals were adequately filled, with homogenous appearance without voids of the canal fillings and obturation lenght from coronal the root apex was regarded acceptable endodontic therapy (16).

When the root canal fillings extruded over the radiographic apex or shorter more than 2 millimeters of apex, the treatment was regarded unacceptable. Root canal filling with voids, insufficient density, empty canals or poor condensation, broken instrument, ledge formation and perforation also considered as unacceptable.

Apical filling extension was classified into 3 groups according to the lenght of the filling: 0-2 mm short, >2 mm short, and beyond the radiographic apex. These parameters were evaluated for each single root canal.

Assessment of Periapical Status

Each single root was evaluated according to Table 1 for periapical status.

Assessment of Coronal Restoration

Also all endodontically treated teeth were evaluated according to the type of the coronal restoration (direct or indirect). The quality of restoration was classified as insufficient if it was partially or completely missing with open edges, overhangs, or secondary cavities. Meantime, presence of post was recorded.

Statistical Analysis

The data were analysed with IBM SPSS (Statistical Package for Social Sciences) 23 software. Calculated values for frequency and percentages were used to create descriptive statistics for categorical variables. The chi-squared test was used to compare the qualitative data. P values under 0.05 were considered significant.

Result

Six hundred eighty nine (58,14%) teeth were from females and 496 (41,86%) from males. The distribution of teeth with endodontic treatment and its relation to sex and age is represented in Table 2. The mean age of patients with periapical lesions and teeth without lesions were found to be significantly different. The mean age of patients with periapical lesions was lower than without lesions (Table 2).

The CBCT-PAI was used to examine the apical status of endodontically treated teeth; scores of 0 indicated no apical lesions, whereas scores of 1-5 indicated apical lesions. Among these teeth, the prevalance of AP was 55.1% (653 teeth) (Table 2). According to sex, woman (52.8%) was found to have lower prevalance of AP than men (58.3%), but the difference was not significant (p>0.05) (Table 2). The periapical lesions was most found between the ages of 18 and 24 years (Table 2).

Maxillary premolars were the most frequently treated teeth (19.9%), followed by mandibular molars (17.9%), maxillary molars (17.3%), and mandibular premolars (16.9%). Mandibular incisors were found to be the least frequently endodontically treated teeth (Table 3). The distribution of RCT according to the type of tooth is detailed in Table 3.

Table 4 presents a summary of all data displayed. Of 1185 endodontically treated teeth, no procedural error was found in 324 teeth (27%) (Table 5). The prevalence AP was found to be 39% (128 teeth) in teeth without procedural errors (Table 4). Of them 73% (861 teeth) had at least one procedural errors (Table 5). The prevalence of AP was found to be 61% in teeth with procedural errors (Table 4). According to this data, statistically difference was found amoung the quality of root canal filling and AP. In teeth with inadaquate root canal filling with at least one procedural error had statistically higher incidence of periapical lesion compared to teeth with adequate root canal fillings with no periapical lesion (p<0.05).

The number of adequate coronal restoration was 569 (Table 5), and 54.5% (Table 4) of them were healthy. The number of inadequate coronal restoration was 616 (Table 5) and 36% (Table 4) of them were healthy.

When lenght of the root canal was investigated, 318 (47.2%) of 673 teeth with adequate root canal fillings, 285 (65.8%) of 433 teeth with short root canal fillings, 50 (63.2%) of 79 teeth with overfilling had periapical lesions (Table 4).

The quality of endodontic treatment and coronal restoration is also summarized in Table 4. Apical lesion was detected in

Table 2. Distribution of root canal treated teeth according to age and gender

Age/gender	Apical periodontitis		Total
	Healthy periapex	Periapex with pathosis	
Female (n=689)	325 (47.2%)	364 (52.8%)	689 (100%)
Male (n=496)	207 (41.7%)	289 (58.3%)	496 (100%)
18-24 yrs (n=123)	32 (26.0%)	91 (74.0%)	123 (100%)
25-34 yrs (n=230)	86 (37.4%)	144 (62.6%)	230 (100%)
35-44 yrs (n=296)	120 (40.5%)	176 (59.5%)	296 (100%)
45-54 yrs (n=331)	174 (52.6%)	157 (47.4 %)	331 (100%)
55 yrs > (n=205)	120 (58.5%)	85 (41.5%)	205 (100%)
Total	**532 (44.9%)**	**653 (55.1%)**	**1185 (100%)**

Table 3. Distrubution of root canal treated teeth according to the tooth group (n=1185)

Tooth	Maxillary	Mandibular
Central incisor	90 (7.6%)	12 (1%)
Lateral incisor	76 (6.4%)	13 (1%)
Canine	89 (7.5%)	49 (4.1%)
Premolar	236 (19.9%)	201 (16.9%)
Molar	206 (17.3%)	213 (17.9%)
Total	697 (58.8%)	488 (41.2%)

48 (29.3%) of 164 teeth with adequate RCT and coronal restoration. However apical lesion was detected in 211 (52.1%) of 405 teeth with inadequate root canal filling but adequate coronal restoration. Statistically significant difference was found in teeth with adequate coronal restoration between quality of root canal filling and incidence of apical lesions (Table 4).

In case of inadequate obturation with inadequate coronal restorations, 314 (68.9%) of 456 teeth had periapical lesions. However, 50% of teeth with adequate obturation and inadequate coronal restorations showed apical lesions. In case of inadequate coronal restoration, statistically significant difference was found between qualtiy of root canal fillings and incidence of AP ($p<0.05$) (Table 4).

Mostly indirect restoration was found in teeth with endodontic post ($p<0.05$). Also, presence of post statistically did not effect AP incidence ($p>0.05$) (Table 4).

Periapical lesion was detected in 31 (65%) of 47 teeth with broken instrument, 123 (78.8%) of 156 teeth with missed canal, 57 (60.6%) of 94 teeth with perforation, 139 (72.3%) of 192 teeth with ledge formation. AP was found in 440 (67.4%) of 702 teeth with nonhomogeneous root canal filling (presenting voids and poor density) (Table 6). The presence of ledge formation, missed canal and non-homogenous root canal filling in endodontically treated teeth had statistically significantly effect on the incidence of AP ($p<0.05$). The presence of perforation and instrument fracture did not demonstrate a statistically significant difference in terms of the distribution of AP ($p>0.05$).

Table 4. Periradicular status of root canal treated teeth relative to diverse factors

Covariate	Healthy periapex	Periapex with pathosis	p-value
Sex			
Female (n=689)	325 (47.2%)	364 (52.8%)	
Male (n=496)	207 (41.7%)	289 (58.3%)	
Quality of coronal restoration			
Inadequate	222 (36.0%)	394 (64.0%)	**0.0001****
Adequate	310 (54.5%)	259 (45.5%)	
Quality of endodontic treatment			
Inadequate	336 (39%)	525 (61%)	**0.0001****
Adequate	196 (60.5%)	128 (39.5%)	
Apical limit of filling			
0-2 mm short	355 (66.7%)	318 (48.7%)	
>2 mm short	318 (48.7%)	285 (43.6%)	**0.0001****
Overfilling	29 (5.5%)	50 (7.7%)	
Post			
Yes	48 (9.0%)	42 (6.4%)	0.094
No	484 (91.0%)	611 (93.6%)	
Combined endodontic treatment and coronal restoration			
Adequate RCT/ adequate restoration	116 (70.7%)	48 (29.3%)	**0.0001****
Adequate RCT/ inadequate restoration	80 (50.0%)	80 (50.0%)	
Inadequate RCT/ adequate restoration	194 (47.9%)	211 (52.1%)	
Inadequate RCT/ inadequate restoration	142 (31.1%)	314 (68.9%)	

Discussion

Epidemiologic studies related to endodontics give information about frequency of AP and prevalance of endodontically treated teeth. Also, the quality of endodontic treatment and errors can be estimated to improve treatment procedures in health care system or universities in the population. With this study, useful

Table 5. Distribution of evaluated variables with root canal filled teeth

Covariate	n	%
Gender		
Female	689	58.15
Male	496	41.85
Quality of coronal restoration		
Adequate	569	48
Inadequate	616	52
Quality of endodontic treatment		
Adequate	324	27
Inadequate	861	73
Apical limit of filling		
0-2 mm short	673	56.8
>2 mm short	433	36.5
Overfilling	79	6.7
Post		
Yes	90	7.6
No	1095	92.4
Periapical status		
Healthy	532	44.9
Diseased	653	55.1

Table 6. Distribution of endodontic technical errors in cases of teeth and its association with periapical status

Endodontic technical errors	Total	Healthy periapex	Periapex with pathosis	p-value
Non-homogeneous filling	702	262 (49.2%)	440 (67.4%)	0.0001**
Non-filled canal	156	33 (6.2%)	123 (18.8%)	0.0001**
Instrument fracture	47	16 (3.0%)	31 (4.7%)	0.127
Perforation	94	37 (7.0%)	57 (8.7%)	0.261
Ledge formation	192	53 (10.0%)	139 (21.3%)	0.0001**

information was provided for the prevalence of AP as well as endodontic treatment outcome in a large subpopulation who were living in urban area of İstanbul, Türkiye.

Although conventional and digital radiographic techniques either panoromic or periapical X-ray has been used for along time to assess the quality of RCT and periapical status of the teeth, some periapical pathosis may be overlooked or misinterpretered by these technique (15). According to Estrela et al., (15) mineral bone loss should be 30-50% for a lesion detected in conventional radiographs. However, studies indicated that CBCT images had higher performance to indentify periapical lesions compared to conventional periapical and panaromic radiograph without superposition of adjacent structures (17,18). Also, the quality of RCT can be evaluated more accurately with 3 D than 2 D analyses of conventional methods (19).

While Orstavik et al. (20) used the periapical index to define AP in two dimensions, Estrela et al. (15) recently developed CBCT-PAI scoring method to analyse periapical lesions three dimensionally. By this method the lesions can be evaluated mesiodistally, buccopalatinally and diagonally with more precisely. Therefore, CBCT PAI scoring method was used in the present study.

The prevalence of AP in endodontically treated teeth was found to be very high in different population (25-64.5%) (21-25). When the studies were evaluated by continents, the highest prevalence of RCT was found in Europe and South America populations. However, African population had the lowest prevalance of RCT. The differences in the age of population, the different level of economic development, and the different access to dental health services may result from the differences between continents (10). Not only in the same continent but also in the same country, different results were declared (19.4-70.1%) depending on socioeconomic factors such as location (urban or rural area) or education level of patient in Türkiye (11-14). In the present study, 1185 of all evaluated teeth received endodontic treatment. Also, 55.1% of the endodontically treated teeth had AP.

Beside geographic and socieconomic factors, age is also found to be an important factor in epidemiologic studies. Higher prevalance of RCT in older population was reported compared to younger population (10). In the present study also, the age group of 45-54 years showed the highest prevalence of RCT. However, the age group of 18-24 years had higher incidence of AP in endodontically treated teeth may be due to decreasing size of the pulp and calcification of ramifications, MB2 canal or lateral canals by the age.

Regarding sex, this study sample consisted of more women than men which might indicate women were more interested in taking dental care in Türkiye. Although less incidence of periapical pathosis was found in women compared to men, the difference was not significant. Other study results collabarated our findings which indicated statistically no relation between gender and periapical pathosis (26).

In literature, high positive correlation was found between prevalence of AP and poor quality of endodontic treatment (2,7,11-14). In the present study of 1185 teeth, 72.7 % of teeth had inadequate RCT. This finding showed that the quality of root canal was low in this subpopulation and found to be much lower than other studies which were performed in Türkiye (11-14). Also, teeth with inadequate RCT showed statistically higher AP prevalance. Furthermore, homogeneity of root canal, canal length, canal errors such as ledge formation or presence of unfilled canal had significant effect on AP prevalence (11-14).

The lenght of the root canal is an important factor on the quality of the root canal. Inadequate root canal debriment with short of filling with no apical closure may lead to bacterial accumualation and this may increase the AP prevalence (27). In the present study, teeth with short fillings showed higher AP prevalence which collobareted other study results (27-29). Also, of 1185 teeth, 79 teeth (6.7%) showed overfillings and 63.2% of these teeth had AP. Overfilling also causes irritation of periapical tissue which may increse the AP incidence (13). Studies related to epidemology or prognosis of endodontology showed that ideal root canal length should be approximately 0.5-2 mm short of apex for healing of periapical tissue (15,30).

Lee et al. (31) indicated that beside apical extent, homogeneity of root canal had also significant impact on periapical healing. Stoll et al. (32) demonstrated higher survival rates in root canal treated teeth with corrected length and homogenous root canal fillings. Related to this finding, in the present study, teeth with non-homogenous fillings had significantly more incidence of AP (62.6%) than teeth with homogenous fillings.

During instrumentation, every error such as zip, perforation, ledge formation, loss of working length will decrease the success of endodontic treatment (27-29,33). Chugal et al. (33) indicated that loss of working lenght with ledge formation would increase the failure of treatment specially in teeth with AP. They found that a millimeter loss of working length increased the chance of failure by 14%. In the present study, 192 teeth had ledge formation and AP was observed in 72.3% of these teeth. Significant correlation was found between ledge formation and AP ($p<0.05$).

Missed canals and inadequate prepared canals always contain microorganisms and this will increase the AP incidence (34). Incidence of missed canal was found to be 42% by Hoen and Pink (35), 23% by Karabucak et al. (36), 12% by Costa et al. (37) and Baruwa et al. (38). In the present study, 13.2% of teeth had missed canal. Also these investigators reported that, 82.8-82.6% of teeth with missed canals had AP (35-38). In parallel to this findings, in the present study, 78.8% of teeth with missed canals had AP. Also, the relationship between missed canal and AP was found to be significant ($p<0.05$).

Broken instrument is a complication which occurs during instrumentation (39) and affects the success of endodontic tretment. The location and the type of the broken instument and maintenance apical patency are all important factors on the

failure of the endodontic treatment (40). In the present study, the prevalance of AP was found to be 65.9% in teeth with broken instrument.

The size and the location of the perforation is important factor on the success of endodontic treatment (41). Also, high incidence of AP (60.6%) was found in teeth with perforation which was similar to findings of de Chevigny et al. (42).

The quality of coronal restoration is also found to be important factor as well as apical sealing in long term of endodontic treatment (43). Ray and Trope (44) emphasized that the technical quality of the coronal restoration might be more important for periapical health than the technical quality of the root filling. However, Kirkevang et al. (45) and Hommez et al. (46) claimed that both coronal and root filling quality was equally important for periapical healing. Tavares et al. (47) found the highest success rate (93.5%) in teeth with both good coronal and apical sealing. Meantime, Cheung and Chan (48) decleared that crown restoration was increased the longevity of endodontically treated teeth.

Stenhagen et al. (49) found that the type of coronal restoration (direct or indirect) did not have a significant effect on the success of RCT in their retrospective study. In the present study also, the type of the restoration was found to have no effect on AP prevalence. Also, presence of post was not found to be significantly related to AP incidence. These findings were in line with the findings of the studies of De Moor et al. (28) and Estrela et al. (15). Unfortunately, it is difficult to assess the quality of crown restoration by CBCT images due to artefacts. Clinical and oral examinations are much reliable methods to examine the quality of coronal restoration.

Study Limitations

Among the limitations of this study was its cross-sectional design. The main disadvantage of a cross-sectional study was the difficulty in determining whether a periapical lesion was healing or not. A radiograph provides only static information about a dynamic process and at the time the radiograph is taken, the size of a periapical lesion may be increasing or healing.

Another disadvantage of a cross-sectional study was the lack information about when endodontic treatments or restorations were performed, the level of education of the clinician performing the procedure and the time elapsed between treatment and the observation period. It was unknown whether endodontic treatments were performed in a similar manner. However, obtaining a large sample size and random selection are among the most significant advantages of this method.

In our study, intraoral examination of coronal restorations was not conducted. Therefore, the type of indirect restoration was unknown. Restorations were evaluated solely based on radiographs. This was among the limitations of our study. Clinical examination would lead to more accurate results in this regard.

Conclusion

This study's results showed that high prevalence of AP with endodontically treated teeth indicated poor technical quality of endodontic treatment. So, continued education and training of general dentists by Health Ministry, Dental Associations and universities is essential.

Ethics

Ethics Committee Approval: Marmara University Clinical Research Ethical Committe was approved this study by no: 2021/07 and dated 07.10.2021.

Informed Consent: Obtained.

Authorship Contributions

Concept: M.G., N.G., Design: M.G., N.G., Data Collection or Processing: M.G., Analysis or Interpretation: M.G., Literature Search: M.G., Writing: M.G., N.G.

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Original Article

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Clinical Importance of the First Trimester Uterine Artery Doppler Measurements in Patients with Hyperemesis Gravidarum

Birinci Trimester Uterin Arter Doppler Ölçümlerinin Hiperemezis Gravidarumlu Hastalarda Klinik Önemi

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ABSTRACT

Objective: To examine the relationship between the first trimester uterine artery doppler measurements, biochemical markers, first trimester antenatal screening test and severity of hyperemesis gravidarum (HG) according to Pregnancy-Unique Quantification of Emesis (PUQE) score.

Methods: A prospective observational study was carried out in a tertiary university hospital, between December 2016 and March 2017. A total of 207 consecutive singleton pregnancies at 11-13.6 weeks of gestations were enrolled. Sociodemographic, clinical characteristics, PUQE scores, biochemical blood, and urine sample results were collected. First trimester antenatal screening test and uterine artery doppler measurements were performed. The severity of HG was made based on the score of PUQE test.

Results: A total 207 pregnant women enrolled in this study. Of the patients, 131 were in group I and 76 in group II. No significant difference was observed in of first trimester screening serum markers, doppler ultrasonography findings and PUQE scores between the two groups (p>0.05). There was no statistically significant difference between the patients in group I and group II in terms of the presence of urinary tract infections. Ketonuria

ÖZ

Amaç: Birinci trimester uterin arter doppler ölçümleri, birinci trimester antenatal tarama testinin biyokimyasal belirteçleri ve Gebeliğe Özgü Bulantı Kusma Şiddetinin Belirlenmesi Ölçeği (PUQE) skoruna göre hiperemezis gravidarum (HG) şiddeti arasındaki ilişkiyi incelemektir.

Yöntemler: Bu prospektif gözlemsel çalışma Aralık 2016 ile Mart 2017 tarihleri arasında üçüncü basamak bir üniversite hastanesinde gerçekleştirildi. 11-13,6 gebelik haftalarında toplam 207 ardışık tekil gebelik çalışmaya kabul edildi. Sosyodemografik, klinik özellikler, obstetrik ve tıbbi öyküler ve antropometrik ölçümler kaydedildi. Daha sonra PUQE skorları, biyokimyasal kan ve idrar örneği sonuçları toplandı. Tüm hastalara birinci trimester antenatal tarama testi ve uterin arter doppler ölçümleri yapıldı. HG'nin şiddeti PUQE testi skoruna göre belirlendi. PUQE test puanına göre hastalar iki gruba ayrıldı. Kontrol grubu (grup I) PUQE puanı <6 olanlar ve çalışma grubu (grup II) PUQE puanı ≥7 olanlar idi.

Bulgular: Bu çalışmaya toplam 207 gebe katıldı. Hastaların 131'i grup I'de, 76'sı grup II'de yer aldı. İki grup arasında birinci trimester tarama serum belirteçleri, doppler ultrasonografi bulguları ve PUQE skorları açısından anlamlı fark izlenmedi (p>0,05). Grup

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ABSTRACT

was detected in three patients (2.4%) in group I and 11 patients (17.2%) in group II (p<0.01).

Conclusion: The result of the study showed no significant relationship between the first-trimester uterine artery doppler parameters, biochemical markers and the severity of HG, which was measured according to PUQE scoring system. However, we did find that PUQE test was an effective test in the detection of patients with ketonuria.

Keywords: First trimester, hyperemesis gravidarum, PUQE test, uterine artery doppler

ÖZ

I ve grup II'deki hastalar arasında idrar yolu enfeksiyonu varlığı açısından istatistiksel olarak anlamlı fark yoktu. Grup I'de 3 (%2,4), grup II'de 11 (%17,2) hastada ketonüri saptandı (p<0,01).

Sonuç: Çalışmanın bulguları, birinci trimester uterin arter doppler parametreleri, biyokimyasal belirteçler ile PUQE skorlama sistemine göre ölçülen HG şiddeti arasında anlamlı bir ilişki olmadığını gösterdi. Ancak PUQE testinin ketonürili hastaların saptanmasında etkili bir test olduğu gösterildi.

Anahtar Sözcükler: Birinci trimester, hiperemezis gravidarum, PUQE testi, uterin arter doppler ultrasonografi.

Introduction

Nausea and vomiting of pregnancy (NVP) are extremely common. Up to 90% of women experience nausea during pregnancy. Studies showed that approximately 27 to 30 percent of women experienced only nausea, while vomiting could be seen in 28 to 52% of all pregnancies (1). However, 0.3-3.6% of pregnant patients suffer from the most severe form of NVP known as hyperemesis gravidarum (HG) (2). HG refers to intractable vomiting during pregnancy, leading to weight loss, electrolyte imbalances, dehydration, and volume depletion, resulting in ketonuria (3). There is no consensus on specific diagnostic criteria, but it generally refers to the severe end of the spectrum regarding NVP (4).

The etiology of HG is unknown, but several theories exist. Levels of human chorionic gonadotropin (hCG) levels peak during the first trimester, corresponding to the typical onset of hyperemesis symptoms (5). Estradiol levels increase in early pregnancy and decrease later, mirroring NVP symptoms. Additionally, nausea and vomiting are side effects of estrogen-containing medications (6).

In patients with severe HG, complications including vitamin deficiency, dehydration, and malnutrition may occur if not treated appropriately. Wernicke encephalopathy, caused by vitamin-B1 deficiency, can lead to death and permanent disability if left untreated (7). Additionally, there have been case reports of injuries secondary to forceful and frequent vomiting, including esophageal rupture and pneumothorax (8). Electrolyte abnormalities such as hypokalemia can also cause significant morbidity and mortality (9).

No single measure can easily define, quantify or evaluate the treatment of HG. An English pregnancy-specific questionnaire, Pregnancy-Unique Quantification of Emesis (PUQE), has been developed to assess the severity of emesis in pregnancy. This questionnaire contains three questions regarding the time-span of nausea, vomiting, and retching, respectively, as well as one question assessing the global psychological and physical quality of life.

To our knowledge, there is a large body of research on the relationship between first-trimester uterine artery Doppler findings and pregnancy complications. However, there is limited research on the use of first-trimester uterine artery Doppler to predict placental abnormalities in cases of HG. Therefore, this study aimed to evaluate the relationship between first-trimester uterine artery Doppler measurements and biochemical markers and PUQE test scores in pregnant women who attended first-trimester prenatal screening.

Methods

This prospective observational study was conducted at the Department of Obstetrics and Gynecology of a Tertiary University Hospital, between December 2016 and March 2017 and comprised singleton pregnant women who attended first trimester prenatal screening. The study was in line with the Helsinki Decleration and approved the Ethics Committee of the Bezmialem Vakıf University (decision no: 17/22, date: 07.12.2016). All participants were informed regarding the study and they gave informed consent prior participation.

Inclusion criteria were: singleton pregnancies between 11-13.6 weeks of gestation, absence of any gastrointestinal, audiovestibular, endocrinological, infectious, and psychological disorders that might cause nausea and vomiting, except pregnancy-induced vomiting, and agreed to participate to the study. Exclusion criteria were: multiple pregnancy, molar pregnancy, fetal structural anormality, and abnormal nuchal fold thickness.

A total of 207 consecutive patients who met the inclusion and exclusion criteria were included in the study. Sociodemographic, clinical characteristics, obstetric and medical histories, and anthropometric measurements were collected. Then, PUQE scores, biochemical blood, and urine sample results were collected. Next, pregnant women underwent first trimester screening and uterine arter doppler ultrasonography using Philips HD11 XE device with 3.5 mHz curvilinear probe. The screening consisted of nuchal translucency (NT) and serum free β-human chorionic gonadotrophin (free β-hCG) and pregnancy-associated plasma protein A (PAPP-A) measurements. Gestational age was determined according to the head-rump distance (CRL) measured in the sagittal plane on abdominal ultrasonography. During the Doppler ultrasonography examination, the probe was placed in the iliac fossa above the level of the inguinal ligament. The uterine artery was

detected where it crossed the external iliac artery by color Doppler imaging. A pulsed Doppler was used to determine the waveform. A resistance-index (RI) and pulsatility-index (PI) were calculated from each uterine artery and the presence or absence of a notch were noted. The same procedure was repeated for the contralateral uterine artery. The urine analysis test was classified according to ketones positivity and urinary tract infection. Those with leukocyte positivity, bacteriuria, or leukocyte esterase positivity were included in the urinary tract infection group.

The 24 h PUQE (10,11) is a self-reported measure of the duration of nausea, episodes of vomiting, and episodes of retching in the last 24 hours on a 5-point scale. A higher score indicates more severe NVP. NVP was evaluated as mild in those with <6 points, moderate in those with 7-12 points, and severe in those with 13 points and above (12). The patients in the mild group were classified as group I, and the patients in the moderate and severe groups were classified as group II.

Statistical Analysis

IBM SPSS 21.0 (Statistical Package for Social Sciences Chicago, USA) was employed to analyze the collected data. When the numerical data fit the normal distribution, analysis between groups was performed using the Student or ANOVA test. The Mann-Whitney test was applied when the data did not conform to the normal distribution. Categorical data were analyzed using the chi-square test. A p-value of <0.05 was considered statistically significant.

Results

A total 207 pregnant women enrolled in this study. Of the patients, 131 were in group I and 76 in group II.

The mean age of the patients was 29.9±4.9 (range: 18-44 years). The mean age of group I patients was 29.7±5.0 and 30.4±4.8 years in group II. The body mass index (BMI) values of all patients ranged from 15.4 to 34.8, with a mean of 24.6±3.7. The mean BMI of the patients in group I was 24.6±3.7 and in group II was 24.5±3.7 kg/m^2. No significant difference was found in demographic and obstetric variables between the two groups. The demographic information and obstetric characteristics of the patients are presented in Table 1.

The mean CRL value of patients in group I was 63.3±7.2 and in group II was 61.8±6.5 mm. There was no significant difference between the two groups in CRL measurements (p=0.1). NT measurements varied between 1.1 and 3.0, with a mean of 1.4±0.2 mm. The mean NT value of the patients in group I was 63.3±7.2 and in group II was 61.8±6.5. There was no statistically significant difference between the two groups in terms of CRL measurements (p=0.1). No significant difference was observed in terms of first trimester screening serum markers, and doppler ultrasonography findings between the two groups. There was no statistically significant difference between the patients in group I and group II in terms of the presence of urinary tract infections. Ketonuria was detected in three patients (2.4%) in group I and 11 patients (17.2%) in group II (p<0.01) (Table 2).

No statistically significant difference was observed between sociodemographic, first-trimester uterine artery Doppler measurements and first-trimester screening ultrasonographic and serum markers between mild NVP, moderate NVP, and severe NVP groups (Table 3).

Discussion

The results of the present study indicated that there was no association between the first trimester uterine artery doppler parameters, biochemical markers of the first trimester antenatal screening test and severity of HG according to PUQE score.

Table 1. Baseline demographic and obstetric characteristics

Variables	Grup I (n=131) Mean ± SD	Grup II (n=76) Mean ± SD	p-value
Age	29.7±5.0	30.4±4.8	0.3
Body mass index (kg/m^2)	24.6±3.7	24.5±3.7	0.8
	Grup I n (%)	**Grup II n (%)**	**p-value**
Primigravid	53 (40.5)	31 (40.8)	0.9
Multigravid	78 (59.5)	45 (59.2)	
	Grup I n (%)	**Grup II n (%)**	
Nullipar	64 (48.9)	n=33 (43.4)	
Primipar	42 (32.1)	n=28 (36.8)	0.7
Multipar	25 (19.1)	n=15 (19.7)	

SD: Standard deviation

Table 2. Comparison of first trimester screening serum markers, doppler ultrasonography findings, and urine analysis test results

Variables	Grup I (n=31) Mean ± SD	Grup II (n=76) Mean ± SD	p-value
PAPP-A	1.15±0.53	1.14±0.57	0.9
β-hCG	1.09±0.75	1.02±0.66	0.5
NT (mm)	1.42±0.25	1.40±0.22	0.6
NT (MoM)	0.88±0.14	0.88±0.12	0.9
CRL	63.3±7.2	61.8±6.5	0.1
Right Uterine Artery PI	1.75±0.55	1.77±0.6	0.7
Right Uterine Artery RI	0.74±0.10	0.75±0.09	0.7
Left Uterine Artery PI	1.92±0.69	1.98±0.72	0.5
Left Uterine Artery RI	0.76±0.09	0.78±0.08	0.1
Mean Uterine Artery PI	1.83±0.49	1.88±0.52	0.5
Mean Uterine Artery RI	0.75±0.07	0.76±0.08	0.2
Bilateral Uterine Artery Notch (+)	16 (12.2)	6 (7.9)	0.3
KetonuriA	3 (2.4)	11 (17.2)	<0.01**
Urinary tract infection	37 (29.1)	22 (34.4)	0.4

PAPP-A: Pregnancy-associated plasma protein A, β-Hcg: β-human chorionic gonadotrophin, NT: Nuchal translucency, RI: Resistance-index, PI: Pulsatility-index, CRL: Head-rump distance, SD: Standard deviation, **p<0.05

Table 3. Comparison of demographic characteristics, first trimester screening serum markers, doppler ultrasonography findings

Variables	Grup I (n=131) Mean ± SD	Grup II (n=69) Mean ± SD	Grup III (n=7) Mean ± SD	p-value
Age	29.7±5.0	30.3±4.8	31.3±4.5	0.5
Body mass index (kg/m^2)	24.6±3.7	24.4±3.7	25.9±3.1	0.5
PAPP-A	1.15±0.53	1.14±0.56	1.12±0.68	0.5
β-hCG	1.09±0.75	1.04±0.68	0.88±0.42	0.7
Right Uterine Artery PI	1.75±0.55	1.76±0.58	1.82±0.79	0.9
Right Uterine Artery RI	0.74±0.10	0.75±0.09	0.74±0.11	0.9
Left Uterine Artery PI	1.92±0.69	1.97±0.72	2.08±0.73	0.7
Left Uterine Artery RI	0.76±0.09	0.78±0.10	0.80±0.09	0.3
Mean Uterine Artery PI	1.83±0.49	0.87±0.51	1.95±0.72	0.7
Mean Uterine Artery RI	0.75±0.07	0.76±0.07	0.77±0.09	0.5
NT (mm)	1.42±0.25	1.41±0.23	1.34±0.12	0.6
NT (MoM)	0.88±0.14	0.88±0.12	0.83±0.06	0.6

PAPP-A: Pregnancy-associated plasma protein A, β-Hcg: β-human chorionic gonadotrophin, NT: Nuchal translucency, RI: Resistance-index, PI: Pulsatility-index, CRL: Head-rump distance, SD: Standard deviation

Prior research revealed that high levels of HCG was associated with placentation abnormalities such as preeclampsia, small for gestational age or HG. A population based cohort study involving over one million pregnant women in Sweden also reported that an elevated risk of placentation dysfunction disorders in women with HG. Therefore, uterin artery doppler ultrasonography examination has been suggested in this patient group (13). In a study evaluating first-trimester Doppler ultrasonography findings in pregnant women with HG, it was found that there was no difference in uterine artery Doppler values, except for uterine artery notch (14).

The literature shows contradictory results between HG and first-trimester biochemical markers. Derbent et al. (15) demonstrated that HG was associated with elevated levels of serum maternal PAPP-A and free β-hCG levels. Similarly, Yildiz et al. (16) demonstrated that higher levels of free β-hCG was related to increased risk for HG. However, researchers also noted that no significant relationship was found in uterin artery doppler findings or serum PAPP-A levels. On the contrary, Madendağ et al. (17) found that serum PAPP-A value was significantly decreased in women with HG. But, they also noted that severe HG had no significant effect on free β-hCG levels (17). In this context, we believe that more research needs to be undertaken on this subject in order to better understand the relationship between HG and PAPP-A, and free β-hCG levels.

Another important finding was that this study showed no link between HG and NT. This finding was in line with the suggestion of Yildiz et al. (16), who suggested that there was no relationship between HG and NT.

There are controversies about the association between first trimester uterine artery doppler values and HG. The findings of the current study were consistent with those of Biyik et al. (14) who also found no association between doppler RI, PI, standard deviation (SD) values and HG. In contrast, a recent study by Kartal et al. (18) demonstrated an association between RI, PI, and SD values of the uterine artery Doppler waveform and HG. A possible explanation for this might be that our cohort consisted of outpatients, Doppler and biochemistry values did not differ significantly between groups.

Study Limitations

One of the limitations of the study was the small number of severe HG patients in the study. We believe that the relationship between Doppler and first trimester parameters and HG severity may be elucidated in future large, multi-center studies. To the best of our knowledge, there are many studies on the relationship between first-trimester uterine artery Doppler findings and pregnancy complications, however, there are only a few studies aimed at predicting placentation abnormalities with first trimester uterine artery Doppler in cases with HG.

Conclusion

In our study, no significant relationship was found between the first-trimester uterine artery doppler parameters, biochemical markers and the severity of HG, which was measured according to PUQE scoring system. However, we did find that PUQE test was an effective test in the detection of patients with ketonuria. Further research is needed to better understand the significance of uterine artery Doppler measurements and biochemical markers in cases with HG.

Ethics

Ethics Committee Approval: The study was in line with the Helsinki Decleration and approved the Ethics Committee of the Bezmialem Vakıf University (decision no: 17/22, date: 07.12.2016).

Informed Consent: All participants were informed regarding the study and they gave informed consent prior participation.

Authorship Contributions

Surgical and Medical Practices: M.M., Concept: M.M., İ.A., B.D., Design: M.M., İ.A., B.D., Data Collection or Processing: M.M., E.K., P.Y., M.M.K., R.B.B., E.M., G.Y., İ.A., B.D., Analysis or Interpretation: M.M., E.K., P.Y., M.M.K., R.B.B., E.M., G.Y., İ.A., B.D., Literature Search: M.M., E.K., P.Y., M.M.K., R.B.B., İ.A., B.D., Writing: M.M., E.K., P.Y., M.M.K., R.B.B., E.M., G.Y., İ.A., B.D.

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Original Article

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Orthodontic Materials Interacting with Fifth Generation (5G) Electromagnetic Waves

Ortodontik Malzemelerin Beşinci Nesil (5G) Elektromanyetik Dalgalarla Etkileşimi

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ABSTRACT

Objective: Public exposure to radiofrequency (RF) fields from fifth generation (5G) and other sources is known to be below human exposure limits. The interaction of RF fields with the human body has been widely documented, with tissue heating being the primary consequence for RF fields above 100 kHz. This study aimed to reveal possible harm to orthodontic patients associated with 5G electromagnetic waves. The possibility of individuals with orthodontic appliances (metallic braces, porcelain braces, thermoplastic appliances) being more affected by 5G was investigated.

Methods: Sixty extracted human teeth were divided into 5 groups. Different types of brackets, arch, and ligature wires were applied to each group. Each group was exposed to 5G electromagnetic waves (3.6 GHz of frequency) for 60 minutes. Temperature measurements were made inside the canine root canals and in saline solution.

Results: Exposure to a 5G electromagnetic field increased the temperatures of the root canal of the tooth and sodium chloride (NaCl) solution surrounding samples. Temperature increase in the canals were as follows; metal self-ligating braces > mini metal braces > porcelain braces > clear aligner > control. The temperature change in the NaCl solution at the 60th minute was close to each other in the self-ligating braces and mini metal braces groups. The temperature rise of the NaCl solution in the control group was also minimal.

ÖZ

Amaç: Normal şartlarda beşinci jenerasyon (5G) ve diğer kaynaklardan gelen radyo frekans (RF) dalgalarına maruziyetin, insanlar için belirlenmiş sınırlarının altında olduğu bilinmektedir. RF dalgaların insan vücudu üzerine etkileri ile ilgili yapılan çalışmalar 100 kHz'in üzerindeki RF alanları için dokular üzerindeki birincil sonucun ısınma olduğunu göstermiştir. Çalışmamızda, 5G elektromanyetik dalgaların ortodonti hastalarına olası etkilerini ortaya çıkarmak amaçlanmıştır. Farklı ortodontik apareyleri (metal braketler, porselen braketler, termoplastik plaklar) olan bireylerin normal bireylere göre 5G'den daha fazla etkilenme olasılığı araştırılmıştır.

Yöntemler: Altmış adet çekilmiş insan dişi 5 gruba ayrıldı. Her gruba farklı tipte braket, ark ve ligatür telleri uygulandı. Her grup 60 dakika boyunca 5G elektromanyetik dalgalara (3,6 GHz frekanslı) maruz bırakıldı. Sıcaklık ölçümleri köpek diş kök kanallarının içinden ve dişin etrafında bulunan salin solüsyonunda yapıldı.

Bulgular: 5G elektromanyetik alana maruz kalan dişlerin kök kanalı ve çevreleyen sodyum klorür (NaCl) solüsyonunun sıcaklıkları kontrol grubuna göre artış gösterdi. Kanallardaki sıcaklık artışı sıralaması metal self-ligating braket > mini metal braket > porselen braket > şeffaf plak > kontrol şeklindeydi. Altmışıncı dakikada NaCl solüsyonundaki sıcaklık değişimi kendinden bağlanan braket ve mini metal braket gruplarında birbirine yakındı. Kontrol grubundaki NaCl çözeltisinin sıcaklık artışı ise minimum düzeydeydi.

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ABSTRACT

Conclusion: The hypothesis that orthodontic materials alter electromagnetic waves is supported by temperature increases. The ferromagnetic density of the orthodontic materials used was shown to be closely connected to temperature increase.

Keywords: Electromagnetic field, ferromagnetic, orthodontic materials, radiofrequency, telecommunication, 5G

ÖZ

Sonuç: Ortodontik materyallerin elektromanyetik dalgaların yoğunluğunu değiştirdiği hipotezi sıcaklık artışları ile desteklenmektedir. Kullanılan ortodontik materyallerin ferromanyetik yoğunluğunun sıcaklık artışı ile yakından ilişkili olduğu gösterilmiştir.

Anahtar Sözcükler: Elektromanyetik alan, ferromanyetik, ortodontik materyaller, radyofrekans, telekomünikasyon, 5G

Introduction

As has been observed in every area in recent years, the volume of mobile phone use and the numbers of base stations have increased with the development of technology in the field of mobile telecommunications. The increasing use of radio frequency (RF) fields above 6 GHz, particularly for the fifth generation (5G) cellular network, has raised public concerns about its potential adverse effects on human health. Public exposure to RF fields from 5G and other sources is below the human exposure limits set by the International Commission on Non-Ionizing Radiation Protection (1). It has been demonstrated that RF electromagnetic waves emitted from mobile phones and base stations might cause health problems like headaches and further increase the risk of developing brain tumours in humans (2). Many concerns have been raised regarding the possible effects of radiation emitted by these devices that are used by people of all ages. In the study conducted by Ionescu et al. (3), it was shown that there was a temporary decrease in pH values of saliva when people were exposed to electromagnetic waves of mobile phones; and these values decreased even further to lower values in the presence of orthodontic wires and braces. In another study conducted, it was discovered that there were temperature changes and electric currents induced by low-frequency magnetic fields generated by electric toothbrushes and dental curing light devices (1-2000 Hz) on tooth surfaces with or without braces. This study concluded that electric current was induced in tooth tissue, irrespective of whether such teeth were bonded to stainless steel or zirconia braces (4).

Many research claims that 5G RF is of higher frequency and more harmful than 4.5G (LTE) that we have used so far and investigating its possible effects on human health emphasize that the effects of 5G have not been investigated enough yet (5,6). 5G technology is planned to be used in every field of our lives worldwide soon. The 5G system expected to bring forth revolutionary developments is intended to be used not only in mobile phones but also in devices requiring high processing power such as driverless vehicles, home internet, virtual reality, smart security cameras, physical and rehabilitation devices, etc. (7). When this system is deployed after the preparation of its infrastructure, exposure to this electromagnetic field will become inevitable for all individuals.

With this study, it was aimed to examine the fifth-generation electromagnetic waves (frequency 3.6 GHz) of mobile telecommunication systems, which was currently a bone of contention due to the insufficient investigation of its possible effects particularly on human health today, in terms of their interaction with orthodontic materials such as metallic braces, porcelain braces and thermoplastic appliances.

Methods

The study was conducted with 60 human teeth (10 molars, 20 premolars, 10 canines, and 20 anterior teeth) extracted in the Department of Oral and Maxillofacial Surgery at Bezmialem Vakıf University Faculty of Dentistry Hospital. Teeth with impaired enamel integrity, or hypoplastic, decalcified, or cracked teeth, which were not suitable for putting braces, were excluded. Patients were informed about the purpose of the study and their written consent was obtained for participation in this study. The ethics committee approval required for the study was received from the Non-Invasive Clinical Research Ethics Board of Bezmialem Vakıf University (approval number: E-54022451-050.05.04-15499, date: 27.04.2021).

Teeth were put in a jar filled with normal saline [0.9% isotonic sodium chloride (NaCl) solution] and stored in a cabinet protected from light until the tests were performed. Sixty teeth were divided into 5 different groups. To simulate the right amount of ferromagnetic effect, each group of 12 teeth included 2 molars, 4 premolars, 2 canines, and 4 anterior teeth. The pink wax was heated and formed into a box shape; and then, the teeth were fixed with a few drops of pink wax in contact with each other.

The boxes were filled with auto-polymerized acrylic resin (STEADY-RESIN S Polymer, SCHEU-DENTAL GmbH, Germany) around the roots, thus leaving the crowns of the teeth exposed. Following polymerization, the acrylic block was removed from the box to make it ready for the application of orthodontic materials. The following groups were prepared:

1st group: Active Self Ligating metal braces (YES metal self-ligating braces, 022 slot size, HUBIT Co., Ltd, Korea), 0.014" NiTi, bands in molars (American Orthodontics, Sheboygan, Wisconsin, USA).

2nd group: Metal braces (mini diamond metal, 0.018" slot size, Ormco, USA), 0.014" Ni-Ti arch wire, SS wire ligature, bands in molars (American Orthodontics).

3rd group: Porcelain braces (Inspire ICE, 0.022" slot size, Ormco, USA), 0.014" Ni-Ti arch wire, SS wire ligature (American Orthodontics).

4th group: 0.030" essix thermoplastic appliance (Dentsply Raintree Essix, York, Pennsylvania., Keystone Industries, USA).

5th group: Control group, did not receive any orthodontic material (Figure 1).

The blocks prepared were placed in a glass box filled with %0.9 isotonic NaCl solution at 22°. All materials and devices to be used in the study were placed in the Electromagnetic Diagnostic and Measurement Laboratory in İstanbul Technical University Technical University, Electrical and Electronic Engineering Faculty. The temperature of the unreflecting room was controlled by the control unit and a constant room temperature of 22 degrees was achieved. The samples were kept at constant 22 degrees for 24 hours before the experiment and homogeneous heat was provided in the samples.

The signal source was an analog signal generator (Agilent Technologies E8257D PSG, 250 kHz-20 GHz, Colorado Springs, USA). High-frequency magnetic fields radiated from the horn antenna (Double Ridged Broadband Horn Antenna, 3.6 GHz- SCHWARZBECK BBHA 9120 A Schönau, Germany), were measured with a spectrum analyzer (Agilent Technologies E7405A EMC ANALYZER, 100Hz-26.5 GHz, California, USA) (Figure 2). Thus, the temperature changes induced in the tooth groups with different orthodontic materials applied were measured using a thermometer (EXTECH SDL200: 4-Channel Datalogging Thermometer, New Hampshire, USA).

In our research, the inputs to the signal source were as follows: Frequency: 3.6 GHz (Start Frequency: 3 GHz, Stop Frequency: 4 GHz), output power: 16.00 dBm. Each group of teeth was exposed to electromagnetic waves for 60 minutes (Figure 3). During such exposure, the thermometer was inserted into the root canals of the canine teeth, which were accessed through the apex in the experimental groups. These tips were placed to measure the temperature increase inside the root canals of the teeth during exposure (Figure 4). Another tip of the thermometer was placed in the saline solution containing the experimental group at a certain level that would be flush with the top of the teeth.

The values measured with the thermometer for each group were recorded for 60 minutes. Thus, it was possible to monitor temperature changes from the first to the last minute of the exposure of teeth to electromagnetic waves. Although the temperature change was fixed for around half an hour for all groups, it was waited in all groups until the 60th minute to ensure that the temperatures were stable.

Results

Exposure to a 5G electromagnetic field increased the temperatures of the root canal of the teeth and NaCl solution surrounding samples. There were gradually increasing temperature changes observed in each group. A total temperature increases of 1.5 °C was measured at the 60th minute inside the root canal of the teeth in group 1 applied with self-ligating metallic braces with the highest metallic content. However, the temperature increases inside the canals were measured as 0.2 °C at the 60th minute in the control group with no orthodontic materials applied (group 5),

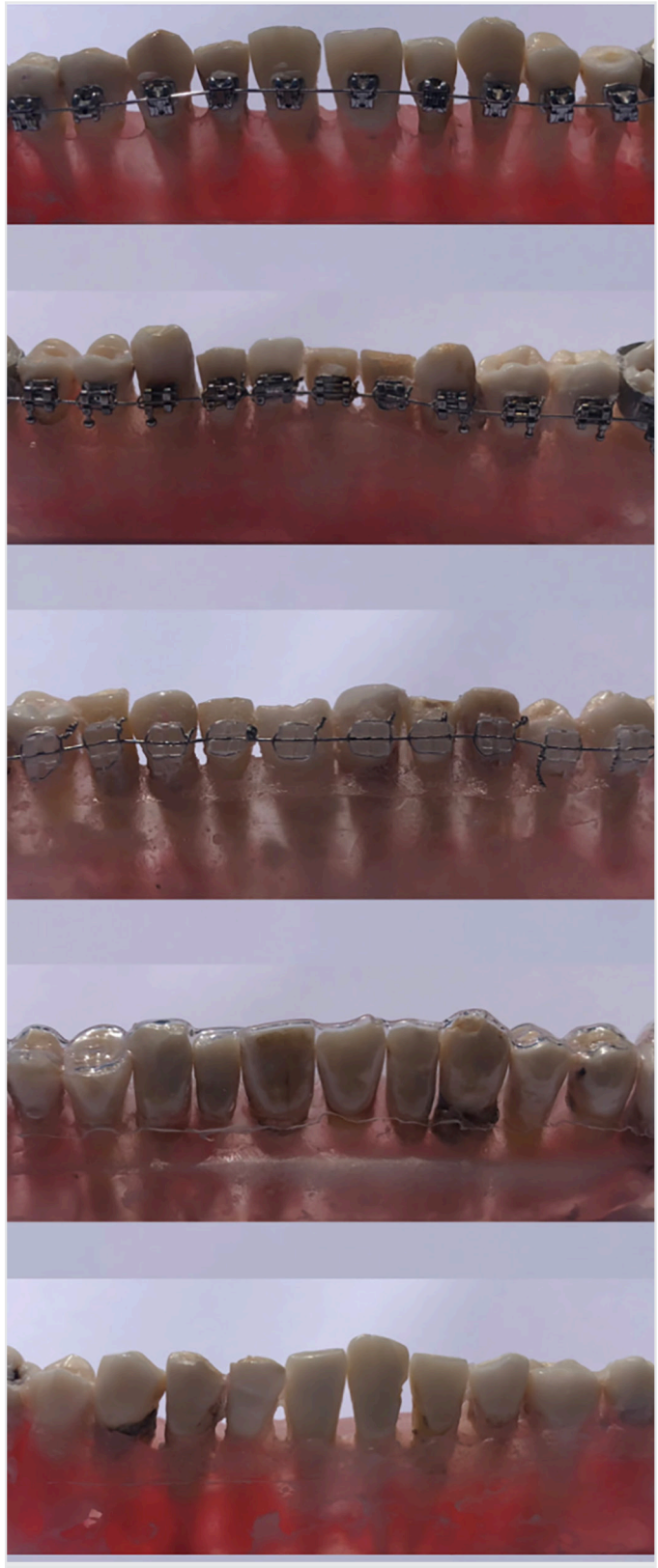

Figure 1. Subject groups (from top to bottom: 1st, 2nd, 3rd, 4th, 5th group)

which reflected the lowest temperature increase measured among all groups. The groups were listed from the group displaying the highest total temperature increase to the group with the lowest temperature increase as follows; group 1 > group 2 > group 3 > group 4 > group 5. The temperature change in the NaCl solution was close to each other for group 1 and group 2 at the 60th minute. The temperature increase of the NaCl solution in the control group was also minimal. Temperature changes of all groups at the 60th minute are given in (Table 1).

Discussion

While the benefits of 5G have been well discussed, and there is no disputing the fact of the need for faster and more reliable wireless communication systems, the implementation of this new technology is raising concerns about the potential health and safety risks associated with exposure to the electromagnetic field emitted by 5G systems (8). The increased use of RF fields above 6 GHz, particularly for the 5G mobile phone network, has sparked public concern about potential health risks. The public's exposure to RF fields from 5G and other sources is below the International Commission on Non-Ionizing Radiation Protection's human exposure limits (ICNIRP). Many researchers have investigated the biological and health effects of RF fields above 6 GHz at exposure levels below the ICNIRP occupational limits (1). But the effects of 5G technologies, which are widely regarded as safe for the public, have never been studied in orthodontic patients. During orthodontic treatment, materials with different ferromagnetic properties are used together in the mouth and the supplier usually does not know the content of

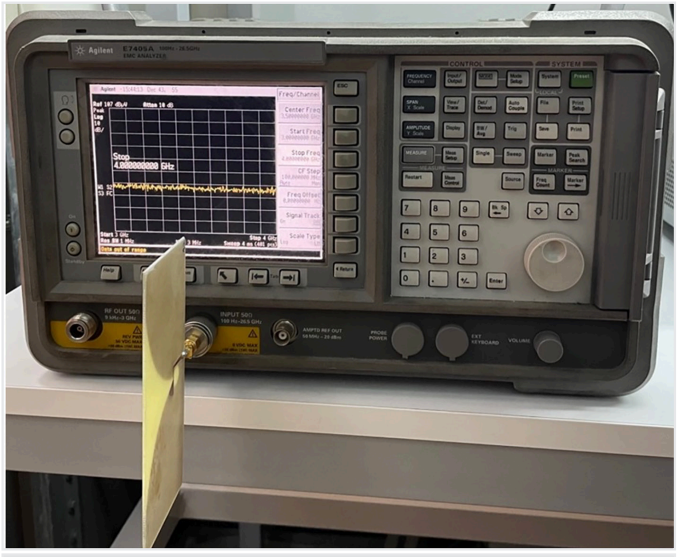

Figure 2. Spectrum analyzer and measurement device

Figure 3. Working system

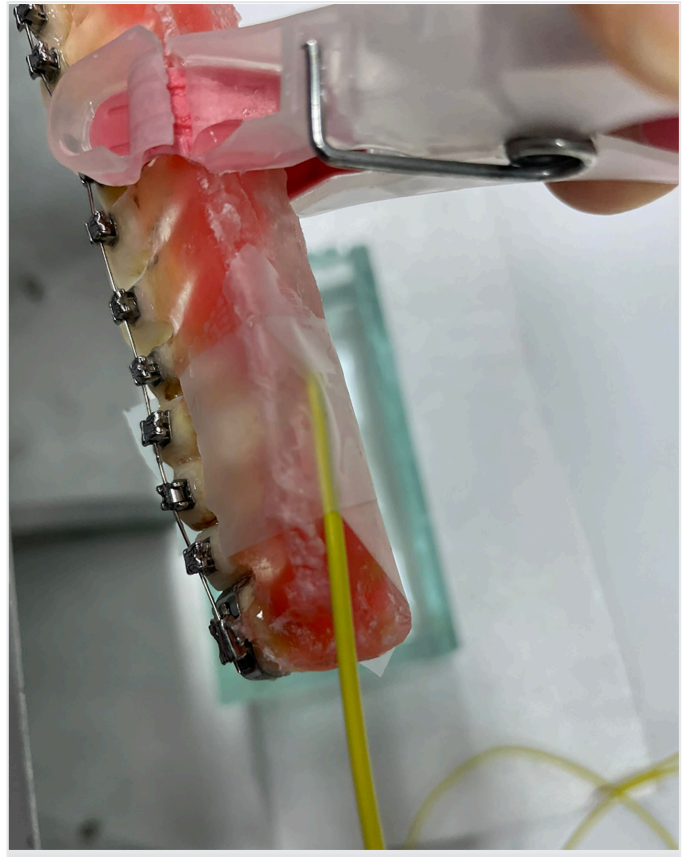

Figure 4. Thermometer tip placement within the root

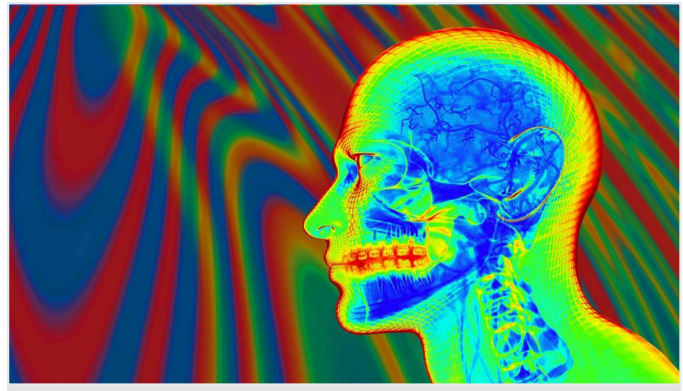

Figure 5. The proposed illustration of a person with braces under RF waves

RF: Radiofrequency

Table 1. Temperature changes of groups

	Group's content	Temperature increase in root canal	Temperature increase in NaCl
Group 1	Self-ligating metal brackets + molar bands + 0.014" NiTi	1.5 °C	1.6 °C
Group 2	Mini metal brackets+ molar bands+ 0.014" NiTi	1.3 °C	1.7 °C
Group 3	Porcelain brackets+ 0.014" NiTi	1.2 °C	0.8 °C
Group 4	0.030" Essix	0.8 °C	1.3 °C
Group 5	Control	0.2 °C	0.4 °C

NiTi: Nickel-titanium, NaCl: Sodium chloride

each material used. The orthodontist may also use components from different vendors combining them on a patient (9). Although stainless steel orthodontic appliances are the most commonly used materials. Titanium, ceramic, and composite materials are also used. Electromagnetic radiation can pass through the structure thanks to the braces' metallic component and the archwire connecting to the various braces. The use of the entire metallic body of orthodontic braces as a radiating antenna element is investigated, and the results have shown that it is a viable option (10). It is shown that residents living near mobile base station antennas experience more headaches, memory changes, tremors, dizziness, depressive symptoms, and sleep disturbance than controls (11).

The temperature increase that occurred in our study was within safe limits (12). However, the effect of ferromagnetic materials working as antennas and altering electromagnetic waves for 24 hours has never been evaluated. during orthodontic treatment, patients take very hot or cold water which shows large differences in the intra-oral temperature and leads to corrosion on archwires (13). The increase in the temperature rises in this study may be more dangerous than those induced by hot nutrients taken in daily life. The heat generated is not due to the balancing of the heat coming from a material taken from outside, but due to constant RF scattering and absorption modified by orthodontic materials. Since the room temperature was kept constant at 22 degrees, the heat produced by the absorption and scattering of RF waves might decrease by the room heat until the heat produced by RF waves reached the equilibrium point and then remained constant. This explained the reason why the intra-root temperature was higher than the saline temperature even though the samples were in an unreflecting room with constant room temperature. At first, the heat increased rapidly. However, the heat increases started to slow down and stabilized after approximately half an hour to 45 minutes, depending on the groups. Therefore, the final values after the stabilization of the heat increases were used. This type of heat increase is commonly referred to as "asymptotic heat increase" and is typically observed in industrial or laboratory environments. In this study, instead of conducting statistical analysis on measurements taken at different times, the decision was made to use the final stabilized value of the heat increases based on the shape and nature of the measured heat increases.

The thermal effect is the most important component in RF exposure over 100 kHz (14). Due to the *in vitro* character of the study, the temperature increase could not be one-to-one with real life. Individuals of all ages will be exposed to 5G for most of each 24 hours. In real life, the penetration depth in soft tissues is minimal (e.g., around 0.85 mm at 30 GHz and 0.5 mm at 60 GHz in cutaneous tissues), thus electromagnetic power absorption is known to be mostly restricted to the skin (15-17). Multilayer skin models have been created to characterize energy absorption and the associated temperature rise (15-18). While these basic models are useful for assessing RF exposure, they do not take into consideration the possibility of children or elderly people wearing metal braces. The thermal time constant of a developing child with a low body mass index wearing an orthodontic appliance containing a high amount of ferromagnetic material, such as a Herbst appliance, may differ from standard animal and human subjects. Our study was the first to draw attention to the question of whether orthodontic treatment patients should be considered a vulnerable population if 5G technologies became widespread (Figure 5).

The findings obtained indicate the interesting fact that the density of the ferromagnetic content of materials is directly proportional to the RF absorption and scattering. This study substantiates the emphasis laid in the literature on the fact that the oral environment with orthodontic appliances is more affected by RF and these interactions should be studied further (4,19,20). This study also showed that the experimental groups with ferromagnetic orthodontic materials were more affected in terms of temperature increase. It has already been shown that cell phone radiations can cause ion release from the appliances of patients undergoing fixed orthodontic treatment and reduce pH levels of saliva (19,20).

Many studies have shown that temperature increase may lead to corrosion; however, the experimental temperatures used are generally above the maximum temperature increase measured in this study (13,21). Radiofrequency may increase the temperature of the surrounding tissues and thus cause dysfunction of facial nerves; the salivary flow rate is increased and there is an alteration of the cytokine expression profile of the salivary gland in heavy cell phone users; and patients with metallic (orthodontic) appliances or restorations should be more cautious as there is the risk of leaching of metallic ions or mercury (19). The mentioned study emphasizing that further human and epidemiological studies are required to evaluate the long-term effect of cell phones on the health of the individual is also justified with the results obtained in this study.

Very few studies regarding the effects of RF on human health were conducted with high frequencies such as 5G (22-24); and most of these studies reached these conclusions by examining quite lower frequency ranges. The conclusion reached in the study indicating

that the higher RF planned to be used in the future might cause a temperature increase in tissues was also in line with the results of this study (22). It seems that exposure levels in areas open to the public will continue to be substantially below the exposure limits defined by organizations that develop worldwide guidelines and standards. The study findings yet do not support a conclusion that harmful health impacts are related to RF exposures, including those from 5G systems. Still, it is also pointed out that future studies should take the necessary safety steps to increase validity (25).

In our study, we tried to simulate the amount of materials affecting the RF waves realistically. The main purpose here is not the amount of heating in the root canal, but to demonstrate whether the behaviour of RF waves causes any change in the heat amount, considering the type of existing appliance. In real life, RF waves deflected by ferromagnetic materials will be absorbed or scattered by the skin, eyes, and brain tissue. In dentistry we can measure this with orthodontic materials because most of the materials are attached to the teeth and they are kept in the mouth for long periods. Although the results obtained are limited, it is the first wet lab study that draws attention to the subject, and the data given here can provide the basis for further dry lab and *in vivo* studies.

Conclusions

Although the temperature increases that were found in the teeth were within the safe ranges considered in the literature, it should be noted that the test was performed *in vitro* in a room with a constant temperature of 22 °C. It was concluded that further *in vitro* and *in vivo* studies were warranted to obtain scientific data on keeping 5G frequencies within reliable limits for patients undergoing orthodontic treatment.

Ethics

Ethics Committee Approval: The ethics committee approval required for the study was received from the Non-Invasive Clinical Research Ethics Board of Bezmialem Vakıf University (approval number: E-54022451-050.05.04-15499, date: 27.04.2021).

Informed Consent: Informed consent was obtained from all participants included in this study.

Authorship Contributions

Surgical and Medical Practices: B.K., H.Y.Ü., E.E., Concept: B.K., H.Y.Ü., E.E., Design: B.K., H.Y.Ü., E.E., Data Collection or Processing: B.K., H.Y.Ü., E.E., Analysis or Interpretation: B.K., H.Y.Ü., E.E., Literature Search: B.K., H.Y.Ü., E.E., Writing: B.K., H.Y.Ü., E.E.

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

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Original Article

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Evaluation of the Physical Properties of Different Bioceramic-Based Root Canal Sealers

Farklı Biyoseramik Esaslı Kanal Dolgu Patlarının Fiziksel Özelliklerinin İncelenmesi

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ABSTRACT

Objective: The aim of this study was to evaluate the physicochemical properties of different bioceramic-based root canal sealers.

Methods: Five bioceramic-based sealers; MTA Fillapex, TotalFill BC Sealer, BioRoot RCS, GuttaFlow Bioseal, Dia-Root Bio Sealer were compared with epoxy resin-based sealer (AH Plus) for this purpose. Ten samples of each sealer were prepared in according to the manufacturers' instructions and evaluated for radiopacity, solubility, flow and dimensional change tests. The data were statistically analyzed using One-Way ANOVA and Tukey post-hoc tests ($p<0.05$).

Results: The AH Plus showed statistically higher radioopacity than all tested bioceramic-based sealers ($p<0.01$). A significantly higher solubility rate was observed for TotalFill BC Sealer ($p<0.01$). Dia-Root Bio Sealer, BioRoot RCS and AH Plus showed solubility rate less than 3% in compliance with ISO standards. TotalFill BC Sealer and MTA Fillapex showed higher flow rates than other tested sealers ($p<0.01$). Dia-Root Bio Sealer, GuttaFlow Bioseal, BioRoot RCS, TotalFill BC Sealer and AH Plus exhibited expansion above 0.1%, while MTA Fillapex showed shrinkage less than 1%.

Conclusion: All the tested sealers met the ISO requirements for radioopacity and flow. Among the tested sealers only MTA Fillapex showed dimensional stability consistent with ISO standards. Dia-Root Bio Sealer, BioRoot RCS and AH Plus exhibited solubility rate in compliance with ISO standards. The recently introduced Dia-Root Bio Sealer demonstrated adequate radioopacity, flow and

ÖZ

Amaç: Bu araştırmanın amacı farklı biyoseramik bazlı kanal dolgu patlarının fizikokimyasal özelliklerinin değerlendirilmesidir.

Yöntemler: Bu amaçla beş biyoseramik esaslı kanal patı; MTA Fillapex, TotalFill BC Sealer, BioRoot RCS, GuttaFlow Bioseal, Dia-Root Bio Sealer, epoksi rezin esaslı kanal patı (AH Plus) ile karşılaştırıldı. Üretici firma talimatlarına uygun şekilde her bir kanal patından 10 adet örnek hazırlandı ve radyoopasite, çözünürlük, akıcılık ve boyutsal değişiklik testleri için değerlendirildi. Veriler tek yönlü ANOVA ve Tukey post-hoc testleri kullanılarak istatistiksel olarak analiz edildi ($p<0,05$).

Bulgular: AH Plus, test edilen tüm biyoseramik esaslı kanal patlarından istatiksel olarak daha yüksek radyoopasite göstermiştir ($p<0,01$). TotalFill BC Sealer diğer kanal patlarına göre anlamlı derecede daha fazla çözünürlük göstermiştir ($p<0,01$). Dia-Root Bio Sealer, BioRoot RCS ve AH Plus patları ISO standartlarına uygun olarak %3'ten daha az çözünürlük göstermiştir. TotalFill BC Sealer ve MTA Fillapex diğer kanal patlarından daha yüksek akıcılık göstermiştir ($p<0,01$). MTA Fillapex %1'den az büzülme gösterirken Dia-Root Bio Sealer, GuttaFlow Bioseal, BioRoot RCS, TotalFill BC Sealer ve AH Plus kanal patları %0,1'den fazla ekspansiyon göstermiştir.

Sonuç: Test edilen tüm kanal patları radyoopasite ve akıcılık açısından ISO standartlarını karşılaşmıştır. Test edilen kanal patları arasında sadece MTA Fillapex ISO standartlarına uygun boyutsal stabilite göstermiştir. Dia-Root Bio Sealer, BioRoot RCS ve AH Plus kanal patları ISO standartlarına uygun çözünürlük oranı

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ABSTRACT

solubility, but its dimensional change rate was found to be higher than required by ISO standards.

Keywords: Bioceramics, Dia-Root Bio Sealer, GuttaFlow Bioseal, MTA Fillapex, physical properties, TotalFill BC

ÖZ

göstermiştir. Yakın zamanda piyasaya sürülmüş olan Dia-Root Bio Sealer uygun radyoopasite, akıcılık ve çözünürlük göstermiştir fakat boyutsal değişiklik oranı ISO standartlarından daha yüksek bulunmuştur.

Anahtar Sözcükler: Biyoseramik, Dia-Root Bio Sealer, fiziksel özellikler, GuttaFlow Bioseal, MTA Fillapex, TotalFill BC

Introduction

The primary objective of an ideal root canal treatment is to achieve three-dimensional filling of the root canal system with inert materials. In an ideal root canal obturation, root canal sealers are employed with a semi-solid or solid core material, to create a leak-proof canal seal to establish a strong bond between the core material and the canal wall, effectively filling irregularities such as isthmuses and accessory canals (1).

Ideal root canal sealers should exhibit specific physical and chemical characteristics such as sufficient radioopacity, adequate film thickness, adherence to the root canal wall, dimensional stability and insolubility in tissue fluids beside biocompatibility (2). To assess the quality of root canal sealers, different physical properties must be investigated including flowability, radioopacity, solubility and dimensional stability. Each of these properties plays a crucial role in determining the efficacy and long-term stability of root canal obturation.

Although so many different endodontic sealers have been in endodontic practice for years, none of the available sealers meet all of these requirements, however AH Plus, an epoxy resin-based sealer comes remarkably close and is widely used as the gold standard regarding to its physical characteristics. Nevertheless, critics have pointed out that a significant drawback of AH Plus is its lack of biocompatibility (3). Concerns regarding the cytotoxicity of resin-based sealers lead researchers to focus on developing new sealers which are biocompatible and bioactive. Therefore bioceramic-based root canal sealers were introduced and garnered attention due to their excellent biological properties. The biological properties of these materials namely their biocompatibility and bioactivity, depend on the formation of hydroxyapatite on their surfaces as result of the production of calcium hydroxide ions (4).

In recent years a bioceramic-based root canal sealer, Dia-Root Bio Sealer (Diadent, Republic of Korea) is introduced to the market. Due to its recent introduction, there is no study in literature assessing the physical properties of Dia-Root Bio Sealer. This study aimed to fill that gap in literature by evaluating the physical properties of Dia-Root Bio Sealer, comparing it with other bioceramic-based root canal sealers (MTA Fillapex, TotalFill BC Sealer, BioRoot RCS and GuttaFlow Bioseal) and a resin-based sealer (AH Plus).

The null hypothesis of this study is that Dia-Root Bio Sealer has better physicochemical properties than other bioceramic-based root canal sealers; MTA Fillapex, TotalFill BC Sealer, BioRoot RCS, GuttaFlow Bioseal and resin-based sealer AH Plus in terms of radiopacity, solubility, flowability and dimensional change.

Methods

In this study, the physical properties of five bioceramic-based sealers including MTA Fillapex, Dia-Root Bio Sealer, GuttaFlow Bioseal, BioRoot RCS and TotalFill BC Sealer were compared with the epoxy resin-based sealer AH Plus (Table 1). The sample size for each test was determined with a power test of significance level α=0.05 and a power of 80%, requiring at least 4 samples per test. Based on previous studies, a sample size of 10 per group was chosen for a total of 60 samples (6 sealers x10 samples each) to ensure adequate statistical power in comparing the bioceramic-based sealers with AH Plus (5,6).

Radioopacity

Ten samples of each sealer (7.5±0.1 mm diameter, 1±0.1 mm height) were prepared according to the manufacturers' instructions. Once the samples had fully set under conditions of 37 °C and 95% humidity, they were subjected to radiography using a dental X-ray machine. This process was conducted alongside an aluminum step-wedge, incrementally graduated by 1 mm, spanning from 1 mm to 10 mm. The focus-object distance was set to 10 cm and radiographs were taken at 70 kVp, 8 mA and 0.17 seconds. The phosphor storage plates were scanned by Dürr Vistascan digital system (Dürr Dental AG, Bietigheim-Bissingen, Germany). The digital images were exported to ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The mean gray value (MGV) was calculated by selecting 3 different areas for each sealer and the aluminum step-wedge. By using regression analysis, a second-degree polynomial was fitted for the gray values of the aluminum steps. The MGV data for each sealer were converted into aluminum step-wedge equivalent thickness (mmAl) with using fitted polynomials.

Solubility

Sample disks (n=10 for each sealer) were prepared using molds (7.5±0.1 mm diameter, 2±0.1 mm height) and set at 37 °C and 95% humidity. After the sealers completely set, they were removed from molds and weighed 3 times using an analytical balance (Ohaus Corp. Pine Brook, NJ USA). The average weights for each sealer were recorded as initial mass (I). Then the samples were placed into 50 mL of distilled water and kept at 37 °C and 95% humidity for 24 h. They were weighed again

3 times and the average weights were recorded as final mass (F). The solubility (S) of each sealer was calculated using the formula S=[(I-F)/I]x100.

Flow

Onto a glass plate, 0.05 mL of freshly mixed material was placed (n=10). After 3 minutes of mixing, another glass plate (20 g) and a 100 g load were placed and kept for 10 minutes on the top of plate. The maximum and the minimum diameter resulting sealer disks were measured using a digital caliper (Mitutoyo Corporation, Japan) with a resolution of 0.01 mm. The mean diameter in mm was recorded as flow rate for each sealer.

Dimensional Change

The cylindrical molds (4 mm diameter, 4 mm height) containing freshly mixed sealers (n=10) were kept at 37 °C and 95% humidity until the specimens were completely set. The flat surfaces of each sample were grinded by a 600-grit wet sandpaper after removing from the mold. The length of each specimen was measured by using a digital caliper (Mitutoyo Corporation, Japan) with resolution 0.01 mm and recorded as initial length (L0). Then the specimens were placed in closed glass flasks containing distilled water and kept in an incubator at 37 °C. The heights of the samples were measured again on the 30th day and recorded as L30. The percentage of dimensional change was calculated with the formula DC=(L30-L0)/L30x100.

Statistical Analysis

Statistical analysis was performed using the SPSS 20.0 program. The data were evaluated statistically using one-way ANOVA and Tukey post-hoc tests at the level of significance ($p<0.05$).

Results

Radioopacity

Table 2 shows the MGV and the equivalent aluminum thickness of the sealers. All the sealers demonstrated radioopacity values above 3 mm of aluminum according to ISO 6876. AH Plus (10.23 mm Al) was found to be statistically the most radioopaque sealer ($p<0.01$). MTA Fillapex was found to be statistically less radioopaque than GuttaFlow Bioseal, TotalFill BC Sealer and AH Plus ($p<0.01$). Statistically no significant difference was found

Table 1. Names, manufacturers and composition of 6 tested sealers

Sealer	Manufacturer	Composition
MTA Fillapex	Angelus, Londrina, PR, Brazil	Base paste: salicylate resin, natural resin, calcium tungstate, nanoparticulated silica, pigments Catalyst paste: diluting resin, MTA, nanoparticulated silica, pigments
Dia-Root Bio Selaer	DiaDent, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Republic of Korea	calcium silicate, calcium aluminate, ytterbium trifluoride, zirconium dioxide, silanamine, 1,1,1-trimethyl-n-(trimethylsilyl)-, hydrolysis products with silica, hydroxypropyl methylcellulose, polyethylene glycol 400 and polyethylene glycol 200, polyoxyethylene (20) sorbitan monooleic acid, light mineral oil
GuttaFlow Bioseal	Coltene/Whaledent,Langenau, Switzerland	Gutta-percha powder, polydimethylsiloxane, platinum catalyst, zirconium oxide, silver, coloring, bioactive glass ceramic
BioRoot RCS	Septodont, Saint-Maur-des-Fossés, France	Powder: tricalcium silicate, zirconium oxide, povidone Liquid: aqueous solution of calcium chloride and polycarboxylate
TotalFill BC Sealer	FKG Dentaire, La Chaux-de-Fonds, Switzerland	calcium silicates, calcium phosphate monobasic, zirconium oxide, tantalum oxide and thickening agents
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	Bisphenol A/F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments, dibenzyldiamine, aminoadamantane, silicone oil

Table 2. Radioopacity, solubility, flow and dimensional change rates of sealers

	MTA Fillapex	Dia-Root Bio Sealer	GuttaFlow Bioseal	BioRoot RCS	TotalFill BC Sealer	AH Plus	p-value
Radioopacity (MGV) and (mmAl) (mean ± standart deviation)	88.11±4.68 3.09±0.07^{c,d}	106±10.9 4.11±0.55^{c,d}	108.22±11.17 4.27±0.91^{c}	96.55±3.97 3.54±0.44^{c,d}	138±5.09 6.47±0.46^{b}	177.33±3.48 10.23±0.26^{a}	<0.01*
Solubility (%) (mean ± standart deviation)	5.57±0.61^{b}	0.06±0.27^{d}	-3.56±0.39^{e}	1.93±0.42^{c}	7.63±0.39^{a}	-0.06±0.03^{d}	<0.01*
Flow (mm) (mean ± standart deviation	28.51±0.95^{a}	25.94±0.75^{b}	22.78±1.22^{c}	26.74±1.87^{b}	29.70±1.01^{a}	23.90±1.67^{c}	<0.01*
Dimensional change (%) (mean ± standart deviation)	-0.60±1.17^{b}	1.53±1.49^{a}	1.96±0.85^{a}	1.74±1.14^{a}	1.72±1.29^{a}	0.57±0.13^{a,b}	<0.01*

*Analysis of variance at the level of significance $p<0.05$. $^{a-e}$: Different letters in the same row indicate significant differences ($p<0.05$).

between radiopacity values of Dia-Root Bio Sealer-GuttaFlow Bioseal (p=0.99), Dia-Root Bio Sealer-BioRoot RCS (p=0.62) and GuttaFlow Bioseal-BioRoot RCS (p=0.42) (Table 3).

Solubility

Table 2 shows the average solubility values (%) for all used sealers. TotalFill BC Sealer (7.63%) was found statistically most soluble than other sealers (p<0.01). No statistically significant difference was found between the solubility rates of Dia-Root Bio Sealer and AH Plus (p=0.98).

GuttaFlow Bioseal (-3.56%) and AH Plus (-0.06%) showed negative solubility and GuttaFlow Bioseal was found less soluble than other sealers (Table 3).

Flow

The mean flow rates of experimented sealers were shown in Table 2. All sealers showed flow rates above 20 mm complying with the ISO requirements. TotalFill BC Sealer (29.7 mm) showed statistically higher flow rate than Dia-Root Bio Sealer, GuttaFlow Bioseal, BioRoot RCS and AH Plus (Table 3), however the difference was not significant between MTA Fillapex (28.51 mm) and TotalFill BC Sealer (p=0.34). Meantime, GuttaFlow Bioseal (22.78 mm) showed statistically lower flow rate than other sealers (p<0.01) but there was no significant difference between AH Plus (23.90 mm) and GuttaFlow Bioseal (p=0.40).

Dimensional Change

Dimensional change rates (%) for each sealer were shown in Table 2. All sealers showed expansion except MTA Fillapex (-0.60 %). GuttaFlow Bioseal (1.96%) exihibited the most expansion rate and AH Plus (0.57%) showed the minimum rate. However, the difference between the expansion rates of the root canal sealers was not statistically significant (Table 3).

Discussion

The radioopacity of root canal sealers is an important property that allows dentists to visualize and assess the quality of a root canal filling on radiograph. It is typically measured in millimeters of aluminum equivalent (mmAl), and higher values indicate greater radiopacity, which makes the material more visible on X-rays. Since the standard of ISO required for minimum radioopacity of root canal sealers was 3 mmAl, all sealers showed adequate radioopacity and met the ISO standards (7). However, it should be noted that the bioceramic-based root canal sealers were found to be less radiopaque than the resin-based sealer AH Plus (10.23 mmAl). The higher radioopacity value of AH Plus might be related to calcium tungstate which was found as an additional radioopacifier beside zirconium oxide. This result was collaborated with previous study results (3,5,6,8-11). TotalFill BC Sealer showed the second highest radioopacity value with 6.47 mmAl. Higher radioopacitiy of TotalFill BC Sealer compared to other bioceramic-based sealers can be explained with its composition. TotalFill BC Sealer contains zirconium oxide and tantalum oxide which are radioopacifiers, whereas BioRoot RCS, GuttaFlow Bioseal and Dia-Root Bio Sealer contain only zirconium oxide (Table 1). The different radioopacifiers may result in different radioopacity values of sealers (10). The radiopacity value of MTA Fillapex (3.09 mmAl) was found to be very close to the minimum threshold specified in ISO standards. In literature the radioopacity value of MTA Fillapex was found to be in range of 2.7 to 8.9 mmAl (12,13). Early versions of MTA Fillapex contained bismuth trioxide, but

Table 3. Comparisons of radioopacity, solubility, flow and dimensional change values among sealers

Sealers	Radioopacity (p value)	Solubility (p value)	Flow (p value)	Dimensional change (p value)
MTA Fillapex-Dia-Root Bio Sealer	0.09	<0.01*	<0.01*	<0.01*
MTA Fillapex-GuttaFlow Bioseal	0.04*	<0.01*	<0.01*	<0.01*
MTA Fillapex-BioRoot RCS	0.72	<0.01*	0.04*	<0.01*
MTA Fillapex-Totalfill BC Sealer	<0.01*	<0.01*	0.34	<0.01*
MTA Fillapex-AH Plus	<0.01*	<0.01*	<0.01*	0.18
Dia-Root Bio Sealer-GuttaFlow Bioseal	0.99	<0.01*	<0.01*	0.95
Dia-Root Bio Sealer- BioRoot RCS	0.62	<0.01*	0.74	0.99
Dia-Root Bio Sealer-TotalFill BC Sealer	<0.01*	<0.01*	<0.01*	0.99
Dia-Root Bio Sealer-AH Plus	<0.01*	0.98	0.01*	0.39
GuttaFlow Bioseal-BioRoot RCS	0.42	<0.01*	<0.01*	0.99
GuttaFlow Bioseal-TotalFill BC Sealer	<0.01*	<0.01*	<0.01*	0.99
GuttaFlow Bioseal-AH Plus	<0.01*	<0.01*	0.40	0.07
Bioroot RCS-TotalFill BC Sealer	<0.01*	<0.01*	<0.01*	1
BioRoot RCS-AH Plus	<0.01*	<0.01*	<0.01*	0.19
TotalFill BC Sealer-AH Plus	<0.01*	<0.01*	<0.01*	0.21

*Tukey post-hoc test at the level of significance p<0.05

due to its discoloring effects on the tooth structure, in the latest version of MTA Fillapex bismuth trioxide was replaced with calcium tungstate (14). This replacement might be the cause of the decrease in radioopacity value of MTA Fillapex which leaded different radioopacity values in different studies. In recent studies, results of the latest version of MTA Fillapex (containing calcium tungstate) reported notably lower radioopacity values ranging from 2.7 mmAl to 5.25 mmAl (4,12,15), in contrast to older studies such as 8.9 mmAl reported by Tanomaru-Filho et al (13).

Root canal sealers should have low solubility for a successful endodontic treatment in long-term. Resolution of sealers shouldn't exceed 3% mass fraction according to the ISO standards (7). In the present study, TotalFill BC Sealer (7.63%) and MTA Fillapex (5.57%) showed significantly higher solubility than the other tested sealers ($p<0.01$). Dia-Root Bio Sealer and BioRoot RCS showed solubility rates 0.06% and 1.93% consistent with ISO requirements. On the other hand, AH Plus (-0.06%) and GuttaFlow Bioseal (-3.56%) showed no dissolution. The high solubility of bioceramic-based sealers may be due to the hydrophilic nature of these materials whereas AH Plus is a hydrophobic material, so its solubility rate is very low compared to bioceramic-based sealers. All the measured solubility rates are in accordance with previous studies with the exception of GuttaFlow Bioseal. The reported solubility rates of TotalFill BC Sealer are ranging from 7.44% to 13.12% (6,11,16,17). Although there are studies reporting that the solubility of MTA Fillapex is less than 3% (15-20), there are also other reports giving the solubility rate of MTA Fillapex higher than 3% up to 25.69% (5,12,21,22). The increasing solubility of MTA Fillapex can be attributed to the change in the composition of the sealer. Tanomaru-Filho et al. (12) used MTA Fillapex, which contained calcium tungstate instead of bismuth oxide and reported the solubility rate of 25.69%. In the case of solubility of BioRoot RCS there are conflicted reports ranging from 1.17% to 37.6 % (5,20). In present study, solubility rate of BioRoot RCS measured as 1.93% as stated in above, which falls in the range of reported solubility rates of previous studies. This wide range of reported solubility rates of BioRoot RCS could be attributed to different experiment settings. Prüllage et al. (20) used stainless ring molds with an internal diameter of 20 mm in contrast to 8 mm specimens found in Siboni et al. (5). The solubility rates of GuttaFlow Bioseal are ranging from -0.75% to 3.03% (15,23). In this study, the solubility of GuttaFlow Bioseal was found to be -3,56% different to the results in previous studies. This might be due to differences in the methods used in the solubility test. In some studies, the samples were kept in distilled water for 7 days instead of 1 day and then kept in a dehumidifier for 24 hours before the solubility test (11,23,24). The conflicting results in the studies can be explained by the duration, the samples spent in distilled water and the differences in the drying methods applied to them before the solubility test.

Flow is an important factor for the root canal sealer to reach spaces such as lateral canals and irregularities that the core material cannot fill. There are different factors such as particle size, composition, shear rate, temperature and time from mixing that affect the flow rate (18). According to the ISO standards, root canal sealers should have a flow rate above 20 mm (7). In the present study, all the tested sealers exhibited a flow rate above 20 mm in compliance with the ISO standards. TotalFill BC Sealer showed the highest flow rate with 29.7 mm, which was in accordance with reported measurements of Kwak et al. (8) and Katakidis et al. (25). All bioceramic-based sealers exhibited greater flow rates than AH Plus except for GuttaFlow Bioseal. Relatively high flow rates of bioceramic-based sealers could be related to nano-sized particles of calcium silicates and zirconium. The lowest flow rate belonged to GuttaFlow Bioseal with 22.78 mm. The reason why GuttaFlow Bioseal has a lower flow rate can be related to the its specific composition which contains relatively large particles compared to other bioceramic-based sealers (26). The measured flow rate was in accordance with Tanomaru-Filho et al. (11) and Lin et al. (27) which reported the flow rate of GuttaFlow Bioseal as 16.88 mm and 25.73 mm respectively. Contrary to these studies, Lopes et al. (15) and Camargo et al. (23) reported the flow rate of GuttaFlow Bioseal as 34.43 mm and 35.4 mm respectively. This difference in flow rates can be attributed to the setting of their experiment. Both researchers used American Dental Association standards in which the volume of experimented sealer was 0.5 mL instead of 0.05 mL (28). The high flow rate in these studies may be related to the higher amount of sealer used.

Dimensional stability of root canal sealers is another important factor in success of endodontic treatment. Although minimum shrinkage is expected, excessive expansion may cause root fractures. According to the ISO standards the maximum expansion rate should be 0.1% and the maximum shrinkage rate should be 1% (7). In the present study, all tested sealers demonstrated higher expansion rates than recommended by ISO with the exception of MTA Fillapex. MTA Fillapex showed a shrinkage rate of 0.60% which fell in the interval defined by ISO standards. In the literature, previous studies have reported different values for the dimensional change rate of MTA Fillapex, ranging from -5.4% to -0.67% (15,18,21). The variation of these reported dimensional change rate was expected considering that some researchers even excluded MTA Fillapex in their measurements. Regarding to this, Lee at al. (10) reported that MTA Fillapex was not completely set in humid incubator even after one month and they had to exclude MTA Fillapex sealer from their study. The dimensional change rates for GuttaFlow Bioseal were reported between -0.68% and 3.23% in different studies (11,15,23,27). The result of the present study was also in this range with the rate of 1.96%. In the literature, there is only one study reporting the dimensional change rate of BioRoot RCS as 1.23% in accordance with the present study (27). Among the tested root canal sealers in the present study, AH Plus showed the most constant mass similar to the previous studies results (6,10,11,18). The dimensional change test of root canal sealers relies on the measurements of height of sample but in real world changes in dimension occur in three dimensions. The width and the breadth of the samples also undergo a dimensional change. Therefore, using techniques such as micro-computed

tomography which can measure the volume change, may yield more meaningful results for dimensional change test.

The null hypothesis was rejected in this study indicating that Dia-Root Bio Sealer did not exhibit better physicochemical properties than other tested sealers regarding radioopacity, solubility, flow and dimensional change. The radioopacity value of Dia-Root Bio Sealer was lower than TotalFill BC Sealer and AH Plus and there was no significant difference among radioopacity values of Dia-Root Bio Sealer, GuttaFlow Bioseal and BioRoot RCS (Table 3). Although Dia-Root Bio Sealer showed lower solubility than other bioceramic-based sealers, there was no significant difference between it and AH Plus (p=0.98). The flowability of Dia-Root Bio Sealer was higher than GuttaFlow Bioseal and AH Plus, but no significant difference was observed between it and BioRoot RCS (p=0.74). In terms of dimensional change, Dia-Root Bio Sealer showed significantly lower values than other bioceramic-based sealers except for MTA Fillapex (Table 2).

Study Limitations

In this study, the physical properties of some bioceramic-based root canal sealers were evaluated *in vitro*. *In vivo* conditions may affect the physical properties of these sealers.

Conclusion

Dia-Root Bio Sealer is a recently introduced bioceramic-based sealer and there is no study in literature regarding its physical properties. This study's results showed that Dia-Root Bio Sealer had adequate radioopacity, solubility and flow complying with ISO standards similar to AH Plus. In addition to this, both sealers exhibited similar dimensional change rates validated by statistical tests. According to the result of this study, Dia-Root Bio Sealer showed comparable physical characteristics with AH Plus and might be preferred in clinical applications.

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Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: N.G., Design: N.G., Data Collection or Processing: D.B.M., N.G., Analysis or Interpretation: D.B.M., Literature Search: D.B.M., Writing: D.B.M., N.G.

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Original Article

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Sleep Problems and Influencing Factors in Children Aged 0-3 Years

0-3 Yaş Grubu Çocuklarda Uyku Sorunları ve Etkileyen Faktörler

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ABSTRACT

Objective: The aim of this study was to identify sleep problems in children aged 0-3 years and the factors influencing them.

Methods: This descriptive correlational study was conducted online with 366 parents of children aged 0-3 years. Children's sleep problems and influencing factors were assessed using a seven-question demographic form, and the Brief Infant Sleep Questionnaire was used to assess infant sleep.

Results: Children aged 0-3 years had a late bedtime (21:30) and were awake more than twice a night. They slept an average of 2 hours during the day and 5 hours of uninterrupted sleep at night. Regardless of gender, they slept a total of 10 hours per night. Children who did not have a regular bedtime (54.2%) and had late sleep onset (51.5%) had more sleep problems. In total, 21.5% of the children were awake more than three times a night, 12.7% were awake for more than one hour at night, 8.8% had a total sleep time of less than 9 hours and 34.4% had sleep problems. Children with a total sleep time of less than 9 hours were more likely to have sleep problems.

Conclusion: Children aged 0-6 months living in extended families and sleeping out of bed had more sleep problems. The results of the study shed light on factors such as the prevalence of sleep problems in children aged 0-3 years, sleep ecology and hygiene.

Keywords: Sleep, child sleep, parents, infant, toddler

ÖZ

Amaç: Bu çalışmanın amacı 0-3 yaş grubundaki çocukların uyku problemlerini belirlemek ve bu problemleri etkileyen faktörleri tespit etmektir.

Yöntemler: Bu tanımlayıcı korelasyonel tipteki çalışma 0-3 yaş arası çocuğu olan 366 ebeveyn ile çevrimiçi olarak yürütüldü. Çocukların uyku sorunları ve etkileyen faktörleri değerlendirmek için yedi sorudan oluşan bir demografik anket kullanıldı ve çocukların uyku durumunu değerlendirmek için Kısa Bebek Uyku Anketi kullanıldı.

Bulgular: Araştırma, çalışmaya dahil edilen 0-3 yaş aralığındaki çocukların geç yattığını (21:30) ve gece boyunca iki veya daha fazla kez uyandığını ortaya koydu. Ortalama gündüz uykusu süresi yaklaşık 2 saat iken, gece boyunca kesintisiz olarak ortalama 5 saat uyudukları görüldü. Cinsiyet fark etmeksizin, çocukların toplamda 10 saat uyudukları belirlendi. Özellikle düzenli yatma zamanı olmayan (%54,2) ve geç yatan çocukların (%51,5) daha yüksek oranda uyku sorunları yaşadığı görüldü. Toplamda çocukların %21,5'i gecede üç kereden fazla uyanık kalmakta, %12,7'si geceleri bir saatten fazla uyanık kalmakta, %8,8'inin toplam uyku süresi 9 saatten az olmakta ve %34,4'ü uyku sorunu yaşamaktaydı. Özellikle toplam uyku süresinin yetersiz olması (9 saatten az) ile uyku sorunları arasında güçlü bir ilişki saptandı.

Sonuç: Araştırma bulguları, geniş ailelerde yaşayan ve belirlenmiş yataklarının dışında uyuyan 0-6 aylık çocuklar arasında uyku sorunlarının daha yüksek bir yaygınlığını ortaya koymaktadır. Bu çalışma, 0-3 yaş grubundaki çocuklarda uyku sorunlarının yaygınlığını, uyku alışkanlıklarını ve hijyen uygulamalarını etkileyen faktörleri aydınlatmaktadır.

Anahtar Sözcükler: Uyku, çocuk uykusu, ebeveynler, süt çocuğu, oyun çocuğu

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Introduction

Sleep is an active neurophysiological process and the primary activity of the developing brain (1). It is a highly dynamic process with high inter-individual variability, particularly in the first year of life. Adequate sleep in infants and children is essential for physical growth, memory, language, executive function, and general cognitive development (2). In studies, 17% (3) and 35% (4) of parents reported that their infants and young children had sleep problems.

Sleep problems are defined as nightly sleep that is at least one standard deviation below normal (5). Sleep disturbance is typically defined as sleep onset latency, short nocturnal sleep, and frequent nocturnal awakenings reported by parents (5). The most common sleep problems are night waking in infancy (40.8%) and difficulty sleeping alone at 2-3 years of age (20.0%) (3). Studies have reported many negative effects of sleep problems on children's development. Late bedtimes and less 24-hour sleep have been associated with anxiety, depression, distractibility, behavioural problems and academic performance (6). Evidence shows that short sleep duration and late bedtimes are associated with childhood overweight and obesity (7). In addition, infant sleep problems can lead to maternal sleep problems (8). This situation may increase the risk of postpartum depression in mothers (9).

Cross-cultural differences have been found in both child sleep problems and parents' perceived distress due to these problems (10,11). Limited studies have examined sleep problems and factors affecting children (12-14) aged 0-3 years in Türkiye. Identifying infant sleep problems reported by mothers during infancy and early childhood, and the factors associated with them, may contribute to the development of early diagnosis and intervention strategies. This study was conducted to determine the sleep problems of 0-3 year old children and the factors affecting them.

Research Questions

What are the sleep habits of 0-3 year old children?

What are the sleep problems of 0-3 year old children?

What factors cause sleep problems in 0-3 year old children?

Methods

Study Design and Sample

This descriptive correlational study was conducted online between January 2021 and January 2022. The study population consisted of parents with a 0-3 year old child living in Türkiye who agreed to participate. The snowball sampling method, a non-probability sampling method, was used in the study. According to the snowball sampling method, individuals were reached through online platforms, intermediaries, and other participants. The study sample consisted of 366 parents who were reached through online platforms who agreed to participate and whose child did not have an acute illness in the past week. Three children with chronic diseases (hypothyroidism, ALL, chronic heart disease) were excluded from the study because their sleep duration and quality would be affected. As a result, 363 parents were included in the study.

Data Collection Tools

Information form included 26 questions about child, family, and sleep habits, as well as demographic questions designed to be consistent with existing literature (12-15).

Brief Infant Sleep Questionnaire (BISQ) was developed by Sadeh (16) to assess sleep behaviors and problems in early childhood. The BISQ consists of 29 questions to be answered by the parent regarding the sleep pattern of a child aged 0-3 years. There are seven variables as sleep criteria in the Turkish BISQ; 1) nighttime sleep duration, 2) daytime sleep duration, 3) number of nighttime awakenings, 4) duration of wakefulness during nighttime hours, 5) time of nighttime sleep onset, 6) settling time, 7) total sleep time (16).

By evaluating these criteria obtained from the BISQ, the total sleep time is calculated by adding the nighttime and daytime sleep time and subtracting the nighttime wake time. If at least one of the following three conditions is observed, the child is considered to have sleep problems; 1) nocturnal awakening more than three times per night, 2) nocturnal awakening for more than one hour, 3) total sleep duration of less than 9 hours (16). In the Turkish validity and reliability study conducted by Dasdemir and Temel (17), the time-dependent invariance of the questionnaire was r=0.35-0.85.

Procedure

The study was conducted online. A total of 366 parents agreed to participate in the study online. Parents were asked to complete the study forms considering their children's sleep status during the previous week. Parents who had a child with an acute illness in the previous week were not included in the study. Parents first completed the child and parent information form and then the BISQ.

Statistical Analysis

The SPSS for Windows (Statistical Package for Social Sciences for Windows, version 23.0) was used to analyze the data. The number percentage distribution was used to describe demographic characteristics, descriptive statistics were used to describe continuous variables (mean, standard deviation, minimum, median, maximum), and the Kolmogorov-Smirnov test was used to assess the conformity of the data with the normal distribution. Categorical variables were analyzed using the chi-square test. The Kruskal-Wallis test was used to compare three or more sets of quantitative data that were not normally distributed. Logistic regression analyses were performed using sleep ecology and demographic variables as predictors of sleep, and a p-value <0.05 was considered significant.

Ethical Issues

Ethical approval was obtained from the Social and Human Sciences Research Ethics Committee of a İstanbul University-Cerrahpaşa, (decision no: 2020/282, date: 05.01.2021). Parents participating in the study were informed online, and sent an informed consent form.

Results

There was a significant difference between the children's age and the family type and having sleep problems ($p<0.05$). A total of 67.2% of children aged 0-7 months and 51.9% of children living in extended families had sleep problems. There was a significant difference between whether the child watched television, played with a phone/tablet, or watched videos/cartoons and whether the child had sleep problems ($p<0.05$). A total of 41.8% of children who did not watch television, 37.2% of those who did not play games with a phone/tablet, and 41.1% of those who did not watch videos/cartoons with a phone/tablet had sleep problems. It was found that there was a significant difference ($p<0.05$) between the place where the children usually slept and the state of having sleep problems, and 42.5% of the children who slept in their parents' bed had sleep problems. There was a significant difference ($p<0.05$) between the children's way of falling asleep, sleeping at the same time, difficulty of falling asleep and sleep problems. A total of 53.3% of the children who slept on their laps, 54.2% of the children who slept at the same time never/1-2 nights per week, and 51.5% of the children who had very late sleep onset times had sleep problems. There was a significant difference between the child's diet and sleep problems ($p<0.05$). Of breastfed children 51.1% had sleep problems. There was no significant difference between gender, maternal work status, maternal education status and sleep problems ($p>0.05$) (Table 1).

Table 1. The comparison of children's demographic characteristics and sleep problems (n=363)

Demographic characteristics			Sleep problems		x^2/p
			Yes	No	
	n	%	n (%)	n (%)	
Age of children (month)					
0-6	70	19.3	34 (48.6)	36 (51.4)	54.591/0.000*
7-12	61	16.8	41 (67.2)	20 (32.8)	
13-24	100	27.5	27 (27.0)	73 (73.0)	
25-36	132	36.4	23 (17.4)	109 (82.6)	
Gender					
Girl	162	44.6	49 (30.2)	113 (69.8)	2.273/0.132
Boy	201	55.4	76 (37.8)	125 (62.2)	
Mother's working status					
Yes	179	49.3	59 (33.0)	120 (67.0)	0.340/0.560
No	184	50.7	66 (35.9)	118 (64.1)	
Mother's education level					
High school or below	76	21	31 (40.8)	45 (59.2)	2.375/0.305
Bachelor	227	62.5	77 (33.9)	150 (66.1)	
Postgraduate	60	16.5	17 (28.3)	43 (71.7)	
Family type					
Nuclear	336	92.6	111 (33.0)	225 (67.0)	3.919/0.048*
Extended	27	7.4	14 (51.9)	13 (48.1)	
Child watching television					
Yes	165	54.5	56 (28.3)	142 (71.7)	7.303/0.007*
No	198	45.5	69 (41.8)	96 (58.2)	
Child playing with a phone or tablet					
Yes	73	20.1	17 (23.3)	56 (76.7)	5.029/0.025*
No	290	79.9	108 (37.2)	182 (62.8)	
Child watching videos/cartoons with a phone or tablet					
Yes	156	43.0	40 (25.6)	116 (74.4)	9.371/0.002*
No	207	57.0	85 (41.1)	122 (58.9)	
Where the child usually sleeps					

Table 1. continued

Demographic characteristics	n	%	Sleep problems Yes n (%)	No n (%)	x^2/p
In his/her room	170	46.9	44 (25.9)	126 (74.1)	
In the parents' room	127	35.0	54 (42.5)	73 (57.5)	10.410/0.005*
In another room of the house	66	18.1	27 (40.9)	39 (59.1)	
Where the child sleeps most of the time					
In his/her own bed	322	88.7	106 (32.9)	216 (67.1)	
In the parent's bed	29	8.0	12 (41.4)	17 (58.6)	3.983/0.137
Other (swing, pram, etc.)	12	3.3	7 (58.3)	5 (41.7)	
The way of falling asleep					
Sucking	132	36.4	53 (40.2)	79 (59.8)	
Rocking/stroller etc.	83	22.8	28 (33.7)	55 (66.3)	
Lap	30	8.3	16 (53.3)	14 (46.7)	
Alone in bed	28	7.7	5 (17.9)	23 (82.1)	13.226/0.010*
In a room with the family	90	24.8	23 (25.6)	67 (74.4)	
Sleeping at the same time at night					
None/1-2 nights a week	48	13.2	26 (54.2)	22 (45.8)	
3-4 nights a week	93	25.6	34 (36.6)	59 (63.4)	
5 nights a week	108	29.8	32 (29.6)	76 (70.4)	11.088/0.011*
Nightly	114	31.4	33 (28.9)	81 (71.1)	
The difficulty level of sleep-onset time					
Very easy	33	9.1	6 (18.2)	27 (81.8)	
A little easy	58	16.0	18 (31.0)	40 (69.0)	
Neither easy nor difficult	148	40.7	43 (29.1)	105 (70.9)	
A little difficult	91	25.1	41 (45.1)	50 (54.9)	14.866/0.005*
Very difficult or difficult	33	9.1	17 (51.5)	16 (48.5)	
Nutrition type					
Breast milk	47	12.9	24 (51.1)	23 (48.9)	
Breast milk + formula	17	4.7	6 (35.3)	11 (64.7)	
Breast milk + complementary food	145	39.9	65 (44.8)	80 (55.2)	
Formula	13	3.6	6 (46.2)	7 (53.8)	
Formula + complementary food	25	6.9	6 (24.0)	19 (76.0)	33.082/0.000*
Complementary food	116	32.0	18 (15.5)	98 (84.5)	
	Mean ± SD			**Min-Max**	
Mother's age	31.14±4.44			21.00-58.00	
Father's age	33.89±4.79			25.00-62.00	

*p<0.05, x^2: Chi-square test, SD: Standard deviation, Min-Max: Minimum-Maximum

The distribution of criteria for determining sleep problems in children according to the BISQ was examined. Of the children, 21.5% were awake more than three times per night, 12.7% were awake for more than one hour per night, and 8.8% had a total sleep duration of less than 9 hours. Considering all these data, it was found that 34.4% (n=238) of the children had sleep problems.

There was a significant difference between the median frequency of nighttime awakenings according to the age groups of the children (p<0.05). The median of children aged 7-12 months was higher than that of other age groups. The median of children aged 0-6 months and 13-24 months was higher than that of children aged 25-36 months. A significant difference was found between the medians of nocturnal wake duration according to the

age groups of children (p<0.05). It was found that the median of children aged 0-6 months was higher than the median of children aged 13-24 and 25-36 months, and the median of children aged 13-24 months was higher than the median of children aged 25-36 months. The median of children aged 7-12 months was higher than that of children aged 25-36 months. There was a significant difference between the medians of uninterrupted nighttime sleep duration according to the age groups of the children (p<0.05). The median of children aged 7-12 months was lower than that of other age groups, and the median of children aged 0-6 months and 13-24 months was lower than that of children aged 25-36 months. There was a significant difference between the medians of total nocturnal sleep duration according to the age groups of the children (p<0.05). The median of children aged 0-6 months was lower than that of children aged 13-24 and 25-36 months. There was a significant difference between the medians of daytime sleep duration according to the age groups of the children (p<0.05). The median of infants aged 0-6 months was higher than the other age groups, and the median of infants aged 7-12 months was higher than that of children aged 13-24 months and 25-36 months. The median of children aged 13-24 months was higher than that of children aged 25-36 months. There was a significant difference between the medians of total sleep duration according to the age groups of the children (p<0.05). The median of children aged 0-6 months, 7-12 months, and 13-24 months was higher than that of children aged 25-36 months (Table 2).

The percentage of explanation of the seven-variable logistic regression model, which predicting the children's sleep problems, was calculated as to be 21%. The model's initial -2 Log likelihood value of the model was 467.495 (Initial -2 Log-Likelihood: 467.495). The -2 Log likelihood value of the generated model was lower as 407.990. Of the two variables that predicted their children's sleep problems, the strongest predictor was the child's age, with sleep problems in 0-6 month/7-12 month children odds ratio (OR): 5.00 [95% confidence interval (CI): 2.610-9.601] times higher. The second predictor variable was where the child usually slept. Sleep problems were OR: 2.24 (95% CI: 1.376-3.648) times higher in children who slept in their parents' bed/in another room (Table 3).

Discussion

Sleep problems in young children are common and often persistent, which can lead to negative outcomes in later life (3). The current literature has reported that the rate of children with sleep problems is increasing daily (18). Considering the results of this study, children aged 0-3 years had late bedtimes (9:30 pm) and and nighttime awakenings more than two times per night. They sleep an average of 2 hours during the day and 5 hours of uninterrupted sleep at night. Regardless of gender, they sleep a total of 10 hours per night. Children aged 0-3 years (11.8) had shorter sleep duration compared to children in Caucasian (13.0) and Asian (12.3) countries, while they had similar sleep duration with children in the Middle Eastern (11.7) countries (19). According to a multinational study, the total sleep duration in 0-3 year old children is 11.6±1.5 hours, and they sleep an average of 2.2±0.7 hours during the day (10). Sadeh (16) showed significant differences between countries and cultures in sleep patterns, parental behaviors, and sleep context. It is believed that the differences in the results are due to the differences that can be seen in the sleep patterns and sleep habits of children living in culturally different countries/regions.

Regarding the adequacy of sleep duration, children in Türkiye tend to sleep close to the recommended time [11 h (toddlers) and 12 h (infants)] for their age group (11.8 h) (20). The results of the current study are consistent with studies of sleep in similar age groups. In the study of Kahraman and Ceylan (13) the mean nighttime sleep duration of children was 9 h and above, the mean daytime sleep duration was 2 h, and the mean total sleep duration was 10.83±3.04 h. Kahraman Berberoğlu and Çalışır (14) reported that the total sleep duration of infants aged 3-12 months was approximately 10 hours. The uninterrupted

Table 2. Children's sleep measurements according to BISQ and age groups (n=363)

	0-36 months	0-6 months (a)	7-12 months (b)	13-24 months (c)	25-36 months (d)	
	Median (IR 25-75p)	Median (IR 25-75p)	Median (IR 25-75p)	Median (IR 25-75p)	Median (IR 25-75p)	KW/p
Number of night wakings	2.00 (1.00-3.00)	3.00 (2.00-4.00)	4.00 (2.00-4.00)	2.00 (1.00-3.00)	1.00 (0.00-2.00)	85.074/0.000* b>a=c>d†
Nocturnal wakefulness (min.)	10.00 (5.00-30.00)	30.00 (10.00-60.00)	15.00 (2.50-60.00)	15.00 (5.00-30.00)	10.00 (0.00-30.00)	35.419/0.000* a>c>d†. b>d†
Uninterrupted sleep duration (hour)	5.00 (3.50-9.00)	5.00 (3.00-7.00)	3.00 (2.75-5.00)	5.25 (3.12-9.87)	8.00 (5.00-10.00)	59.777/0.000* b<a=c<d†
Nocturnal sleep duration (hour)	10.00 (9.00-11.00)	9.00 (8.00-11.00)	10.00 (9.00-10.75)	10.00 (9.50-11.00)	10.00 (9.12-11.00)	13.840/0.003* a<c=d†
Daytime sleep duration (hour)	2.00 (1.50-3.00)	4.00 (3.00-5.00)	2.50 (2.00-3.25)	2.00 (1.50-2.00)	1.50 (0.31-2.00)	147.264/0.000* a>b>c>d†
Total sleep duration (hour)	11.83 (10.75-12.91)	12.66 (11.00-14.00)	12.41 (11.00-13.58)	11.91 (10.75-12.89)	11.25 (10.41-12.00)	30.990/0.000* a=b=c>d†

*p<0.05, KW: Kruskal-Wallis test, †: P-value obtained as a result of Bonferroni correction p<0.008, BISQ: Brief Infant Sleep Questionnaire

Table 3. Regression model for some demographic characteristics predicting children's sleep problems (enter method)

Sleep problems Nagelkerke R^2=0.209
(0: No, 1: Yes)

Independent variables	β	S.H	Wald	SD	p	Exp (β) %95 Cl
Constant	-2.004	0.381	27.665	1	0.000	0.135
Age (0: 13-24 month/25-36 ay, 1: 0-6 ay/7-12 month)	**1.611**	**0.332**	**23.498**	**1**	**0.000**	**5.006** **(2.610-9.601)**
Watching TV (0: No, 1: Yes)	0.251	0.286	0.769	1	0.380	1.285 (0.733-2.253)
Playing games with a phone/tablet (0: No, 1: Yes)	0.039	0.365	0.011	1	0.916	1.039 (0.508-2.127)
Watching videos/cartoons with a phone/tablet (0: No, 1: Yes)	-0.133	0.306	0.188	1	0.665	0.876 (0.481-1.595)
Falling asleep (0: Alone in bed/room, in bed with family, 1: While suckling, rocking/stroller, etc., on lap)	0.083	0.292	0.081	1	0.776	1.087 (0.613-1.927)
Sleeping at the same time (0: Every night, 5-nights a week, 1: Never/1-2 nights a week, 3-4 nights a week)	0.251	0.255	0.969	1	0.325	1.286 (0.780-2.120)
Where the child usually sleeps (0: In his/her room/bed, 1: In the parents' bed, in another)	**0.807**	**0.249**	**10.508**	**1**	**0.001**	**2.240** **(1.376-3.648)**

SD: Standard deviation, CI: Confidence interval

sleep duration was 4 hours at night and 2 hours during the day. Taşdemir and Temel (12) stated that the average total sleep duration in the first 3 years of life was 11.32±3.0 hours, the uninterrupted sleep duration at night was 4 hours, and the daytime sleep duration was 2.33 hours.

Establishing a regular bedtime routine is a crucial parental practice to promote healthy sleep (21). Late sleep onset or bedtime negatively affects nighttime sleep duration (22). In the current study, children who did not have regular bedtime (54.2%) and had late sleep onset (51.5%) had more sleep problems. Consistent with this study in the literature, common sleep problems in young children include nocturnal insomnia, difficulty falling asleep, number of nighttime awakenings, and late sleep onset (18).

In the current study, 21.5% of the children had night waking more than three times a night, 12.7% had night waking for more than one hour, 8.8% had total sleep duration less than 9 hours, 34.4% had sleep problems. At the same time, it was found that children with a total sleep duration of less than 9 hours had more sleep problems. Wearick-Silva et al. (23) reported that 58.6% of children aged 0-3 years had sleep problems during the Coronavirus disease 2019 pandemic. They found that children most commonly had problems with night waking more than three times per night (23.1%), night waking (39.4%), and total sleep time less than 9 hours (30.7%). The number of nocturnal awakenings in the current study is similar to that of Wearick-Silva et al. (23). However, other results were higher in Wearick-Silva et al. (23) because research data were collected early in the pandemic (7^{th} week of quarantine). In the present study, data were collected later in the pandemic.

Sleeping in their own bed or elsewhere, especially with their parents, affects children's sleep differently. While some parents have reported that co-sleeping with their child reduces the child's sleep quality and crying duration, some studies have suggested that bed-sharing may be associated with more awakenings and shorter sleep duration during the night (24). Studies have reported that children who sleep in the same bed with their parents rather than in their own bed have more sleep problems (18,19). In the current study, sleep problems were 2.24 times more common in children who slept in their parents' bed or in another room. In total, 53.3% of the children who slept on their laps and 42.5% of the children who slept in their parents' bed had sleep problems. The results of the current study are therefore compatible with other studies.

Emond et al. (25) reported that increased screen exposure in infants aged 3-12 months was associated with decreased nighttime sleep. Various studies have shown a significant impact of screen time on sleep problems in children, resulting in problems such as short sleep duration, poor sleep quality, and irregular bedtimes (26-28). Screens of touch-sensitive handheld devices have been found to contribute to increased screen time for children and to disrupt sleep by distracting them (26). In a study of the same age group, 33.3% of children given tablets or phones experienced sleep problems (13). In the current study, 28.3% of children who watched television, 23.3% of children who played games on phones or tablets, and 25.6% of children who watched cartoons

on phones or tablets reported experiencing sleep problems. The lower prevalence of sleep problems in our study compared to the literature was likely due to the higher educational level of the mothers (62% with a bachelor's degree).

While the need for sleep is highest in the neonatal period, this rate gradually decreases in subsequent months and ages. Valla (29), in the study of sleep problems in 1,555 infants aged 6-12 months, found that 3-14% of infants aged 6-24 months had sleep problems reported by their parents at a maximum of 6 months and at least 24 months. In the study by Yılmaz-Kurt et al. (30), the highest rate of sleep problems was reported in children aged 0-12 months (65.1%). Similar to the literature, in our study, 48.6% of infants aged 0-6 months and 67.2% of infants aged 7-12 months had sleep problems.

There are different sleep-wake cycles, especially in the neonatal period (31). In the current study, there was a significant difference between the age groups of the children and the medians of the frequency of nocturnal wakings, duration of nocturnal awakenings, and uninterrupted nocturnal sleep, total nocturnal sleep, daytime sleep, and total sleep. The decrease in daytime sleep duration is mainly explained by the age of the child (16). In the current study, total sleep duration in children was 12.66 hours in the first six months and 11.91 hours between 13 and 24 months. A systematic review (32) found this rate to be 12.9 hours for the first six months and 12.6 hours for the 24 months. Paavonen et al. (33) found sleep duration of 13.7 hours in children aged 0-6 months and 11.9 hours at 24 months. While the total sleep duration by age obtained in the current study was similar to that of Galland et al. (32), it was lower than that of Paavonen et al. (33). The sample size, different definitions of sleep problems in different cultures, and different measurement instruments used may affect the frequency of sleep problems in children (34). Based on this, the different results obtained in the current study might be because the 0-6 month group was smaller in proportion, cultural differences, and individual sleep had different normative characteristics.

Study Limitations

The sampling method of this study was the first limitation. Despite the large number of participants and data from different provinces, the study participants could not be generalized to the Turkish population. The second limitation was that the participants were not proportionally sampled from all regions of the country. The third limitation was the use of online platforms for data collection. This provided access to all segments of society. However, this might allow only those interested in the topic to participate in the study. Accordingly, the fourth limitation was that parents who did not have an internet connection or a smartphone did not have access to this study.

Conclusion

In conclusion, this study found that children aged 0-6 months living in extended families and sleeping outside their beds had more sleep problems. The results of the study provide insight into factors such as the prevalence of sleep problems in children aged 0-3 years, sleep ecology, and hygiene. The results are expected to contribute to studies on the diagnosis, evaluation and prevention of sleep-related problems that may occur at an early age. In the future, it is recommended to carry out studies that test attempts to prevent sleep problems in children aged 0-3 years.

Nurses, who play an active role in the development of health, can identify and evaluate situations that may cause sleep problems in children, in collaboration with the family, at all levels of the health system, using standardized measurement tools appropriate for different cultures. Depending on the cause, this study can also support the planning of counseling and education services for families. These counseling and education services should focus on normative sleep duration, healthy sleep habits, sleep ecology, and hygiene according to age groups.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Social and Human Sciences Research Ethics Committee of a İstanbul University-Cerrahpaşa, (decision no: 2020/282, date: 05.01.2021).

Informed Consent: Parents participating in the study were informed online, and sent an informed consent form.

Authorship Contributions

Concept: M.A., N.K., K.Ş., S.B., Design: M.A., N.K., K.Ş., S.B., Data Collection or Processing: M.A., N.K., K.Ş., S.B., Analysis or Interpretation: M.A., N.K., K.Ş., S.B., Literature Search: M.A., N.K., K.Ş., S.B., Writing: M.A., N.K., K.Ş., S.B.

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Original Article

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Effect of Eight Weeks of Reformer Pilates on Shoulder Proprioception Dynamic Stability and Functionality

Sekiz Haftalık Reformer Pilatesin Omuz Propriosepsiyonu Dinamik Stabilitesi ve Fonksiyonu Üzerine Etkisi

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ABSTRACT

Objective: This study aimed to compare shoulder proprioception, dynamic stability, and upper extremity function between Reformer Pilates practitioners and asymptomatic individuals.

Methods: The study included twenty individuals who practiced Reformer Pilates for eight weeks (25.45±2.56) as the case group, and twenty asymptomatic individuals (25.70±1.80) of similar age and gender, who did not participate in any exercise program, as the control group. Shoulder proprioception was assessed using the Clinometer mobile app [4.3.1 (1412091) on IOS] using an active joint position sense. The function, dynamic balance, and stability of the upper extremity and trunk were evaluated with the Y balance test - upper quarter. The dynamic stability of the shoulder was evaluated with the Closed Kinetic Chain Upper Extremity Stability test.

Results: Shoulder joint position sense ($p<0.001$, $d=1.64$ to 3.07), upper extremity functionality ($p<0.001$, $d=1.49$ to 2.35), and dynamic stability ($p<0.001$, $d=2.82$) were found significantly better in the Reformer Pilates group.

Conclusion: Reformer Pilates practitioners have better shoulder joint position sense, upper limb functionality, and dynamic stability.

Keywords: Pilates training, shoulder joint, proprioception, joint instability, sedentary behavior

ÖZ

Amaç: Bu çalışmanın amacı, Reformer Pilates uygulayıcıları ile asemptomatik bireyler arasında omuz propriyosepsiyonu, dinamik stabilite ve üst ekstremite fonksiyonunu karşılaştırmaktır.

Yöntemler: Sekiz hafta boyunca Reformer Pilates yapan 20 birey (25,45±2,56) olgu grubu olarak ve benzer yaş ve cinsiyetteki herhangi bir egzersiz programına katılmayan 20 asemptomatik birey (25,70±1,80) kontrol grubu olarak dahil edildi. Omuz propriyosepsiyonu Clinometer mobil uygulaması [iOS'ta 4.3.1 (1412091)] kullanılarak aktif eklem pozisyon hissi ile değerlendirilmiştir. Üst ekstremite ve gövdenin fonksiyonu, dinamik dengesi ve stabilitesi üst çeyrek Y dengesi testi ile değerlendirildi. Omzun dinamik stabilitesi Kapalı Kinetik Zincir Üst Ekstremite Stabilite testi ile değerlendirildi.

Bulgular: Reformer Pilates grubunda omuz eklemi pozisyon hissi ($p<0,001$, $d=1,64$-$3,07$), üst ekstremite fonksiyonu ($p<0,001$, $d=1,49$-$2,35$) ve dinamik stabilitesi ($p<0,001$, $d=2,82$) anlamlı derecede daha iyi bulundu.

Sonuç: Reformer Pilates uygulayıcıları daha iyi omuz eklemi pozisyon hissine, üst ekstremite fonksiyonuna ve dinamik stabilitesine sahiptir.

Anahtar Sözcükler: Pilates eğitimi, omuz eklemi, propriyosepsiyon, eklem instabilitesi, sedanter yaşam

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Introduction

The shoulder is one of the most commonly used joints for everyday tasks such as eating, drinking, grooming, and using technology. The shoulder is prone to overuse injuries, including subacromial impingement syndrome, rotator cuff tendinitis, biceps tendinitis, and frozen shoulder (1). Shoulder disorders are the third most common disease of the musculoskeletal system (2) and the second most common musculoskeletal issue encountered in physical therapy (3). To improve musculoskeletal health and prevent these diseases regular physical activity is recommended (4). Regular physical activity is a key factor for aging well and preventing chronic diseases (5) such as diabetes (6), hypertension, and dementia (7). In recent years, physical activities that involve large muscle group movements such as yoga, tai chi, and Pilates have become more popular (8).

Pilates is a mind-body exercise that is used in rehabilitation and is becoming more and more popular as a method of rehabilitation. Pilates exercises increase the strength of the deep core stabilization muscles and the control of the mind over the movements of the body and limbs (9). It improves flexibility, muscle strength, endurance, cardio-respiratory function, range of motion, attention, and enjoyment of life (10). In addition, it reduces the risk of injury and chronic disease (11). Pilates includes varied exercises that are performed with rhythm and controlled breathing while maintaining core stabilization. Core stabilization is part of the synchronized upper limb movements that make up the kinetic chain. In the kinetic chain, all parts of the upper extremity are considered as a single kinetically connected functional unit. This coactivation between the proximal and distal muscles of the upper extremity allows the successful performance of daily activities (12).

Activities of daily living require a synergistic coordination of the upper extremity and the core region. The core region's muscles are activated before the upper extremities' muscles, even in a movement that requires only the use of the upper extremity (13). Core stabilization provides force, velocity, and momentum transfer to the shoulder. The shoulder is a transferring point that effectively transfers the energy generated in the core to the upper extremity. There is an important relationship between strength and endurance of core and shoulder function (14). The core stabilization exercises are beneficial for healthy aging and injury prevention (15). Pilates is one of the exercises that have a beneficial effect on core stabilization and balance (16). Pilates is a therapeutic technique for disabilities (17) and a preventive program for healthy individuals (18). There are studies that demonstrate the beneficial therapeutic effects of Pilates on muscle strength, core stabilization, risk of falls, balance, gait, and cardiorespiratory fitness (19). However, there are only a few studies that have examined the effects of Pilates on specific joints or functions, such as the shoulder (20). The aim of this study was to compare shoulder proprioception, dynamic stability and function between Reformer Pilates practitioners and non-Pilates practitioners in asymptomatic participants. We hypothesized that those who practiced 8 weeks Reformer Pilates would have better shoulder's proprioception, dynamic balance, and function than those who did not.

Methods

Design

This study was designed case-control. It was carried out at the North Sport Institution between June and December 2021 and conducted in accordance with the Declaration of Helsinki. Before starting the study, the approval was obtained from the Üsküdar University Non-Interventional Research Ethics Committee (approval number: 61351342/ŞUBAT 2021-12, date: 25.02.2021). The participants were informed about the study, and their written consent was obtained.

Participants

This study was designed to compare shoulder proprioception, dynamic stability and upper extremity function between Reformer Pilates practitioners and individuals who did not participate in any exercise or physical activity program. The study was conducted in İstanbul, a metropolitan area in Türkiye. We enrolled individuals between the ages of 20 and 55 who regularly participated in 8-week Reformer Pilates exercises. A total of 25 individuals were evaluated for eligibility and 2 individuals were excluded because they had a shoulder injury and 3 individuals were excluded because they had been practicing Pilates for many years. We continued with 20 individuals (13 female, 7 male) in the case group.

We enrolled 20 individuals (12 female, 8 male) who did not participate any excises or regular physical activity program in the same city as a control group by inviting them through brochures and social media. We used the following exclusion criteria for both the case and control groups: Participants with a history of shoulder trauma, with limited shoulder range of motion, and who consulted a physician in the previous 6 months for musculoskeletal, neurologic, or rheumatologic conditions (such as pain, edema or increased temperature) were excluded. We also excluded individuals with a history of previous surgery or fracture, a complaint related to the shoulder region, and those who were regular participations in any type of exercise or sport program.

Intervention

Reformer Pilates exercises were performed one-on-one under the supervision of a physiotherapist at the center three times a week for 8 weeks. The physical therapist was certified in Reformer Pilates by the Türkiye Gymnastics Federation. The sessions lasted 60 min, including 10 min of warm-up and 40 min of reformer exercises, followed by 10 min of cool-down. Warm-up exercises included breathing, mini squat, roll down, twist stretch, shoulder circles, hip roll, seated hip stretch. Reformer Pilates provided a variety of exercises such as the footwork (leg series), tendon stretch, running, and supine arm work, including parallel pull, lateral pull (arm series), drawing down, shoulder bridge, feet in straps, arm work, hundred, short box arm work, pulling the strap long box, side split, cat series, side split, and mermaid. Cool-

down exercises included breathing, cat stretch, chest stretch, seated hip stretch, swinging, toy soldier, cobra.

The first Pilates session covered the basics: breath control and positioning of the pelvic floor, chest, shoulders, head, and neck. Reformer exercises were performed on springs, ropes, and a sliding platform with variable resistance. We adjusted the difficulty of the exercises by increasing the resistance of the springs and adding different positions. All participants started at level 1 and gradually progressed to level 3. They were asked to inform the physiotherapist if they felt any discomfort. The average number of weekly sessions attended by the Reformer Pilates group over an 8-week period was 2.82±1.03.

Outcome Measures

Demographic data such as gender, age, weight, height, the dominant hand, and health status of all participants were obtained by face-to-face interviews. Shoulder joint proprioception sensation was measured using a mobile phone application called "Clinometer". The dynamic balance, functionality, and stability of the upper extremity and trunk were evaluated with the Upper Extremity Y Balance test (YBT-UQ), and the dynamic stability of the shoulder was evaluated with the Closed Kinetic Chain Upper Extremity Stability test (CKCUEST).

Shoulder Joint Position Sense

Shoulder joint position sense was measured using a smartphone application Clinometer [4.3.1 (1412091) on IOS]. The application was a low-cost method that could be easily accessed by everyone, and was used together with an inclinometer and goniometer, with proven validity and reliability in Turkish population by Keleş et al. (21). The inter-user reliability value of the Clinometer application was found to be 0.98 (22).

The active angle repetition method was used for the proprioception evaluation. The evaluation was made as 45º for internal rotation and 45º-75º for external rotation (23). The participant was lying on her back in the 90º flexion and abduction position. The smartphone was fixed between the participant's shoulder and elbow. The test was explained in detail before the participant started the measurement. The participants were first taught the value of each angle with their eyes open. Then, the participants' eyes were covered with black tape and asked to perform the movement with their eyes closed. During the measurement, when the participant's arm reached the target angle. The participant was asked to wait 10 seconds and feel the movement. Later, the arm was brought back to the starting angle. Measurements were started after the target angle was felt three times. The participant was instructed to stand at the previously felt target angle and say "OK". A five-second rest interval was given between measurements. Measurements were repeated six times. The absolute value of the difference between the angle read in the clinometer and the measured target angle value was recorded (21). The test was performed bilaterally. Absolute values of deviations from the target angle were taken.

The Upper Extremity Y Balance Test (YBT-UQ)

The YBT-UQ was used to evaluate the functionality, dynamic balance, and stability of the trunk and upper extremities. The YBT-UQ was shown to be reliable in the Turkish population to measure shoulder dynamic balance (24). Initially, the participant was asked to take a push-up position with her arms shoulder-width apart. The participant was allowed to experiment before starting the test. During the test phase, the participant was asked to reach the maximum possible points in 3 different directions: nondominant hand medial (0º), inferior lateral (45º), and superior lateral (45º), and the distances were recorded. The same test was repeated for the dominant hand. Within the scope of this test, the upper extremity lengths of the participants were also measured. The measurement was made in the anatomical position, with the arms of the person in the 90º abduction position and recording the distance in cm between the spinous process of the 7th cervical spine and the tip of the middle finger. The total score obtained using the participant lying in 3 different directions was divided by the length of the upper extremity multiplied by 3. The result obtained was multiplied by 100 and recorded as the test result. The formula [mean maximum reach (cm)/limb length (cm)] X 100 was used. The test was applied to both sides. The calculation was made by taking the average of the dominant and non-dominant values (25).

The Closed Kinetic Chain Upper Extremity Stability Test (CKCUEST)

Dynamic stability of the shoulder was evaluated with CKCUEST. The CKCUES test is a reliable tool for assessing upper extremity function in asymptomatic individuals (26). Participants took a push-up position and placed their hands-on strips that were 1.5 inches (3.81 centimeters) wide, and 36 inches (91.44 centimeters) apart. With one hand fixed on the ground, the other hand was lifted, and the band under the fixed hand was touched back to the starting point. The test was done bilaterally. The number of repetitions was recorded for 15 sec. and 3 attempts were made. Between trials, the participant was given a rest of 45 sec. Three trials were averaged (26).

Statistical Analysis

Statistical Package for Social Science (SPSS) 26.0 was used to analyze the data obtained in our study. We considered $p<0.05$ statistically significant. We used the G* power analysis program to calculate the sample size. Using shoulder proprioception as the primary outcome, we calculated that for a 5% alpha error and 80% 1-D error, there should be at least 18 patients in both groups for an effect size of 0.85 (10). We calculated the effect size so that a difference of 3.2°±1.3° in the case group and 5.3°±3.2° in the control group would be significant (27). The conformity of the data to the normal distribution was evaluated using the Kolmogorov-Smirnov/Shapiro-Wilk tests. Categorical demographic variables between groups were analyzed with the chi-square test. Shoulder proprioception, balance, and functionality scores of the groups were compared with the independent t-test as they showed a normal distribution. When the comparison was

found to be significant, an effect size analysis was performed. Cohen-d value was used in the effect size analysis because the number of cases in the two groups was equal. In calculating the effect size, the small effect was considered as 0.2≥, the medium effect was 0.5≥, and the large effect was 0.8≥. The relationship between shoulder proprioception, function and dynamic stability was calculated by Pearson correlation coefficient. Correlation coefficient score between 0 and 0.19 was considered as very low correlation, between 0.2 and 0.39 as low correlation, between 0.4 and 0.59 as moderate correlation, between 0.6 and 0.79 as high correlation, between 0.8 and 1.0 as very high correlation.

Results

The demographic characteristics of the case and control groups were not significantly different (Table 1).

The Reformer Pilates group had significantly better shoulder proprioception scored with a large effect size in 45° internal rotation and 45°-75° external rotation with both dominant and nondominant sides (p<0.001 and d score ranging 1.61 to 3.07) (Table 2).

The Reformer Pilates group had significantly better shoulder functionality and balance with a large effect size on both the dominant and nondominant sides (p<0.001 and d score ranging 1.49 to 2.35) (Table 2).

The Reformer Pilates group had a significantly higher upper extremity stability score with a large effect size on both the dominant and non-dominant sides (p<0.001 and d=2.82) (Table 2).

Table 1. Sociodemographic data of the participants

Variables	PG (n=20) Mean (SD)	CG (n=20) Mean (SD)	p
Gender			
Female	13	12	0.744×²
Male	7	8	
Dominant side			
Right	18	18	0.698×²
Left	2	2	
Age (year)	25.45 (2.56)	25.70 (1.80)	0.91ᵗ
BMI (kg/m²)	20.66 (1.87)	22.29 (1.98)	0.43ᵗ
Average Participation in Session (per/week)	3.12 (1.56)	NA	

Data expressed as mean (SD: Standard deviation), BMI: Body mass index, CG: Control group, PG: Pilates group, x²: Chi-square test, t: t-test

Table 2. Comparison of shoulder proprioception functionality and stability of Pilates and sedentary participants

Proprioception (°)	PG (n=20) Mean (SD)	CG (n=20) Mean (SD)	Test statistics p	Cohen-d
45° IR dominant	2.23 (1.09)	4.03 (0.60)	<0.001ᵗ	-2.04
45° IR non-dominant	2.88 (1.10)	4.29 (0.56)	<0.001ᵗ	-2.65
45° ER dominant	1.52 (0.79)	3.18 (0.40)	<0.001ᵗ	-3.07
45° ER non-dominant	2.13 (0.76)	3.40 (0.38)	<0.001ᵗ	-1.61
75° ER dominant	1.82 (0.67)	3.55 (0.43)	<0.001ᵗ	-2.11
75° ER non-dominant	2.54 (0.66)	3.68 (0.44)	<0.001ᵗ	-2.03
UE functionality				
M dominant	104.67 (1.84)	101.41 (1.41)	<0.001ᵗ	1.98
M non-dominant	102.98 (1.65)	96.05 (4.48)	<0.001ᵗ	1.85
SL dominant	70.19 (5.46)	61.03 (4.35)	<0.001ᵗ	1.49
SL non-dominant	68.90 (6.10)	56.93 (3.87)	<0.001ᵗ	2.05
IL dominant	75.81 (4.65)	68.80 (4.75)	<0.001ᵗ	2.35
IL non-dominant	73.10 (3.94)	64.48 (5.26)	<0.001ᵗ	1.85
CKCUEST				
Number of touches	17.89 (1.29)	14.28 (1.27)	<0.001ᵗ	2.82

Data expressed as mean (SD: Standard deviation), CKCUES: Closed Kinetic Chain Upper Extremity Stability, CG: Control group, ER: External rotation, IL: Inferolateral, IR: Internal rotation, M: Medial, SL: Superolateral, PG: Pilates group, t: t-test, UQYBT: Upper Quarter Y Balance test, UE: Upper extremity

There was no significant correlation between proprioception and shoulder function and dynamic stability in the case group except left external rotation at 75º and dynamic stability, whereas a positive, moderate to very high correlation (p<0.001, r score ranging -463 to -826) was found in the control group in all parameters (Table 3).

Discussion

The study compared shoulder proprioception, shoulder dynamic stability and upper limb functioning between individuals with and without regular Reformer Pilates. In our study, the participants, who did regular reformer plates for 8 weeks, had better shoulder proprioception, dynamic stability, and upper extremity function compared to control group.

There is some evidence in the literature that open and closed kinetic chain exercises have a positive effect on shoulder abduction and external rotation proprioception (28-30). In our study, Reformer Pilates participants had significantly better shoulder proprioception with a large effect size. To the best of our knowledge, our study is the first to investigate shoulder proprioception in asymptomatic individuals performing Pilates. Salles et al. (27) investigated the effect of strengthening exercises on shoulder proprioception in asymptomatic individuals and found that strengthening exercises performed at constant intensity were most effective for shoulder repositioning proprioception. In their research, the intervention group demonstrated about 2º shoulder rotation repositioning deviation after post-exercise, in comparison to the control group which exhibited approximately 5º deviation (27). The comparable results of the case and control groups in two studies support the idea that regular Reformer Pilates supports the repositioning proprioception. Proprioception is defined as the body's ability to transmit a sense of position, interpret processed information, and respond to stimuli, consciously or unconsciously (31). Proprioception provides the sensory information necessary for effective neuromuscular control. The sense of proprioception helps to increase movement control and coordination (32). We found that individuals who performed Reformer Pilates exercises had more controlled and coordinated shoulder movements. Reformer Pilates exercises may be recommended to support shoulder proprioception in asymptomatic individuals.

Our study shows that Reformer Pilates practitioners has a significantly better the dynamic stability of the shoulder. Reformer Pilates includes strength and endurance exercises for the core and shoulder stabilizer muscles. Core stability supports the upper extremities' motor control (33). A study of female handball players found that they significantly increased their ball-throwing speed with core stabilization training (34). Another study of baseball players showed that athletes who received six weeks of core training improved their throwing performance and upper extremity closed kinetic chain balance (35). Reformer Pilates may strengthen the kinetic chain between the upper extremity and core. Thus, it may have provided more torque and momentum transfer to the upper extremity and increased the dynamic stability of the shoulder. It should be noted that practice for 8 weeks of Reformer Pilates is sufficient to achieve better dynamic shoulder stability.

Core stabilization exercises, including closed kinetic chain activities, contribute to upper extremity function. The progressive and dynamic trunk stabilization exercises are effective in improving upper extremity function in athletes (36). However, there are few studies in the literature focusing on exercise performance to assess upper extremity-specific functions in asymptomatic healthy participants. We found that upper extremity function was better in individuals doing 8 weeks of Reformer Pilates than in those doing no exercise. Zengin Alpozgen et al. (37) showed that 8 weeks of Pilates training significantly improved shoulder function in individuals with breast cancer. Pilates participants

Table 3. Relationship between shoulder proprioception and shoulder functionality and stability in case and control group

	CKCUEST		UQYBT (Right)		UQYBT (Left)	
	PG p (r)	CG p (r)	PG p (r)	CG p (r)	PG p (r)	CG p (r)
45° IR dominant	0.182 (-0.311)	0.002** (-0.638)	0.152 (-0.332)	<0.001** (-0.821)	0.439 (-0.183)	<0.001** (-0.792)
45° IR non-dominant	0.298 (-0.245)	0.003** (-0.536)	0.736 (-0.080)	<0.001** (-0.789)	0.172 (-0.318)	<0.001** (-0.826)
45° ER dominant	0.554 (-0.141)	0.040* (-0.463)	0.214 (-0.290)	0.001** (-0.669)	0.951 (-0.015)	0.002** (-0.652)
45° ER non-dominant	0.869 (-0.039)	0.007** (-0.583)	0.683 (-0.097)	0.001** (-0.593)	0.293 (-0.247)	0.002** (-0.660)
75° ER dominant	0.546 (-0.143)	0.014* (-0.542)	0.015 (-0.537)	<0.001** (-0.732)	0.250 (-0.270)	0.001** (-0.694)
75° ER non-dominant	0.979 (-0.006)	0.001** (-0.702)	0.684 (-0.097)	0.001** (-0.703)	0.019* (-0.520)	<0.001** (-0.764)

CKCUES: Closed Kinetic Chain Upper Extremity Stability, CG: Control group, ER: External rotation, IR: Internal rotation, UQYBT: Upper Quarter Y Balance test, PG: Pilates group, r: Pearson correlation coefficient, *: p<0.05, **: p<0.01

had better upper limb function, which might be due to the closed kinetic chain trunk stabilisation exercises in Pilates (17). There is literature data showing that Pilates exercises improve function and independence in daily life in clinical conditions such as chronic back pain (38), geriatric population (11), multiple sclerosis (39). In line with existing literature, our study demonstrates that asymptomatic Pilates practitioners exhibit better shoulder function.

Proprioception is critical for smooth and well-coordinated movement and is linked to both balance and functions (40). In our study, there was a moderate to high significant positive correlation between shoulder proprioception and function and dynamic stability in the control group, while no significant correlation was found in the case group. Suner-Keklik et al. (41) investigated the effects of online Pilates exercises on trunk proprioception and endurance. They found significant improvements in both parameters with similar effect sizes. However, in our study, the Pilates group demonstrated better shoulder proprioception with a higher effect size when compared to stability and function skills. It could be said that individuals in the Pilates group showed more individual variation in proprioception, stabilization, and functional skills compared to the control group.

Study Limitations

To the best of our knowledge, this is the first study to compare the effectiveness of Reformer Pilates on shoulder proprioception, dynamic stabilization, and function in asymptomatic individuals. The main limitation of this study was that although individuals practicing Reformer Pilates were prospectively enrolled in the study, their baseline testing was not performed prior to the intervention. Therefore, only one measurement was available. In future studies, it is recommended that the design be prospective and that pretest-posttest evaluations be conducted. Another limitation concerns habit control of the participants. When participants start Reformer Pilates, they can adopt other behavioral changes that will improve their health. Although participants were told to maintain their current diet and not participate in any other exercise programs, there was no way to rigidly control this.

Conclusion

Our study shows that regular Reformer Pilates practitioners have significantly better shoulder proprioception, dynamic stability, and upper extremity function with large effect sizes. The current study suggests that Reformer Pilates exercises can be recommended as an exercise program for asymptomatic individuals to obtain better shoulder proprioception, stabilization and function.

Ethics

Ethics Committee Approval: Permission for the research was obtained from the Üsküdar University Non-Interventional Research Ethics Committee (approval number: 61351342/ŞUBAT 2021-12, date: 25.02.2021).

Informed Consent: The participants were informed about the study, and their written consent was obtained.

Authorship Contributions

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

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Original Article

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Morphological, Fractal, and Textural Features of the Mandible in Familial Mediterranean Fever Patients: A Case-control Study

Ailevi Akdeniz Ateşi Olan Hastalarda Mandibula'nın Morfolojik, Fraktal ve Dokusal Özellikleri: Olgu Kontrol Çalışması

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ABSTRACT

Objective: Familial Mediterranean fever (FMF) is an inflammatory disease and chronic inflammation may affect bone turnover and metabolism. This study aimed to compare the morphological, fractal, and textural features of the mandibular bone in FMF patients with healthy controls on panoramic radiographs.

Methods: Fifty patients with FMF and, age- and sex-matched 50 healthy controls were included in the study. Morphological evaluation of the mandibular cortex on digital panoramic images of a total of 100 individuals was performed using the mandibular cortical index (MCI). For fractal dimension (FD) and texture analysis of trabecular bone, regions of interest with a size of 50x50 pixels were selected from the trabecular bone region between the roots of the second premolar and first molar teeth. The box-counting method was applied to calculate the FD. Since the pixel gray-scale levels of these regions showed different distributions, pre-processing was performed with histogram equalization for texture analysis. First-order and gray-level co-occurrence matrix-based second-order features of panoramic images were calculated and their textural characterizations were obtained.

Results: The MCI values of the mandibular cortex did not significantly differ between the case and control groups (p>0.05).

ÖZ

Amaç: Ailevi Akdeniz ateşi (FMF) inflamatuar bir hastalıktır ve kronik enflamasyon kemik döngüsünü ve metabolizmasını etkileyebilir. Bu çalışmanın amacı panoramik radyografiler üzerinde mandibular kemiğin morfolojik, fraktal ve dokusal özelliklerini FMF hastaları ve sağlıklı bireylerle karşılaştırmaktır.

Yöntemler: Çalışmaya FMF tanısı alan 50 hasta ve yaş ve cinsiyet açısından uyumlu 50 sağlıklı kontrol dahil edildi. Toplam 100 hastanın dijital panoramik görüntüleri üzerinde mandibular korteksin morfolojik değerlendirmesi mandibular kortikal indeks (MKI) kullanılarak yapıldı. Trabeküler kemiğe ait fraktal boyut (FB) ve doku analizi için, ikinci küçük azı ve birinci büyük azı dişlerinin kökleri arasındaki trabeküler kemik bölgesinden 50x50 piksel büyüklüğünde ilgi alanları seçildi. FB hesaplanmasında kutu sayma yöntemi uygulandı. Bu bölgelerin piksel gri-skala düzeyleri farklı dağılımlar gösterdiğinden doku analizi için histogram eşitleme ile ön işleme yapıldı. Panoramik görüntülerin birinci derece ve gri seviye eş oluşum matrisi tabanlı ikinci derece özellikleri hesaplanarak dokusal karakterizasyonları elde edildi.

Bulgular: Mandibular korteks MKI değerleri olgu ve kontrol grupları arasında anlamlı farklılık göstermedi (p>0,05). Trabeküler kemiğe ait FB değerleri olgu grubunda 1,43, kontrol grubunda 1,44

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ABSTRACT

FD values for the trabecular bone were 1.43 in the case group and 1.44 in the control group, and there was no significant difference between them (p>0.05). First and second-order textural features of trabecular bone did not differ statistically significantly between the case and control groups (p>0.05).

Conclusion: Morphological, fractal, and textural features of the mandibular bone did not differ on panoramic radiographs between FMF patients and healthy controls.

Keywords: Familial Mediterranean fever, mandible, fractal, entropy, panoramic radiography

ÖZ

olup aralarında anlamlı fark yoktu (p>0,05). Trabeküler kemiğin birinci ve ikinci derece dokusal özellikleri olgu ve kontrol grupları arasında istatistiksel olarak anlamlı farklılık göstermedi (p>0,05).

Sonuç: Mandibular kemiğin morfolojik, fraktal ve dokusal özellikleri FMF hastalarında ve sağlıklı kontrollerde panoramik radyografiler üzerinde farklılık göstermemektedir.

Anahtar Sözcükler: Ailesel Akdeniz ateşi, mandibula, fraktal, entropi, panoramik radyografi

Introduction

Familial Mediterranean fever (FMF) is the most common autoinflammatory disease worldwide, characterized by self-limiting recurrent episodes of fever, polyserositis, and sometimes erysipelas-like dermatological findings (1,2). Populations of Mediterranean and Middle Eastern origin, such as Armenians, Greeks, Turks, Italians, and Arabs, are frequently affected by FMF (3). Symptoms appear in the first decade in 60-70% of cases and before the age of twenty in 80-90% (4). Thus, the majority of patients are diagnosed in the first two decades. FMF is caused by point mutations in the *MEFV* gene, which is located on the short arm of chromosome 16 and encodes the Pirin protein, and is inherited in an autosomal recessive manner (5). The frequency of the disease in Türkiye is approximately 1/1,000 and the carrier rate is 1:5 (6). Although the etiology is not fully understood, proinflammatory cytokines such as interleukin-6 (IL-6), IL-8, IL-12, and tumor necrosis factor-α (TNF-α) are elevated during FMF attacks (7). Colchicine treatment usually prevents attacks and inflammation (6).

Caries and periodontal diseases have been reported to be common in FMF (8). Temporomandibular joint arthritis associated with FMF is a rare maxillofacial finding (9) and recurrent aphthous ulcers may also be seen in these patients (8). Recurrent oral aphthous ulcers are rare mucocutaneous manifestations, may accompany attacks, and are thought to be related to dysregulation in cellular immunity, although the etiology is not clearly explained. Colchicine treatment has been reported to be effective on oral ulcers (8).

Methods such as mental index, gonial index, antegonial index, panoramic mandibular index, and mandibular cortical index (MCI) are used for radiographic evaluation of mandibular bone quality and density by examining panoramic radiographs, which are frequently used in dental practice (10). MCI, also known as Klemetti index (11), is more practical than other methods because it does not require any measurement. Scoring is done according to the degree of resorption in the mandibular cortex. Fractal analysis, a mathematical method, has been used frequently in recent years to analyze the complex structure of mandibular trabecular bone architecture. Its important advantages are that it is an easily accessible method, it is not affected by variables such as projection geometry and radiodensity, and it provides objective data about the internal trabecular structure. As the fractal dimension (FD) increases, the complexity of the examined structure increases (12).

The texture is the repetition of a pattern or patterns over a region. These patterns may be fine, coarse, smooth, random, or striped in terms of quality (13). Disease-induced textural changes in radiological images of patients and normal control groups can be used as a marker for disease diagnosis. Statistical features can be obtained by using the cumulative and neighboring pixel behavior of grayscale-level pixel distributions in the regions of interest (ROI). For this purpose, first-order statistical (FOS) features and gray-level co-occurrence matrix (GLCM) based second-order statistical features are frequently used in the literature (14-17). FOS features calculate cumulative mean, variance, skewness, kurtosis, energy, and entropy values considering the grayscale-level color distribution in the histogram of the image (18). GLCM, on the other hand, can produce higher-quality features by considering the textural relationships arising from pixel neighborhoods. The GLCM defined by Haralick et al. (19) can calculate many statistical properties such as energy, correlation, entropy, homogeneity, and contrast, which can model texture changes according to pixel neighborhood orientation degree and pixel distance values (20). This method compares the grayscale-level differences between two different pixels at different locations. In recent years, several studies conducted with magnetic resonance imaging (MRI) (20) and computed tomography (21) have shown promising results using GLCM texture features for benign-malignant differentiation of lesions in bone and prostate, and for the detection of bone metastases. In a study by Yildirim et al. (22), bone mineral density (BMD) of the lumbar spine, femoral neck, and total femur determined by dual-energy X-ray absorptiometry (DXA) was compared between subjects with FMF and healthy subjects, and it was shown that BMD was lower in FMF patients. Researchers have reported that bone mass reduction may be related to the chronic inflammatory feature of the disease and chronic inflammation may affect bone turnover and metabolism. FMF is a common disease in the Turkish population and there is insufficient data in the literature regarding its effect on the trabecular and cortical structure of the jaw bones. The aim of this study was

to determine the changes in mandibular cortical morphology, trabecular bone microarchitecture, and textural properties of FMF patients compared with the healthy control group using panoramic radiographs.

Methods

Sample Selection and Study Design

The ethical approval for the study was obtained from Necmettin Erbakan University Faculty of Dentistry (decision no: 2023/257, date: 23.02.2023). The study was carried out by retrospectively collecting panoramic radiographs in the database of individuals who were admitted to the Dentomaxillofacial Radiology clinic for dental examination between 2021 and 2022 and who had a diagnosis of FMF in their medical history. All panoramic radiographs within the scope of the study were obtained with a 2D Veraviewpocs (J MORITA MFG corp, Kyoto, Japan) digital panoramic device with irradiation parameters of 70 kV, 5 mA, and 15 s. Individuals with a history of FMF constituted the case group and systemically healthy individuals who matched the case group in terms of age and gender constituted the control group.

Patients with FMF (all cases consisting of colchicine users with various dosages: 0.5-3 mg/day), and their age- and sex-matched, systemically healthy subjects aged ≥18 years with teeth were included. The presence of maxillofacial pathologies visualized on panoramic radiographs, radiographs of edentulous individuals, and panoramic radiographs that were not diagnostically adequate due to patient positioning or irradiation errors were excluded from the study.

Morphological Evaluation

The MCI scores of 100 patients (50 cases and 50 controls) were evaluated twice at 14-day intervals by the same observer (M.T.) with 11 years of experience in oral radiology. The Kappa value for intraobserver agreement was calculated as 0.94. In MCI, bone resorption in the cortical region extending from the distal foramen mentale to the antegonial region is analyzed. According to this index (11):

C1 (Normal Mandibular Cortex): The margins are equal and sharp on both sides of the cortex.

C2 (Moderately Resorbed Mandibular Cortex): The endosteal margins of the cortex show half-moon-shaped defects (lacunar resorption) and the margins are observed as 1-3 layers.

C3 (Severely Resorbed Cortex): Cortical cortices are severely porous with dense endosteal debris.

In the MCI index, panoramic radiographs are evaluated and graded separately for right and left, and then a single grade is assigned for each panoramic radiograph. In determining the final class, the class with more morphologic destruction is preferred to the class with less destruction (Figure 1).

Image Pre-processing

The panoramic radiographs saved in *.tiff format were first resized for homogeneous study (2943x1435 pixels). Then, selected ROI from the trabecular bone region between the second premolar and first molar roots were cut with the ImageJ program with equal row and column sizes (50x50 pixels) (Figure 2).

Histogram equalization was performed on 50 FMF (+) and 50 FMF (-) ROI images with 50x50 row and column sizes to increase the contrast of textural changes and to examine them at an equal pixel grayscale-level range. Histogram-equalized images were used to obtain FOS and GLCM features.

Fractal Analysis

The same regions of the case and control images were cut and the FD analysis of the trabecular bone was calculated with the ImageJ program using the box-counting technique described by White and Rudolph (23) (Figures 3, 4). Measurements were calculated by the same observer (M.T.).

First Order Statistics

The FOS is a texture feature extraction method obtained without considering the relationship in pixel neighborhoods. With the FOS feature extraction method, mean, kurtosis, variance, skewness, entropy, and energy values are calculated based on the histogram representing the frequency of the pixel distribution in the image. The histogram is a statistical definition of the number of times the pixel intensity values are repeated throughout the image. In two-dimensional space, the image size is expressed in width and height. By multiplying the width and length, the total pixel number of the ROI region can be calculated.

Gray-level Co-occurrence Matrix

While the FOS features provide simple statistical properties of grayscale-level pixel values in the image, they do not give differences due to inter-pixel neighborhoods. Therefore, high-order texture statistics, such as GLCM, can characterize textural differences due to neighborhoods between pixels. There are

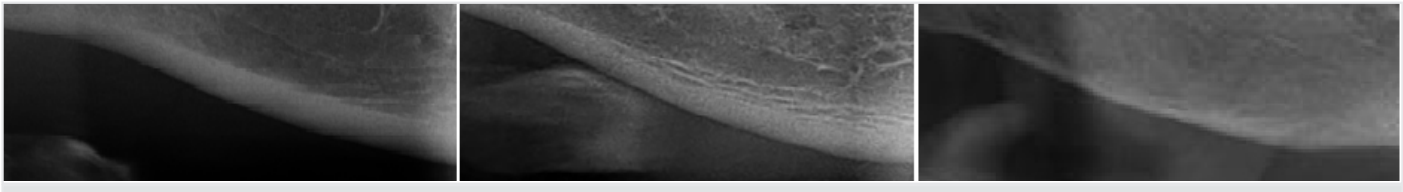

Figure 1. MCI description on cropped panoramic images (left to right: C1, C2, C3)
MCI: Mandibular cortical index

two important parameters for the GLCM method, also known as second-order texture statistics: Pixel neighborhood distance (D) and pixel neighborhood orientation degree (θ). A pixel of interest in the image has 8 neighbors. These neighborhoods consist of horizontal 0°, vertical 90°, right 45°, anti-diagonal 135° directions, and their four opposite directions in terms of degrees. Neighborhood distance is a measure of how far from the pixel of interest it is to a neighboring pixel that needs to be processed (15-17). In Figure 4, pixel values in a region of the FMF ROI obtained after histogram equalization and neighborhood orientations of a pixel of interest are given. Table 1 shows the pixel neighborhood orientation angles and offset values of the pixel distance of interest.

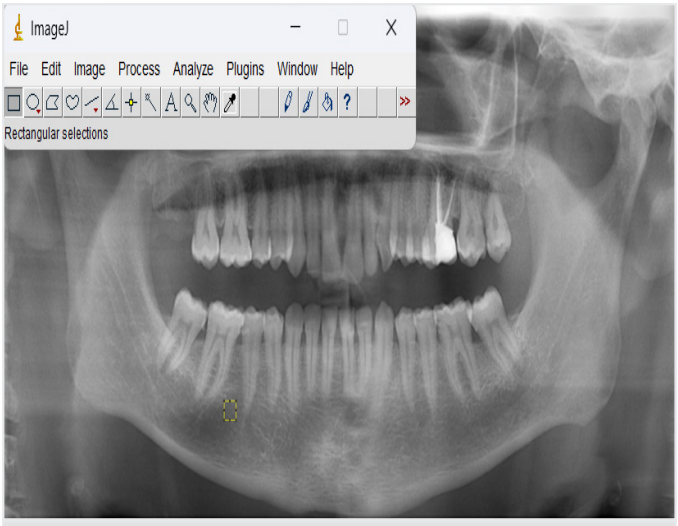

Figure 2. The selected ROI (50x50 pixel dimensions)
ROI: Regions of interest

In this study, 19 second-order statistics were obtained by forming co-occurrence matrices from FMF (+) and FMF (-) ROI images according to four different θ angles and 1 offset value [0° (0 1), 90° (-1 0), 45° (-1 1), 135° (-1 -1)] (19,24-28). These statistics are given in Table 2.

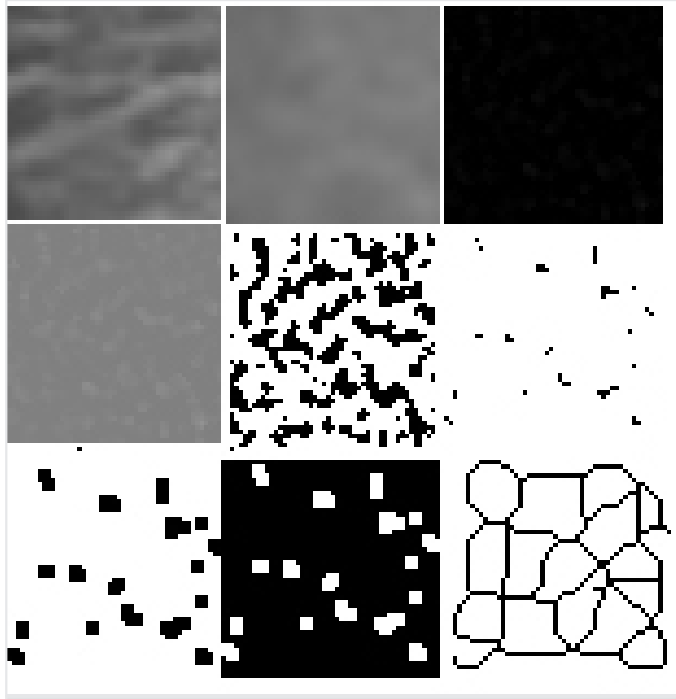

Figure 3. (a) ROI selection, (b) Gaussian filter (c) Subtraction of the ROI from the original image (d) Addition of 128 gray-scale values to each pixel location (e) Binarization (f) Erosion (g) Dilatation (h) Invertion (i) Skeletonization
ROI: Regions of interest

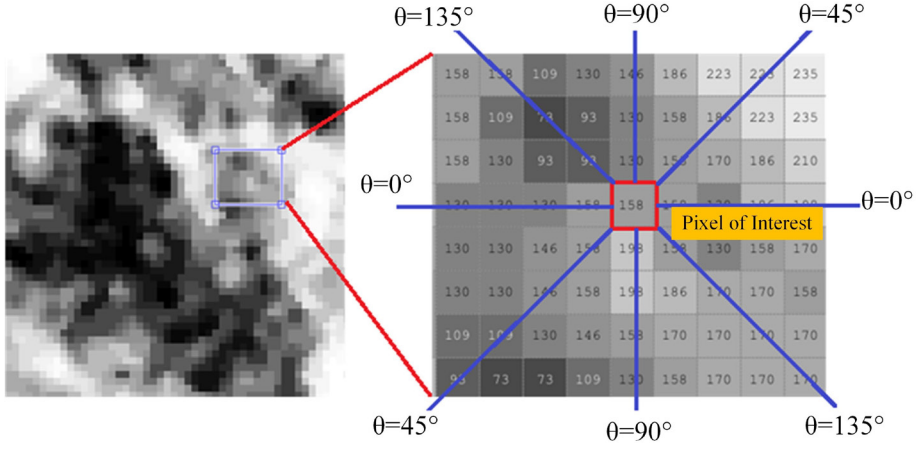

Figure 4. FMF ROI image obtained after histogram equalization, and neighborhood orientations relative to a pixel point of interest in a region within the image
FMF: Familial Mediterranean fever, ROI: Regions of interest

Table 1. Pixel neighborhood orientation angles and offset representations of the pixel distance of interest

The Orientation Angel (θ)	Offset (Distance)
0° (horizontal)	[0 D]
45° (diagonal)	[-D D]
90° (vertical)	[-D 0]
135° (anti-diagonal)	[-D -D]

Statistical Analysis

The data obtained were evaluated using SPSS v21.0 (IBM Corp, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine the normality of data. For statistical significance between two independent groups, independent samples t-test, Mann-Whitney U test, and Spearman correlation analysis were performed. The significance between categorical data was

Table 2. Second-order features obtained with GLCM and statistical results

		FMF(-)	FMF(+)		FMF(-)	FMF(+)		FMF(-)	FMF(+)		FMF(-)	FMF(+)	
Features	Stats	(0.1)	(0.1)	p	(45.1)	(45.1)	p	(90.1)	(90.1)	p	(135.1)	(135.1)	p
1. autoc	Mean	25.036	24.956	0.616	24.608	24.582	0.819	24.869	24.891	0.656	24.654	24.607	0.589
	Std	0.509	0.604		0.611	0.685		0.525	0.617		0.595	0.698	
2. contr	Mean	0.841	0.931	0.753	1.620	1.663	0.618	1.105	1.108	0.711	1.529	1.617	0.409
	Std	0.391	0.408		0.619	0.677		0.372	0.417		0.565	0.652	
3.corrp	Mean	0.920	0.911	0.960	0.845	0.841	0.827	0.895	0.895	0.977	0.854	0.846	0.707
	Std	0.038	0.038		0.061	0.064		0.037	0.039		0.056	0.061	
4. cprom	Mean	723.138	711.352	0.374	672.246	664.316	0.299	704.180	698.546	0.130	676.992	666.018	0.164
	Std	43.571	34.592		49.215	38.249		41.047	29.528		49.300	36.062	
5. cshad	Mean	0.434	0.418	0.249	0.489	0.210	0.278	0.402	0.047	0.366	0.475	0.371	0.282
	Std	4.405	6.832		4.454	6.758		4.376	6.745		4.459	6.536	
6. dissi	Mean	0.554	0.585	0.936	0.843	0.844	0.414	0.665	0.654	0.542	0.819	0.835	0.452
	Std	0.150	0.159		0.176	0.209		0.126	0.148		0.170	0.202	
7. energ	Mean	0.055	0.054	0.326	0.041	0.042	0.716	0.049	0.050	0.931	0.042	0.042	0.920
	Std	0.010	0.009		0.007	0.008		0.007	0.007		0.007	0.008	
8. entro	Mean	3.165	3.196	0.444	3.455	3.443	0.803	3.296	3.277	0.919	3.433	3.437	0.930
	Std	0.196	0.189		0.172	0.193		0.153	0.161		0.168	0.179	
9. homop	Mean	0.751	0.741	0.847	0.652	0.655	0.277	0.710	0.717	0.536	0.658	0.657	0.454
	Std	0.054	0.058		0.050	0.065		0.043	0.051		0.051	0.062	
10. maxpr	Mean	0.107	0.105	0.763	0.092	0.091	0.230	0.101	0.101	0.453	0.093	0.092	0.327
	Std	0.012	0.011		0.011	0.009		0.010	0.009		0.012	0.010	
11. sosvh	Mean	25.277	25.347	0.789	25.027	25.156	0.781	25.069	25.122	0.465	25.102	25.028	0.437
	Std	0.515	0.551		0.578	0.626		0.525	0.586		0.546	0.623	
12. savgh	Mean	8.989	8.986	0.533	8.986	8.989	0.265	8.985	8.993	0.318	8.986	8.989	0.276
	Std	0.100	0.125		0.105	0.132		0.105	0.130		0.105	0.132	
13. svarh	Mean	60.290	60.050	0.988	58.991	58.931	0.860	59.686	59.782	0.861	59.094	58.985	0.956
	Std	1.673	1.743		1.707	1.788		1.586	1.686		1.699	1.821	
14. senth	Mean	2.656	2.658	0.424	2.686	2.685	0.855	2.674	2.670	0.887	2.686	2.685	0.891
	Std	0.036	0.029		0.016	0.014		0.023	0.018		0.018	0.015	
15. dvarh	Mean	0.841	0.931	0.753	1.620	1.663	0.618	1.105	1.108	0.711	1.529	1.617	0.409
	Std	0.391	0.408		0.619	0.677		0.372	0.417		0.565	0.652	
16. denth	Mean	0.933	0.970	0.887	1.191	1.191	0.331	1.049	1.043	0.360	1.171	1.185	0.468
	Std	0.164	0.176		0.151	0.187		0.128	0.155		0.146	0.176	
17. inf1h	Mean	-0.462	-0.444	0.789	-0.321	-0.324	0.391	-0.398	-0.404	0.515	-0.331	-0.327	0.608
	Std	0.090	0.094		0.078	0.098		0.069	0.083		0.076	0.091	
18. inf2h	Mean	0.916	0.909	0.966	0.847	0.845	0.800	0.893	0.894	0.951	0.854	0.848	0.744
	Std	0.032	0.033		0.053	0.056		0.031	0.034		0.049	0.054	
19. indnc	Mean	0.941	0.939	0.882	0.914	0.914	0.360	0.931	0.932	0.532	0.916	0.915	0.462
	Std	0.014	0.015		0.015	0.019		0.012	0.014		0.015	0.018	

autoc: Autocorrelation, contr: Contrast, corrp: Correlation, cprom: Cluster Prominence, cshad: Cluster Shade, dissi: Dissimilarity, energ: Energy, entro: Entropy, homop: Homogeneity, maxpr: Maximum probability, sosvh: Sum of sqaures:Variance, savgh: Sum average, senth: Sum entropy, dvarh: Difference variance, denth: Difference entropy, inf1h: Information measure of correlation1, inf2h: Information measure of correlation2, indnc: Inverse difference normalized (INN), , FMF: Familial Mediterranean fever, GLCM: Gray-level co-occurrence matrix

evaluated with chi-square test. The evaluation of the test results was made according to a 0.05 significance level.

Results

There were 54 women and 46 men in the case (n=50) and control (n=50) groups consisting of 100 individuals. The age range of the patients matched in terms of age and gender was 18-71 years with a mean age of 30±13 years. MCI scores related to the mandibular cortex did not differ significantly between the case and control groups (p>0.05) (Table 3).

The FD values for mandibular trabecular bone were 1.43 in the case group and 1.44 in the control group, and there was no significant difference between them (p>0.05). All the patients in the case group consisted of individuals using colchicine. The distribution of the individuals according to the colchicine doses they used daily is given in Table 4. There was no significant correlation between colchicine doses and FD (p>0.05).

The FOS properties and statistical values of trabecular bone are given in Table 5. There was no statistically significant difference between the case and control groups (p>0.05).

In Table 2, second-order features obtained with GLCM and statistical results are given. There was no statistically significant difference between the case and control groups (p>0.05).

Discussion

The FMF is a common disease in the Turkish population (3) and BMD has been reported to be decreased in FMF (22). Based on this, we aimed to investigate possible differences related to FMF in the jaw bones of these patients. For this purpose, MCI was used to evaluate cortical bone, FD, first and second-order image features (GLCM) were used to examine trabecular bone on panoramic images.

Fractal analysis is used to evaluate the effects of various drugs (bisphosphonate, corticosteroid, antiepileptics, aromatase inhibitor, selective serotonin reuptake inhibitors, proton pump inhibitors), systemic diseases and conditions (sickle cell anemia, thalassemia, type I and II diabetes mellitus on the jaws, osteogenesis imperfecta, chronic renal failure, osteoporosis) (29-31). In the study conducted by Bayrak et al. (29), panoramic radiographs were used, 59 patients with thalassemia major

Table 3. The distribution of the sample according to MCI scores

	MCI (number of subjects)			Total	x²
	1	2	3		
Control, n	36	13	1	50	
	72.0%	26.0%	2.0%	100.0%	
Case, n	27	21	2	50	p>0.05
	54.0%	42.0%	4.0%	100.0%	
Total, n	63	34	3	100	
	63.0%	34.0%	3.0%	100.0%	

MCI: Mandibular cortical index

Table 4. The distribution of individuals in the case group according to daily colchicine usage doses

	The number of patients	Valid percentage %
0.5 mg	11	22.0
1 mg	3	6.0
1.5 mg	25	50.0
3 mg	11	22.0
Total	50	100.0

Table 5. FOS features and statistical values

	FMF (+)		FMF (-)		
FOS	Mean	Std	Mean	Std	p-value
Mean	129.5411	0.6000	129.4133	0.6088	0.887
Variance	5396.4161	68.8685	5419.3532	55.2592	0.235
Skewness	0.0031	0.0081	0.0019	0.0073	0.534
Kurtosis	1.8050	0.0127	1.8047	0.0120	0.776
Entropy	4.3089	0.5517	4.3280	0.5402	0.882
Energy	0.0601	0.0241	0.0590	0.0232	0.653

FOS: First-order statistics, FMF: Familial Mediterranean fever

and 59 controls were included, and FD was measured in 4 separate ROIs. It was stated that FD was lower in the cases in 2 of the 4 selected ROIs, and there was no significant difference between cases and controls in the other 2 ROIs. In their study investigating lactation-induced bone loss, Coşgunarslan et al. (31) selected 3 different ROIs, including cortical and trabecular bone, on panoramic radiographs. While FD was significantly lower in the case group in the 1st and 2nd ROIs selected from the trabecular bone, no difference was found between the case andcontrol groups in the ROI selected from the cortical bone. Based on the findings of these studies, it appears that selecting different ROIs may affect the results.

In the medical field, GLCM textural features can be used for brain tumor classification using MRI images (32), brain cancer diagnosis using histopathological images (33), malignant-benign differentiation of liver tumors using US images (34), classification (35) and early detection of benign-malignant breast masses using mammography images (36), skin tissue analysis for allergic, viral, bacterial and fungal skin diseases (37). It has been used in many fields such as examination of changes in the parotid gland after radiotherapy using the ultrasound (US) (38), prostate cancer classification using prostate biopsy sections (39), early diagnosis of lung cancer using computed tomography (CT) images (40), diagnosis of skin melanomas using dermoscopy images (41), diagnosis of esophageal cancer using positron emission tomography images images (42), benign-malignant differentiation of thyroid nodules using US images (43), detection of cervical cancer using colposcopy images (44) and many others. GLCM textural feature studies in the field of dentistry are very limited. In the study by Kavitha et al. (45) mandibular cortical width and GLCM features were calculated on panoramic radiographs for the detection of osteoporosis in Korean women. They reported that the use of all three together instead of a single feature had higher accuracy in the diagnosis of osteoporosis. Another study (46) showed that GLCM features were successful in detection of caries in intraoral images. Veena et al. (47) examined GLCM features (entropy, contrast, homogeneity, energy, and correlation) in terms of dental caries and cysts using panoramic radiographs and reported that these features might be helpful in diagnosis.

Various studies are investigating a more economical solution by examining textural features instead of DXA, which is the traditional method for evaluating bone quality related to osteoporosis. Kawashima et al. (48) used GLCM texture analysis for the detection of osteoporosis on non-contrast head CTs. It was shown that many regions in the skull base and maxillofacial bones had different GLCM texture characteristics between individuals with normal BMD and patients with osteoporosis. They stated that quantitative analysis of the microarchitecture in cancellous bone on non-contrast head CT images could be used as a new indicator in the diagnosis of osteoporosis. In another study (49) femur radiographs were used for texture analyses. It was concluded that tissue information contained in the trabecular bone structure visualized on radiographs could predict whether an implant anchor could be used and determine local bone quality from preoperative radiographs. The lack of textural difference between the case and control groups in the current study may be related to the imaging method used and the selected mandibular trabecular bone structure being different from other studies.

In the study by Hwang et al. (50) the diagnosis of osteoporosis was investigated by calculating FD and GLCM values on panoramic radiographs. Four different ROIs were selected and it was reported that the ROI selected from the mandibular cortex showed more strut features (quantification of the structural elements of the bone) than the medullary bone in the comparison of patients with and without osteoporosis. While individuals with osteoporosis showed lower FD in the ROI region selected from the endosteal region of the bone, no difference in FD was observed between the two groups in the trabecular bone region. In the present study, no significant difference related to FMF was found in the ROI selected from the medullary bone in FD and GLCM calculation. The only study in the literature on FMF in which FD was calculated from panoramic radiographs was conducted with pediatric patients aged 5-15 years. In this study (30) there was no difference in FD and MCI between healthy and FMF children. In addition, there was no correlation between the duration of colchicine use (in months) and FD. The findings of the present study were similar. FD and MCI were similar in adults with FMF and healthy individuals. There was no significant correlation between the dose (mg) of colchicine used and FD. FMF did not affect FD values and MCI of mandibular trabecular bone.

MCI has well-defined cut-off values, as the score from C1 to C3 increases, the porosity of the endosteal margin of the cortex increases. In the literature, the results of studies on MCI are conflicting. MCI was recommended as a feasible tool to screen initial BMD loss (osteopenia). The sensitivity and specificity of MCI for osteopenia were 0.81 and 0.48, respectively. The sensitivity and specificity of MCI for osteoporosis were, 0.35 and 0.88, respectively (51). Conversely, the findings of the present study showed that there was no difference in terms of MCI scores between FMF cases and controls. Similar to our results, Pacheco-Pereira et al. (52) revealed that MCI did not differ between patients with familial adenomatous polyposis and the control group and FD values were lower in the cases. They concluded that MCI was not useful for the analysis of the cortical bone pattern and FD was a promising tool for bone structure evaluation in dental panoramic radiographs. The age and gender distributions of the samples examined in the studies may affect the results obtained. Additionally, all individuals examined in this study used colchicine. It has been reported that colchicine inhibits bone resorption by reducing the number of osteoclasts and thus prevents osteoporosis (53). Accordingly, MCI values may not have differed between FMF case and control groups.

In the study conducted by Yildirim et al. (22), lumbar and femoral BMD values were found to be significantly lower in individuals with FMF than in healthy individuals, as a result of measurements made with DXA. In the mentioned study, 28

patients with FMF and 30 controls were evaluated. The mean age of the sample was older than the present study (35.1 for patients and 36.6 for controls). Contrary to this study, no difference was found between patients with FMF and healthy individuals in our examinations. It should be taken into consideration that the measurements were performed in the mandible, which was a different region, and the medullary structure of the femoral and lumbar bones might be different compared to the mandible. Additionally, the age and gender distribution of the sample might have an impact on differences in results.

Study Limitations

With its anti-inflammatory, anti-oxidative, anti-apoptotic, and bone-protective effects, colchicine treatment has been reported to have a prophylactic effect in preventing alveolar bone loss (54). These positive effects of colchicine in individuals with FMF might be the reason why there was no difference in FD and GLCM values between the case and control groups in this study. A limitation of the present study was that it was a retrospective study and there might be other possible systemic diseases of the individuals that were not yet diagnosed. Although the number of patients examined in this study was limited due to the relatively low number of patients receiving regular FMF treatment in adulthood, this study was planned to be improved with the inclusion of new patients.

Conclusion

Morphologic features of the mandibular cortex and fractal and textural features of the trabecular bone did not show a difference on panoramic radiographs between FMF patients and healthy controls. Further studies with different imaging techniques and image processing methods are needed.

Ethics

Ethics Committee Approval: The ethical approval for the study was obtained from Necmettin Erbakan University Faculty of Dentistry (decision no: 2023/257, date: 23.02.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: M.T., M.Ü.Ö., Design: M.T., M.Ü.Ö., Data Collection or Processing: M.T., M.Ü.Ö., Analysis or Interpretation: M.T., M.Ü.Ö., Literature Search: M.T., M.Ü.Ö., B.Ö., Writing: M.T., M.Ü.Ö., B.Ö.

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Case Report

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Unilateral Optic Disc Neovascularization in a Patient with Optic Disc Pit Depending on Proliferative Diabetic Retinopathy

Optik Pitli Bir Olguda Proliferatif Diyabetik Retinopatiye Bağlı Unilateral Optik Disk Neovaskülarizasyonu

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ABSTRACT

Optic disc pit is an excavation of the optic disc that usually develops congenital or rarely acquired, mostly located in the inferotemporal part of the disc. It is usually asymptomatic but sometimes might be symptomathic with maculopathy development. It is not well known at how much the optic pituitary influences the optic nerve structure or how it leads to other pathologic susceptibilities. In this case report, a patient with diabetic retinopathy who had unilateral optic pit and advanced disc neovascularization in the same eye was examined.

Keywords: Optic disc pit, diabetic retinopathy, neovascularization

ÖZ

Optik pit, konjenital veya nadiren edinsel olarak gelişen, çoğunlukla optik diskin inferotemporalinde yer alan, genelde asemptomatik seyreden, fakat bazen makülopati gelişimi ile semptom verebilen bir optik disk ekskavasyonudur. Optik pitin optik sinir yapısını ne derece etkilediği ya da optik diske ait başka patolojilere yatkınlığa ne ölçüde öncülük ettiği tam olarak bilinmemektedir. Bu olgu sunumunda tek taraflı optik piti olan ve aynı gözde ileri derecede disk neovaskülarizasyonu gelişen diyabetik retinopatili bir hasta irdelenmiştir.

Anahtar Sözcükler: Optik disk piti, diyabetik retinopati, neovaskülarizasyon

Introduction

First described as "optic disc depression" in a 62-year-old female patient in 1882, the optic pit is an oval, gray-white colored pit that develops congenital or is rarely acquired, and mostly located inferotemporal of the optic disc. Its incidence is less than 1/10000, and the incidence of co-occurrence in both eyes is 10-15% (1). Gender does not affect the incidence of the optic pit. Although mostly sporadic, unilateral cases have also been suggested to be autosomal dominant inherited (2). In most of the cases, the diagnosis is made during routine ophthalmologic examination.

The frequency of maculopathy seen as serous retinal detachment, cystoid macular edema, or schisis in the inner retinal layers in patients with optic pit varies between 25% and 75% in various publications (3,4). It is not known exactly to what extent the optic pit affects the optic nerve structure or to what extent it predisposes to other pathologies of the optic disc. In this case report, a patient with diabetic retinopathy (DRP) with unilateral optic pit and disc neovascularization (NV) in the ipsilateral eye will be discussed.

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Case Report

In the routine control examination of a 55-year-old female patient with known type II diabetes for 10 years and followed in our clinic for DRP for two years, her visual acuity was 10/10 in both eyes. Examination findings of anterior segment, intraocular pressure, eye movements, pupillary reactions and color vision were normal. In the fundus examination, retinal changes consistent with DRP were observed (Figure 1). In addition, optic pit located in the temporal part of the optic disc in the left eye and abnormal vascularization at the optic disc of the same eye (at the normal optic disc tissue, just below the pit region) were observed (Figure 1B). There wasn't abnormal vascularization in the optic nerve head of the right eye (Figure 1A). Bilateral DRP findings were detected in fluorescein angiography performed on the same day. Ischemic areas close to each other were observed in the nasal and temporal quadrants of both eyes (Figure 2). In addition, there was prominent disc NV in the left eye, and leakage from these neovascular vessels into the vitreous was occurring in the late phase of angiography (Figure 2D). While NV was not observed in the retinal areas other than the disc in the left eye, NV was not found on the disc or any other retinal area in the right eye (Figure 2C, D). Macular sections evaluated by optical coherence tomography (OCT) were normal in both eyes, and no accompanying diabetic maculopathy or optic pit maculopathy was detected.

Discussion

Optical pit is generally asymptomatic, it is one of the cavitary disc anomalies such as optic coloboma and morning glory syndrome. It is an optic disc excavation that can give symptoms with the development of maculopathy in the last decade. Optic pit, which is a pathology which, formation mechanism is still not clearly explained, has been accepted as a subgroup of optic colobomas for many years and has been attributed to the inability of the optic fissure to close completely during development (1). This opening has also been shown to be associated with the subarachnoid space. However, the fact that optic pits have not been shown to form in the inferonasal part, that they are usually unilateral and sporadic, that iris and choroidal colobomas are not accompanied, are the weak points of the hypothesis that the optic fissure does not close. Histologically, optic pit is a herniation of the dysplastic retina from a defect in the lamina cribrosa to the subarachnoid space.

Depending on the structure of the pit, it may have a direct connection with the subretinal space or the subarachnoid space. Hypotheses about the source of subretinal or intraretinal fluid in optic pit maculopathy also provide clues about the connection between the optic pit and the subretinal or subarachnoid space. In a recent study with spectral domain OCT, direct passage of fluid from the optic pit to the subretinal area has been demonstrated (5).

It is thought that the optic pit may affect the disc structure and may lead to other disc pathologies related to this. The most well-known of the innocent changes caused by the optic pit in the optic disc is the wider optic disc in 85% of optic pit cases (6). In some cases, choroidal atrophy is observed. It has been suggested that these peripapillary changes are a predisposing factor for peripapillary choroidal NV (7). Optic pit cases accompanied by peripapillary subretinal NV have been reported in the literature (8). However, to the best of our knowledge, the association of disc NV and optic pit, which is a sign of proliferative DRP, has not been defined.

The development of NV in DRP occurs as a result of the shift of the angiogenic/antiangiogenic factor balance in favor of angiogenesis. Angiogenesis is associated with the severity of

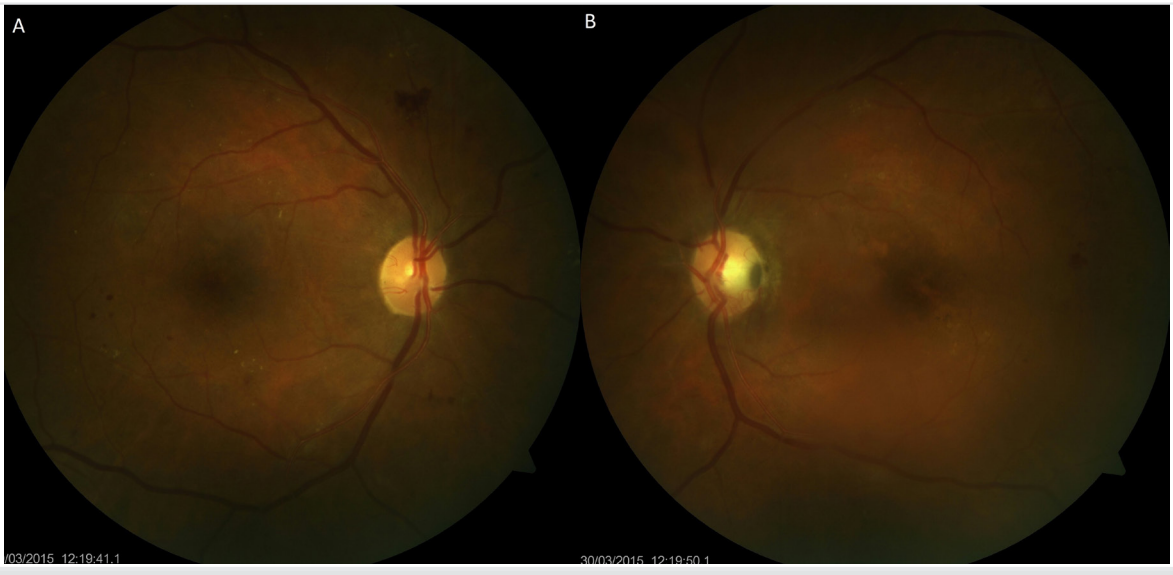

Figure 1. Fundus photographs of the patient's right (A) and left (B) eyes with diabetic retinopathy are shown. Neovascular vessels at peripapillary region and optic pit are seen in the left eye

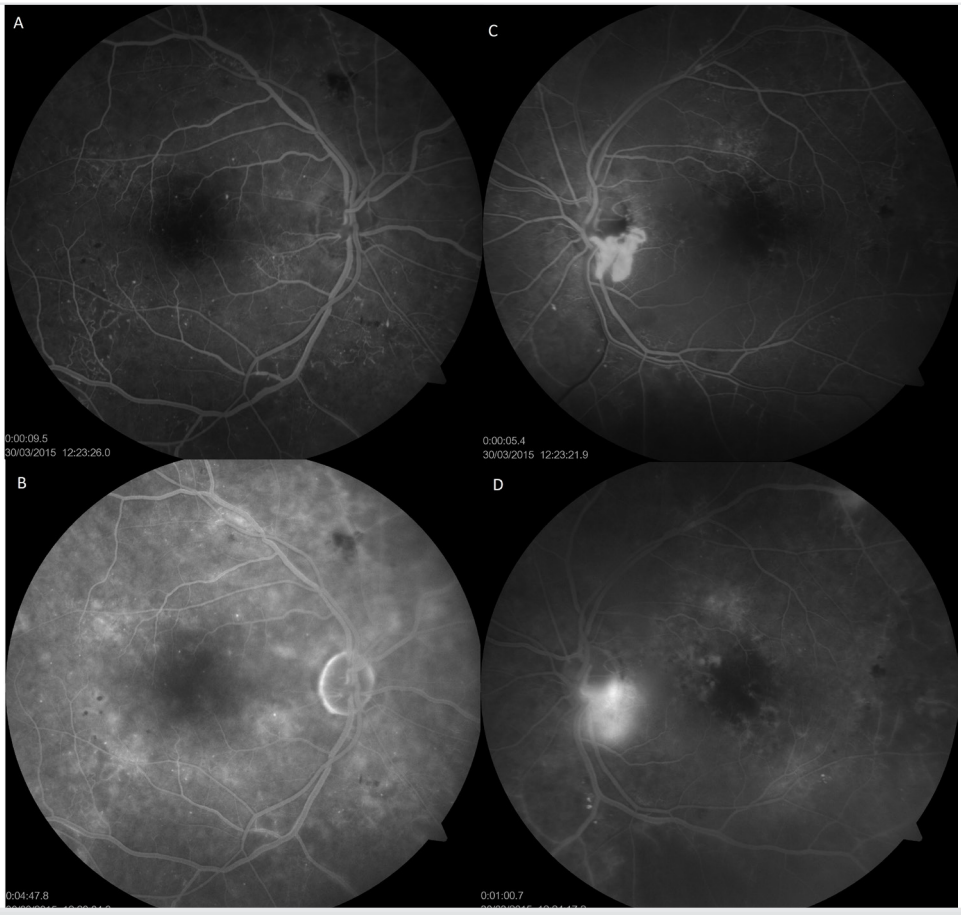

Figure 2. Fluorescein angiography images of the patient's right (A,B) and left (C,D) eyes are given. A and C belong to early phase, B and D belong to late phases. Hyperfluorescence of neovascular vessels is observed at early and long phases in the left eye

retinal ischemia (9). Accordingly, similar rates of disc NV are expected in eyes with a similar degree and amount of ischemic area on both sides. Although retinal ischemia was observed at similar rates angiographically in our case, disc NV developed only in the eye with optic pit. Lin et al. (10) showed that the incidence of DRP decreased in patients with high myopia. It has been shown that thinning of the vessels in this region due to the increase in axial length explains the negative relationship between DRP and high myopia in these patients. In our case, the localized increase in axial length in the optic pit region would be expected to have prevented the development of NV in this eye, similar to myopic patients. However in our case, NV was observed in the eye with optic pit without NV in the other eye. This may be due to the fact that the vessels in the optic pit region are thinner than normal and cause more severe localized ischemia in the normal region just below.

This case suggests that the optic pit may have a facilitating effect on the development of disc neovascularization, both anatomically and histologically.

Ethics

Informed Consent: Informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: A.Ş., C.E.A., Concept: A.Ş., C.E.A., H.Ö., Design: A.Ş., H.Ö., Data Collection or Processing: A.Ş., C.E.A., Analysis or Interpretation: A.Ş., C.E.A., H.Ö., Literature Search: C.E.A., Writing: A.Ş., C.E.A.

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Review

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Possible Effects of Immunosuppressive Therapy on Male Fertility and Pregnancy Outcomes After Paternal Exposure in Kidney Transplant Patients

Renal Transplant Hastalarında İmmünosupresif Tedavinin Erkek Fertilitesi ve Paternal Maruziyet Sonrası Gebelik Sonuçlarına Olası Etkileri

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ABSTRACT

Kidney transplantation is a crucial treatment for improving the quality of life of patients with renal failure. Immunosuppressive drugs are necessary to prevent organ rejection and are vital for the success of the transplantation. However, there is limited information on the potential adverse effects of these drugs on male fertility. Observational studies suggest that paternal drug exposure, as well as maternal drug exposure, may contribute to the risk of teratogenicity. This presents challenges in managing the treatment of men on chronic medication who are planning to conceive. The purpose of this article was to raise awareness among clinicians of this issue by examining the impact of immunosuppressive drugs used in renal transplant patients on paternal fertility and teratogenicity. Although further studies are required to understand the long-term effects of these drugs, it is recommended that options such as sperm banking should be considered in patients who are planning to have children and are considering immunosuppressive therapy.

Keywords: Kidney transplantation, male, fertility, paternal exposure, teratogens, pregnancy

ÖZ

Renal transplantasyon, renal yetmezlikli hastalar için yaşam kalitesini belirgin şekilde artıran önemli bir tedavi seçeneğidir. Transplantasyon sonrası hastalara verilen immünosupresif ilaçlar, organ rejeksiyonunu önlemek için vazgeçilmezdir ve transplantasyon başarısında önemli role sahiptirler. Ancak, bu ilaçların paternal maruziyetinin erkek fertilitesi üzerinde olası olumsuz etkileri hakkında bilgiler sınırlıdır. Bunun yanında yapılan gözlemsel çalışmalar, teratojenite riskinde sadece maternal ilaç maruziyetinin değil, aynı zamanda paternal ilaç maruziyetinin de rol oynayabileceğini desteklemektedir. Bu durum, bebek sahibi olmayı planlayan kronik ilaç kullanan erkeklerde tedavi yönetiminde güçlüklere yol açmaktadır. Makale, renal transplant hastalarında kullanılan immünosupresif ilaçların paternal teratojenite ve fertilite üzerine etkilerini değerlendirerek, klinisyenlerin bu konudaki farkındalığını artırmayı hedeflemektedir. Bu ilaçların uzun dönem etkilerini anlamak için daha kapsamlı çalışmalara ihtiyaç duyulmakla birlikte çocuk sahibi olmayı planlayan ve immünosupresif tedavi alması planlanan hastalarda, sperm bankacılığı gibi seçeneklerin de göz önünde bulundurulması önerilmektedir.

Anahtar Sözcükler: Renal transplantasyon, erkek, fertilite, paternal maruziyet, teratojenler, gebelik

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Introduction

Today, kidney transplantation has become an important treatment option that significantly improves the quality of life of patients with renal failure. Immunosuppressive drugs given to post-transplant patients are essential to prevent organ rejection and are one of the most important factors in improving transplant success (1). The etiology of two-thirds of congenital anomalies in newborns is not yet known. However, unknowingly being exposed to or forced to use drugs during pregnancy is one of the important factors blamed. With the increasing awareness of drug use during this period, the effects of maternal drug exposure on the infant have been investigated in detail by observational studies. Recent studies support that not only maternal drug exposure but also paternal drug exposure may play a role in the risk of teratogenicity (2). Scientific data on the effects of paternal exposure on the infant are still limited (3). However, paternal exposure to drugs is known to be harmful to the baby during pregnancy, and the intense anxiety about this may complicate the treatment management of men who are chronic drug users and planning to have a baby (4). Another more important concern for men regarding medication is the issue of fertility. The possible adverse effects of immunosuppressive drugs given to male renal transplant recipients on male fertility may cause anxiety and compliance problems in long-term drug use. However, there are various clinical studies on the adverse effects of some immunosuppressive drugs on sperm morphology, motility and sperm count (5); more comprehensive and long-term studies are still needed for many drugs.

Currently, there is limited information on the potential of these pharmacological agents to cause male infertility and possible fetal anomalies after paternal exposure following kidney transplantation. This lack of information is causing confusion and anxiety among clinicians and patients.

Clinical and Research Consequences

Calcineurin Inhibitors

Cyclosporin-A: Cyclosporins are fungal metabolites that function as immunosuppressants. One of these compounds, cyclosporine A, is clinically used after tissue and organ transplantation, in treating lupus, and in ocular inflammation associated with keratoconjunctivitis sicca. There have been no extensive human reports of possible adverse effects of cyclosporine exposure on male reproduction. However, in prepubertal male rats, chronic administration of low doses of cyclosporine caused impaired testosterone production, spermatogenesis and fertility. In mature male rats treated with cyclosporine A at 30 mg/kg orally for 28 days, decreased testicular weight and damage to germ cells were observed (6). In a study evaluating sex hormone levels in male patients receiving cyclosporine (n=21) and tacrolimus (n=16) after kidney transplantation, hormone levels were found to be normal (7).

Partners of three out of four male renal transplant patients on cyclosporine could conceive (8). Potential effects on fertility are thought to be associated with higher doses. Therefore, if low serum drug levels can be achieved while maintaining allograft function, pregnancy may be attempted (9). A study of male renal transplant recipients found that erectile dysfunction was more common with cyclosporine than with other immunosuppressants (10).

In a study in which paternal cyclosporine use was analysed, therapeutic abortion and congenital malformation rates of 152 pregnancies were evaluated. Of 152 pregnancies, two resulted in therapeutic abortion, and four resulted in congenital malformations (11). Although information on the paternal use of cyclosporine is limited, it has not been shown to increase the risk of adverse teratogenic pregnancy outcomes (12).

Tacrolimus: Tacrolimus is a macrolide derived from Streptomyces and used as an immunosuppressant in transplant patients. It is also used topically for the short-term treatment of atopic dermatitis (6). No decrease in basal or human chorionic gonadotropin- stimulated testosterone was observed in male rats treated with tacrolimus at a daily dose of 2 mg/kg. Daily administration at 1 mg/kg dose did not affect testicular weight and histology. Culturing Leydig cells with tacrolimus up to 1 mg/L did not result in impaired viability or decreased basal or stimulated testosterone production (13). There are not enough studies on the effect of tacrolimus on human fertility. However, studies are reporting an increase in sperm quality after switching to tacrolimus in patients with impaired sperm quality during sirolimus use (5). Normal sex hormone levels have been reported in male patients using cyclosporine and tacrolimus after kidney transplantation.

Six different studies, including case series and case reports, analysed the effects of paternal use of cyclosporine, tacrolimus or sirolimus on pregnancy outcomes. No association between tacrolimus and adverse pregnancy outcomes was reported (5). In another study evaluating the rates of therapeutic abortion and congenital malformations in 255 pregnancies with paternal tacrolimus use, 8 of 255 pregnancies resulted in therapeutic abortion, and 10 resulted in congenital malformations (11).

Clinical data on the paternal use of tacrolimus and its effect on teratogenic pregnancy outcomes are limited. Based on the limited data, tacrolimus is compatible with paternal exposure in pregnancy (14).

Anti-proliferative (Anti-metabolite) Agents

Mycophenolate: Mycophenolate mofetil is an immunosuppressant used in organ transplantation, rheumatoid arthritis and lupus. Following oral administration, mycophenolate mofetil is converted to the active metabolite mycophenolic acid (6). Mycophenolate sodium is frequently used in renal transplant patients. According to the product label, mycophenolate in male rats causes no adverse effects on fertility at oral doses lower than the recommended dose for renal and heart transplant patients based on body surface area (15).

Information on the effects of mycophenolate on male fertility or pregnancy outcomes following paternal exposure is limited;

however, available data do not indicate safety concerns (4,12). In a study in which paternal mycophenolate mofetil use was analysed, therapeutic abortion and congenital malformation rates of 313 pregnancies were evaluated. Of these pregnancies, eight resulted in therapeutic abortion, and nine resulted in congenital malformations (11). The manufacturer recommends that sexually active male patients and their partners use effective contraception for 90 days following the last dose of the patient's treatment. The recommendation, also based on animal data, is that semen should not be donated for 90 days after the last dose of mycophenolate treatment. Nevertheless, based on limited human data, mycophenolate may be considered for men with musculoskeletal and rheumatic diseases who plan to have children (16,17).

Azathioprine: Azathioprine is an antimetabolite agent used in immunosuppression, metabolised to mercaptopurine and 6-thioguanine. Azathioprine and its metabolites are cytotoxic purine analogues. Numerous clinical data are available. In 23 men with inflammatory bowel disease, semen quality did not decline after 1-4 years of treatment with azathioprine, and six of the patients had seven healthy children while on this drug regimen (18). Among 164 male renal transplant patients receiving long-term immunosuppressive therapy with azathioprine and other drugs, no adverse effects on fertility were observed (19). A prescription registry study of the infants of 54 men who were prescribed azathioprine or mercaptopurine before their wives became pregnant identified four infants with congenital anomalies (20).

A teratology information service study followed up 115 pregnancies fathered by men treated with azathioprine or 6-mercaptopurine. In 101 of these pregnancies, the father had drug exposure at the time of conception (21). There were 9 spontaneous abortions and 11 children with congenital anomalies. Three of the children had major anomalies (ventricular septal defect, horseshoe kidney and motor defect), and eight had minor anomalies (umbilical hernia, small haemangioma, hip dysplasia, xanthoma and persistent foramen ovale). This series of results was consistent with the general population experience. In 37 pregnancies fathered by men treated with azathioprine for inflammatory bowel disease, there was no increase in adverse pregnancy outcomes (22). No adverse effects on cancer, autism spectrum disorders, psychoses (including schizophrenia) or attention deficit hyperactivity disorder were found in 735 children whose fathers used azathioprine/6-mercaptopurine three months before conception (23). In another study, therapeutic abortion and congenital malformation rates were evaluated in 59 pregnancies with paternal azathioprine use. Of these, two resulted in therapeutic abortion, and four resulted in congenital malformations (11).

Available data have not shown that azathioprine adversely affects male reproductivity or increases the risk of adverse teratogenic pregnancy outcomes when used within three months prior to conception (4,11,24).

mTOR Inhibitors

Sirolimus: It is an mTOR kinase inhibitor macrolide antibiotic used as an immunosuppressant. Like tacrolimus, sirolimus interacts with interleukin-2 (IL-2), but the interaction mechanism is different for the two drugs. According to the product label, male rats exposed to a dose level 10 times the human dose relative to body surface area showed a decrease in sperm count. Male monkeys were given sirolimus for four weeks at therapeutic human dose levels and developed testicular tubule degeneration (25). Rats exposed to these levels for eight weeks showed impaired spermatogenesis (26). In another rat study, the adverse effects of sirolimus on the testis appeared to be reversible by discontinuation of the drug (27).

There are also human studies suggesting that sirolimus treatment is associated with decreased testosterone production, sperm count and fertility in renal transplant patients (6). Fifteen studies involving 492 patients (263 cases and 229 controls) were analysed in a systematic review conducted in 2020, all of whom received sirolimus or cyclosporine for organ transplantation (mostly kidney transplantation). In 11 of these studies, sperm quality abnormalities and reproductive-related hormonal changes (low testosterone and high FSH/LH levels) were reported after exposure to sirolimus. Some of these studies reported that infertility due to sirolimus treatment was reversible (5).

In one study, seven pregnancies with paternal sirolimus use were evaluated for therapeutic abortion and congenital malformations. Therapeutic abortion and congenital anomalies were not observed (11). There are insufficient studies to assess the risk of paternal use of sirolimus and its teratogenic effect on pregnancy outcomes.

Everolimus: It is a mammalian mTOR inhibitor that inhibits T and B cell activation. It is used for the prevention of transplant rejection, treatment of some cancers and seizure control in patients with tuberous sclerosis complex. Male rats treated with everolimus at a dose of 5 mg/kg/day developed infertility due to decreased sex organ weights and seminiferous epithelial abnormalities. Complete recovery was not observed after 13 weeks of treatment interruption (6).

Male transplant patients given everolimus at two doses (1.5 or 3 mg/day) showed increases in mean testosterone, FSH and LH values compared to baseline. Since there was no control group in this study, it was impossible to conclude whether the changes were clinically significant (28). A 30-year-old male patient who received everolimus after kidney transplantation presented with infertility, and sperm concentration was found to be less than 0.5 million/mL. Semen analysis was not performed before treatment. Three months after the treatment was stopped, the sperm concentration increased to 130 million/mL, and the patient's wife became pregnant one month later (29).

In a study, five pregnancies with paternal everolimus use were evaluated in terms of therapeutic abortion and congenital malformations. No congenital anomaly was observed in these

five pregnancies, and therapeutic abortion was observed in 1 of them (11). There are insufficient studies to assess the risk of paternal use of everolimus and its effect on teratogenic pregnancy outcomes. However, patients whose partners may become pregnant should be advised to use effective contraception during treatment and for four weeks after the last everolimus dose (30).

Prednisolone, Methylprednisolone: Prednisolone, the active metabolite of prednisone, is a glucocorticoid. It is used in treating autoimmune connective tissue-vascular diseases and as an immunosuppressant. Although short-term treatment with corticosteroids has been reported to be used to treat immunological infertility in men, some researchers have not found this treatment effective. It has been reported that long-term, high-dose glucocorticoid use may impair spermatogenesis, and recovery may require as long as six months. However, it does not affect chromosome number or sperm morphology (6). Although asthenospermia and oligospermia are adverse effects of the drug, the frequency of these adverse effects has not been reported (30).

In a study in which paternal glucocorticoid use was analysed, therapeutic abortion and congenital malformation rates of 298 pregnancies were evaluated. Of these pregnancies, ten resulted in therapeutic abortion, and nine resulted in congenital malformations (11). There are insufficient studies to evaluate the teratogenic effect on pregnancy outcomes in fathers who use prednisolone and methylprednisolone.

Basiliximab: Basiliximab, an IL-2 receptor antagonist, is used to prevent acute organ rejection in patients with kidney transplantation. There are no studies on the effect of basiliximab on fertility, paternal use and its effect on teratogenic pregnancy outcomes (6).

Dacliximab: Dacliximab (Daclizumab) is a monoclonal antibody that binds to the IL-2 receptor. Dacliximab was used in the treatment of multiple sclerosis and as an immunosuppressive agent after transplantation. However, it was withdrawn from the market due to its association with inflammatory brain disorders (6). No adverse effects on sperm count, motility, morphology, serum testosterone, organ weights or testicular histopathology were found at dose levels of dacliximab up to 200 mg/kg every two weeks for 60 days in monkeys (31). The effect on fertility has not been evaluated. There are no studies on the paternal use of dacliximab and its effect on teratogenic pregnancy outcomes.

Muromonab-CD3 (OKT3):

The monoclonal antibody OKT3 (Orthoclone, Muromonab CD3) is a mouse-derived antibody used to treat allograft rejection. However, it was withdrawn from the market in 2010 due to numerous side effects, better-tolerated alternatives and declining utilisation (32). There are no studies on the paternal use of Muromonab CD3 and its effect on teratogenic pregnancy outcomes.

Antithymocyte Globulin, Antilymphocyte Serum: Antithymocyte globulin (anti-lymphocyte serum) is a purified immunoglobulin G used to prevent and treat acute organ rejection after transplantation. The product label indicates that it does not alter hormone concentrations or mating behaviour when administered to male monkeys at doses up to 40 mg/kg/day. Fertility impairment occurred in five female and one male patient immunoablated with cyclophosphamide and antithymocyte globulin prior to stem cell transplantation. A relationship between cumulative cyclophosphamide dose and infertility has been established, but the effect of antithymocyte globulin is unclear (6). There are no studies on the paternal use of antithymocyte globulin and its effect on teratogenic pregnancy outcomes.

Alemtuzumab: It is a human IgG monoclonal antibody against the cell surface CD52 glycoprotein. CD52 is present in human and rodent reproductive tissues. Treatment of mice carrying the humanised CD52 gene with alemtuzumab at a dose seven times higher than the human dose based on plasma concentration (area under the curve) resulted in a less than 10% decrease in sperm count and up to 3% abnormal sperm forms. However, there was no effect on reproductive capacity (6).

A sub-study of 13 male patients receiving alemtuzumab treatment found no evidence of aspermia, azoospermia, increased motility or morphological abnormalities (33). However, there is still insufficient clinical safety data on the effect of alemtuzumab on fertility. Similarly, there are no studies on paternal exposure and its teratogenic effect on pregnancy outcomes.

Belatacept: Belatacept is a fusion protein consisting of the Fc fragment of human IgG1 immunoglobulin. The drug blocks T-cell activation and is used to prevent graft rejection. Fertility was not affected in male or female rats at exposures up to 25 times the human dose (6). Serum testosterone, FSH, LH and inhibin were measured before and after transplantation in 53 male patients with chronic renal failure. Four of these patients were reported to be taking belatacept (34). However, this study did not establish a relationship between belatacept and male fertility because the results of patients using belatacept were not discussed separately. In a study, six pregnancies with paternal belatacept use were evaluated in terms of therapeutic abortion and congenital malformations. Therapeutic abortion and congenital anomalies were not observed in these six pregnancies (11). There are insufficient studies to assess the risk of paternal use of belatacept and its effect on pregnancy outcomes.

Conclusion

In conclusion, scientific data on the effects of many immunosuppressant agents on paternal teratogenicity and fertility are still limited. Since there are not enough human studies on most drugs, data from animal studies have been utilised to give an idea. It should be kept in mind that the results obtained in animal studies cannot be directly adapted to humans but may be guiding.

This study aimed to raise awareness about the effects of immunosuppressive agents used in renal transplant patients on male fertility and pregnancy outcomes after paternal drug

exposure. Based on this, sperm banking may be considered as an option in patients who are planning to have a child and in whom immunosuppressive treatment is planned to be initiated.

Ethics

Authorship Contributions

Concept: E.H., İ.Y., Design: E.H., İ.Y., Y.Ö.İ, Data Collection or Processing:E.H., İ.Y., Analysis or Interpretation: İ.Y., Y.Ö.İ, Literature Search:E.H., İ.Y., Writing: E.H., İ.Y., Y.Ö.İ.

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Review

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Approach to Fibromyalgia and the Role of Phytotherapy in Treatment

Fibromiyaljiye Yaklaşım ve Tedavide Fitoterapinin Rolü

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ABSTRACT

Fibromyalgia (FM) is characterized by chronic widespread pain accompanied by fatigue, poor sleep quality, and numerous accompanying conditions. Its prevalence worldwide is around 2.7%, and it is more common in women. Although its epidemiology and pathophysiology cannot be precisely explained, it is known that various factors coexist. Over the years, guidelines containing various criteria have been established for the diagnosis of the disease. The goal of treatment in FM is to improve the patient's quality of life and minimize symptoms as much as possible. The success of treatment in FM is limited. Many patients seek alternative treatment methods, including diet and lifestyle changes. Recently, medical nutritional therapies and phytotherapy products have been at the forefront of research in this area. Phytotherapy products can be added alone or in combination with other treatment methods and can enhance the success of treatment. In this article, the epidemiology, pathophysiology, diagnostic methods, pharmacological and non-pharmacological methods used in the treatment of FM syndrome will be discussed, and the most widely used phytotherapeutic products will be addressed.

Keywords: Fibromyalgia, phytotherapy, pharmacological treatments, non-pharmacological treatments, etiopathogenesis

ÖZ

Fibromiyalji (FM), kronik yaygın ağrının yanı sıra yorgunluk, kötü uyku kalitesi ve çok sayıda eşlik eden hastalıkla karakterizedir. Dünya çapında yaygınlığı %2,7 civarındadır ve kadınlarda daha sık görülür. Epidemiyoloji ve patofizyolojisi kesin olarak açıklanamamakla birlikte çeşitli etmenlerin birliktelik gösterdiği bilinmektedir. Hastalığın tanısı için yıllar boyunca çeşitli kriterleri içeren kılavuzlar oluşturulmuştur. FM tedavisinde amaçlanan hastanın yaşam kalitesini artırmak, semptomları mümkün olduğunca azaltmaktır. FM'de tedavinin başarısı sınırlıdır. Birçok hasta diyet ve yaşam tarzı değişiklikleri de dahil olmak üzere alternatif tedavi yöntemleri aramaktadır. Bu noktada son zamanlarda araştırmaların odağında tıbbi beslenme tedavileri ve fitoterapi ürünleri yer almaktadır. Fitoterapi ürünleri tek başına ya da kombine şekilde diğer tedavi yöntemlerine eklenebilmekte ve tedavinin başarısını artırabilmektedir. Bu yazıda FM sendromunun epidemiyolojisi, patofizyolojisi, tanı yöntemleri, tedavide kullanılan farmakolojik ve non-farmakolojik yöntemlere değinilecek ve en çok kullanım alanı bulan fitoterapötik ürünler ele alınacaktır.

Anahtar Sözcükler: Fibromiyalji, fitoterapi, farmakolojik tedaviler, non-farmakolojik tedaviler, etiyopatogenez

1. Definition of Fibromyalgia

Fibromyalgia (FM) is a syndrome characterized by chronic musculoskeletal pain. The main symptoms of this disease are muscle and joint stiffness, insomnia, fatigue, mood disorders, cognitive dysfunction, anxiety, depression, general sensitivity, and inability to adequately perform normal daily activities. FM is also associated with specific diseases such as infections, diabetes, rheumatic, psychiatric or neurological disorders. There are similarities with neuropathic pain in terms of clinical findings, pathophysiology and neuropharmacology. Although FM is not a musculoskeletal system disease, most of the symptoms occur in this system (1,2). It is known that hypersensitivity is observed in some anatomical areas called tender points in FM syndrome

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(3,4). FM, which was initially perceived primarily as a rheumatic disorder, is now recognized as a pain processing disorder and central nervous system sensitization (5,6).

2. Epidemiology and Etiology

It has been stated that the prevalence of FM is between 0.2% and 6.6%, 2.4% to 6.8% in women, 0.7% to 11.4% in urban areas, and 0.1% to 5.2% in rural areas (7). Although the incidence varies depending on the diagnostic criteria used in a prevalence study; it was found to be 3.6-5.6% in Türkiye, 2.64% in Europe and 2.7% worldwide (8,9). Studies on FM report that it is 9-10 times more common in women than in men (2,10).

Although the etiopathogenesis of FM syndrome is not clear, its causes include genetic, neurological, psychological, sleep-related factors and immunological factors (2,11).

Various hypotheses have been put forward about the emergence of the disease. There are studies revealing the relationship between immune system disorders, sleep disorders, neuroendocrine disorders, peripheral and central nervous system abnormalities and FM symptoms; however, the findings of these studies are not sufficient to determine the mechanism of formation of the disease (2,4,12,13).

2.1. Genetic Factors

The heritability of FM is estimated to be approximately 50%, indicating that genetics has an important role in etiopathogenesis (14). The probability of having FM in first-degree relatives of patients with FM is 8 times higher than in the healthy population (15). However, it has been observed that the risk of any sensitivity and chronic pain in family members of patients with FM is higher than healthy controls (16). Recent genetic studies focus on specific gene polymorphisms, especially in the serotonergic, dopaminergic and catecholaminergic systems. In patients with FM, it has been shown that the disease is worse in those carrying serotonin transporter, dopamine 4 receptor, serotonin 5-HT2A receptor (T/T phenotype) and catecholamine o-methyl transferase polymorphisms and gene alleles with increased monoamine oxidase activation (4,17). The *TRPV2* gene was found to be responsible for the decrease in pain threshold in FM (18). A study by D'Agnelli et al. (19) shows that potential candidate genes associated with FM are SLC64A4, TRPV2, MYT1L, and NRXN3, and a gene-environment interaction involving epigenetic changes is suggested as a triggering mechanism. Additionally, it has been shown that FM exhibits a hypomethylated DNA pattern in genes related to stress response, DNA repair, autonomic system response, and subcortical neuronal abnormalities (19).

In a study investigating the risk of FM in siblings, the risk of recurrence in siblings was found to be 27%, and in this study, a genome scan was performed and a suggestive link to FM was shown in a region on chromosome 17p11.2-q11.2. The best signal was found to be at the D17S2196 mark on chromosome 17p11.2-q11.2 (20).

2.2. Endocrine Factors

FM is more common among women than men (2). Although the reasons behind this gender dominance are not fully understood, they may include hormonal, genetic, and psychosocial factors (21). Hormonal fluctuations, especially during reproductive periods such as menopause, are thought to affect the severity and prevalence of symptoms in women. In one study, pain was found to be most severe on days when progesterone level was low and cortisol level was high. Additionally, an inverse relationship has been detected with testosterone level (2).

Recently, emphasis has been placed on the relationship between FM and sex hormones. Studies have shown that there is a relationship between estrogen and substance P, and serotonin, and that these two neurotransmitters are modulated by estrogen in the brain (22,23). It is thought that neuroendocrine disorders caused by dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis may be related to the etiopathogenesis of FM (2,12). In another study, it was found that the low cortisol level in patients with FM was more pronounced in patients with high depression scores (9,24).

Circulating somatomedin C levels were found to be lower in patients with FM compared to the control group. Low somatomedin C levels cause persistent impairment of growth hormone (GH) secretion and, as a result, cause dysregulation in the HPA axis response and a decrease in GH secretion (9,25).

2.3. Neurotransmitter Dysregulation

Neurotransmitter dysregulation is important in the pathophysiology of FM and affects the processing of pain signals in the central nervous system. Several neurotransmitters, such as serotonin, norepinephrine, and dopamine, play a role in the altered pain perception observed in patients with FM (26). Low serotonin levels have been observed in patients with FM, and a positive and significant relationship has been found between serotonin levels and tender points (2). Dopamine has been associated with mental disorders frequently observed in FM.

It has been determined that dopamine, norepinephrine and serotonin levels decrease in the cerebrospinal fluid in patients with FM, while glutamate and substance P levels increase. It has been shown that abnormal pain perception related to FM is associated with increased substance P levels and low serotonin levels (27). Although low serotonin causes a decrease in pain threshold, it may also shed light on the relationship between FM and sleep and cognitive disorders (9,28).

2.4. Immunological Factors

Emerging evidence suggests that abnormalities in immune function, including immune system dysregulation and increased levels of inflammatory cytokines, may contribute to the pathophysiology of FM (29).

In addition, the fact that FM frequently accompanies autoimmune diseases suggests that there may be an immunological factor in its etiology (30). FM is common in autoimmune diseases such as

rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome. It has been observed that tumor necrosis factor-α, interleukin (IL)-8, and IL-10 levels are high in patients diagnosed as having FM, and a correlation has been found between these cytokine levels and other FM symptoms, especially pain (5,9,17). The fact that FM may develop after human immunodeficiency virus, coxsackie virus, and parvovirus infection suggests that there may be an immunological mechanism in the etiology (31).

2.5. Psychiatric Disorders and Sleep Disorders

There are studies arguing that psychiatric disorders exist in patients with FM before diagnosis and accompany them throughout life (9,32). Psychiatric disorders are more common in FM compared to other rheumatic diseases. The most common psychiatric diseases were found to be depressive disorder (20-80%) and anxiety disorder (13-65.8%) (2,33). It has been found that pain sensitivity increases even more in patients with FM with depressive symptoms (34). It has been determined that abnormalities in alpha and delta wave patterns in deep sleep are common in sleep disorders in patients with FM (35). It has been observed that poor sleep quality is associated with increased both pain intensity and fatigue (5,12). The most common sleep-related complaints in patients with FM are daytime sleepiness, frequent awakenings, nocturnal restlessness, and involuntary leg movements (36). It has been observed that sleep disturbance affects the pain threshold, but there is a mutual interaction between pain and the sleep process (9,12,37).

2.6. Central Sensitization

Central sensitization has an important place in understanding the amplification of pain signals in FM. This involves an abnormal response of the central nervous system to stimuli, leading to an exaggerated and prolonged experience of pain. Changes in the function of N-methyl-D-aspartate (NMDA) receptors are associated with imbalances in excitatory and inhibitory neurotransmitter systems (38).

2.7. Concomitant Conditions

FM often occurs with other medical and psychiatric conditions, reflecting the complex and interconnected nature of the syndrome (2). Rheumatic diseases, psychiatric disorders, chronic fatigue syndrome and sleep disorders may occur together with FM (39). Additionally, a link has been found between obesity and increased risk, development and severity of symptoms.

3. Pathophysiology of Fibromyalgia

Although the pathophysiological factors of FM are not well known, it is thought to be related to a pain processing problem in the brain. In many cases, patients become hypersensitive to pain. Constant hypervigilance to pain is also associated with psychological problems (40). The main changes observed in FM are dysfunction in mono-aminergic neurotransmission. It leads to an increase in the levels of excitatory neurotransmitters such as glutamate and substance P and a decrease in the levels of serotonin and norepinephrine at the level of descending anti-nociceptive pathways in the spinal cord. Other abnormalities observed are dopamine dysregulation and altered activity of endogenous cerebral opioids. Taken together, these components appear to explain the central pathophysiology of FM (41).

Peripheral abnormalities may contribute to increased nociceptive tonic support in the spinal cord, resulting in central sensitization. Other factors that appear to play a role in the pathophysiology of FM are neuroendocrine factors, genetic predisposition, oxidative stress, and environmental and psychosocial changes.

Chronic pain is defined as pain that lasts longer than three months or is recurring. In chronic pain syndromes, pain is usually the only complaint. In syndromes such as FM or non-specific low back pain conditions, chronic pain may be perceived as a disease in itself and described as "chronic primary pain". Although the exact pathogenesis is still unclear, this type of pain persists despite receiving adequate treatment and in the absence of any signs of inflammation, prompting the search for evidence of central sensitization. It is now clear that FM is related to neural hypersensitization and reduced conditioned pain modulation (42). Patients with FM have a lower pain threshold, resulting in a generalized state of hyperalgesia and/or allodynia. This indicates that there may be an increase in pain or a problem with sensory processing in the central nervous system.

Various studies have revealed findings indicating neurological damage (43). Clinical studies based on functional magnetic resonance imaging (fMRI) have confirmed a central neuronal change in nociceptive processes. In particular, following the same amount of pressure stimulation, patients with FM have greater neuronal activation in pain processing areas of the brain than control subjects (44). Subtle differences in fMRI results in studies of FM or other chronic pain conditions are due to the fact that the pain-triggering stimulus is not normalized and therefore the intensity may differ with each scan. fMRI studies have also been useful in determining the role of psychological factors in pain processing in FM and in examining the degree of connectivity between various brain regions (45). The degree of connection between these regions depends on the intensity of spontaneous and continuous pain (46). For example, in patients with FM, a decrease in the connection between anti-nociceptive areas in the brainstem is observed following a painful stimulus. Additionally, increased glutamate levels have been observed in the brains of FM patients in clinical studies. When proton magnetic resonance spectroscopy was used, it was stated that these levels increased in the main areas of pain processing such as the insula (47,48).

Increasing evidence shows that neurogenic inflammatory processes occurring in peripheral tissues, spinal cord and brain are also responsible for the pathophysiology of FM (49). In fact, the release of biologically active agents such as chemokines and cytokines leads to the activation of the innate and adaptive immune system. All of these correspond to many of the peripheral clinical features reported by patients with FM, such as swelling and dysesthesia, and these may also affect central symptoms such as cognitive changes and fatigue. Additionally, physiological mechanisms related to stress and emotions are thought to be upstream drivers of neurogenic inflammation in FM (50).

The role of stress in exacerbating FM symptoms has been widely described epidemiologically, both through self-reports and clinical surveys. Despite the inconsistency between different studies regarding possible changes in plasma cortisol levels in patients with FM, dysregulation of circadian variation is frequently observed. In particular, a flattening of the plasma cortisol concentration curve is observed during the day. In addition, decreased cortisol secretion in response to adrenocorticotropic hormone tests has also been described. The HPA axis involves neurotransmitter and neuroendocrine response systems to stress and may be activated in FM. This system may explain some of the symptoms seen in FM (51,52).

4. Diagnostic Biomarkers

The diagnosis of FM is currently based solely on a complete clinical evaluation.

Among the 1990 American College of Rheumatology (ACR) FM Syndrome Diagnostic Criteria; The criteria for widespread chronic body pain (ongoing for at least 3 months) and tender points (pain in at least 11 of the 18 defined tender points) were changed in 2010. In the 2010 ACR diagnostic criteria, tender point examination was abandoned and instead of a symptom-based evaluation, a common pain index and symptom severity scale (SSS) that patients filled out themselves were developed. The first diagnostic criteria were as follows;

1. Widespread pain index (WPI) ≥7, SSS ≥5 or WPI: 3-6 and SSS ≥9,

2. Chronic symptoms (>3 months),

3. There is no other disease that can explain the pain.

Later, in 2016, the 2010 ACR diagnostic criteria were renewed, and the areas of pain evaluated in the WPI were divided into regions, and pain in at least 4 of these 5 regions was added to the diagnostic criteria. These 5 regions are as follows: upper right region, lower right region, upper left region, lower left region, axial region. The presence of other painful disorders does not exclude this diagnosis (2,5,53-55).

Individual phenotypic variability and the coexistence of other pathologies make clinical examinations inadequate for definitive diagnosis, making it impossible to decide on universal criteria for this disease. Moreover, specific biomarkers are not yet available, and therefore research has been directed towards the search for new indicators for the objective diagnosis of affected individuals through the identification of genetic, environmental and epigenetic factors underlying the pathophysiology of FM.

There is great interest in using a simple blood test to diagnose FM. Therefore, various attempts have been made to identify unique serological markers. These findings, as well as genetic testing, are often contradictory, and no clinical tests have been confirmed to date (34). Among the parameters to be checked for diagnosis: autoantibodies (antipolymer antibody, antiserotonin, antiganglioside and antiphospholipid), neuropeptides (plasma neuropeptide Y and substance P), inflammatory cytokines (IL-8), glutamate.

Various functional neuroimaging techniques and studies show measurable changes in patients with FM. One of the first functional neuroimaging techniques to be used to study FM was single photon emission computed tomography (SPECT). This method provides measurement of regional cerebral blood flow (rCBF), which reflects neural activity in the brain following the injection of a radioactive tracer. Similar to SPECT, positron emission tomography (PET) uses radioactive tracers but has higher temporal and spatial resolution. fMRI, which has higher temporal and spatial resolution than SPECT or PET, provides more accurate results in both of these areas (56).

5. Clinical Findings

The most common symptom at presentation is chronic musculoskeletal pain. Pain is usually present on both sides of the body, above and below the waist, and along the spine (57,58). It may be in the form of widespread body pain or it may be regional. Patients may describe the pain as burning, aching, or stinging. Stressors, comorbidities, chronic fatigue, and temperature changes are among the factors that worsen pain (59).

Another common symptom is fatigue. There is a state of deep fatigue, especially in the morning, that often continues throughout the day, regardless of the amount or quality of sleep (2,60). Sleep disorders are common. Difficulty falling asleep, frequent awakenings, deterioration in sleep quality, and feeling unrested are often observed (59). Most of them have paresthesia complaints such as numbness and tingling in any part of the body, often in the extremities (61). It is thought that the cognitive disorders seen in FM are often associated with chronic pain (62). Cognitive complaints such as forgetfulness, inability to concentrate, and difficulty finding words are common, and this condition is called "fibro fog" (2,63). Patients may also experience a variety of other symptoms, such as headache, irritable bowel syndrome, temporomandibular joint disorders, anxiety, and depression (64-66).

6. Differential Diagnosis

The first things that come to our mind in differential diagnosis are; rheumatic diseases such as myofascial pain syndrome, chronic fatigue syndrome, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, polymyalgia rheumatica, Sjögren's syndrome, myositis, chronic viral infections, osteoarthritis, pain of psychogenic origin, hypothyroidism, depression and neuropathies (67,68).

7. Treatment

The aim of FM treatment should be to improve the patient's quality of life, reduce symptoms as much as possible, and enable the patient to perform daily activities. There is no treatment method that completely eliminates the symptoms or provides complete recovery. Although there is no single effective treatment method for this disease, of which etiology is multifactorial,

a multidisciplinary treatment approach is applied in clinical practice by using both drug and non-drug treatment methods (69).

7.1. Non-pharmacological Treatment Methods

Exercise, cognitive behavioral therapy, occupational therapy, hyperbaric oxygen, acupuncture, massage, yoga, tai chi, and aerobics have been found effective in relieving symptoms.

Lifestyle changes such as regular, low-impact exercise, walking, swimming or cycling, creating a comfortable sleeping environment and maintaining good sleep hygiene, stress management, deep breathing exercises, a balanced diet, reducing caffeine or avoiding certain trigger foods are recommended for patients.

7.2. Pharmacological Treatment Methods

Pharmacological treatment options with proven effectiveness in the treatment of FM;

- Painkillers; Paracetamol is the most used. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce inflammation and pain but have limited effectiveness.

- Seratonin reuptake inhibitors (SSRI); medications such as fluoxetine (Prozac), sertraline (Zoloft), paroxetine (Paxil), fluvoxamine (Luvox), and escitalopram (Lexapro).

- Seratonin noradrenaline reuptake inhibitors (SNRI); Effective dose of duloxetine is 60 mg. Milnacipran relieves pain and improves mood.

- Tricyclic antidepressants (TCA); Amitriptyline and nortriptyline are used to improve sleep patterns and reduce pain.

- Tramadol is an opioid pain medication used to treat moderate to moderately severe pain.

- Antiepileptics (pregabalin and gabapentin); Pregabalin shows its anxiolytic, analgesic and antiepileptic effects by binding to calcium channel receptors of presynaptic neurons in the brain and reducing the release of neurotransmitters such as substance P and glutamate. It reduces neuropathic pain and improves sleep patterns.

- Muscle relaxants; Cyclobenzaprine relieves muscle spasm and improves sleep quality

- 5-HT3 receptor antagonists; Treatments such as Dolasetron, Granisetron, Ondansetron, Tropisetron and Palonosetron are also used.

However, the 3 drugs that received FDA approval are Pregabalin, Duloxetine, and Milnacipran (70).

7.3. Phytotherapy and Supportive Treatments in Fibromyalgia

7.3.1. Herbal Ingredients

7.3.1.1. Capsaicin

Latin name: *Capsicum annuum L.*

Active ingredient: Capsaicinoids

Mechanism of action: Capsaicin is an alkaloid substance that gives red hot pepper its hotness. It is thought that capsaicin blocks the transmission of pain signals by affecting nerve endings. Capsaicin is a vanilloid derivative of vanillic acid and interacts with receptors located in peripheral nerves. Vanilloid receptor-1, to which capsaicin binds, shapes the flow of sodium, potassium and calcium into the cell, causing depolarization in the neuron and the secretion of neurotransmitters. Studies have been conducted to clarify that capsaicin has a direct relationship with the sensation of pain. Capsaicin is included in the group of painkillers that act through primary sensory neurons. It has been reported that by eliminating the sensitivity of these neurons, they can be used in posthepatic neuropathy, diabetic and other neuropathic pain.

Scientific studies:

In a randomized controlled study, the effectiveness of local application of 0.025% capsaicin in the treatment of FM was tested compared to placebo cream. Forty-five patients were randomly selected into groups so that capsaicin, control and placebo groups could be examined comparatively. After 4 weeks of this double-blind treatment, patients were evaluated for pain, tenderness, and sleep quality. Pain and sleep quality were assessed using a visual analogue scale (VAS), while tenderness was measured using a dolorimeter. A significant improvement in sensitivity has been associated with capsaicin. However, no improvement in pain or sleep quality was seen (71,72).

7.3.1.2. Chlorella

Latin name: *Chlorella pyrenoidosa*

Active ingredient: In addition to high levels of provitamin A, protein, vitamins, minerals, monosaccharides, polysaccharides, agar and antioxidants.

Mechanism of action: It is a single-celled green algae that grows in freshwater sources. It has the highest chlorophyll content among known plants and also contains high concentrations of many vitamins and minerals, as well as fiber, nucleic acids, amino acids, enzymes and other substances. Chlorella is generally known to have antioxidant properties. Antioxidants help protect cells against damage from oxidative stress. Conditions such as FM have been linked to increased oxidative stress. For this reason, Chlorella is used as a treatment support with its antioxidant properties.

Scientific Studies

Nutritional supplementation with *Chlorella pyrenoidosa* was associated with improvement in FM symptoms in two studies by the same research group. In the first study, an uncontrolled open-label study, participants with FM supplemented their diet with 10 g of Chlorella and 100 mL of Chlorella extract daily for 2 months. The average number of tender points decreased significantly.

In a larger, well-designed, randomized placebo-controlled, double-blind crossover clinical trial in 37 patients with FM, a

significant reduction in the number of tender points was observed in the treatment group. When this decrease was compared to the slight increase in the placebo group, a significant increase in function was also observed (73,74).

7.3.1.3. Ganoderma (Reishi Mushroom)

Latin name: *Ganoderma lucidum*

Active ingredient: Triterpenoids, polysaccharides and proteoglycans

Mechanism of effect: Ganoderma lucidum is a type of mushroom commonly known as the "mushroom of immortality" and has medicinal properties such as strengthening effects, increasing life energy and strengthening heart function. Ganoderma has been used in traditional Chinese medicine for thousands of years to promote health, lasting youth, vitality and longevity. Modern medical studies have shown that this mushroom has a wide range of bioactivities, including anti-inflammatory, anti-oxidant, anti-glycemic, anti-ulcer, anti-cancer and immunostimulatory effects. Ganoderma has also been used in the treatment of various chronic diseases such as hepatopathy, nephritis, hypertension, arthritis, migraine, insomnia, bronchitis, asthma, diabetes and cancer (75).

Scientific studies: One randomized, double-blind clinical study evaluated the effect of Ganoderma Lucidum supplementation on physical performances in patients with FM. Sixty four women with FM were divided into two groups to receive 3 grams of G. Lucidum twice daily (n=32) or 6 grams of C. siliqua daily (n=32). At the end of the study period, an improvement in aerobic endurance, walking speed, and body flexibility (chair sit-and-reach test, 6-minute and 20-minute walk test) was observed in patients with FM over 6 weeks (76).

7.3.1.4. Turmeric

Latin name: *Curcuma longa L.*

Active ingredient: Curcuminoids

Mechanism of action: Anti-inflammatory, antioxidative, antinociceptive.

It has been found in *in vivo* and *in vitro* studies that the anti-inflammatory effect is due to the curcumin substance found in plant rhizomes. It limits the expression of 5-lipoxygenase and COX-2, an enzyme that plays a role in most inflammations, and inhibits thromboxane B2, suppresses the activation of the transcription factor NF-kB, TNF, IL-1, IL-6, IL-8. It has been noted that it limits the expression of inflammatory cytokines such as and chemokines and many cell surface adhesion molecules associated with inflammation, and therefore shows anti-inflammatory activity. Curcuma longa is considered to be the most effective compounds in reducing pain in osteoarthritis in the short term.

Scientific studies: Turmeric is one of the most common herbs proven to provide relief from the chronic pain of FM. Its mechanism-based effectiveness in neuropathic pain has been demonstrated in many experimental models (77).

7.3.1.5. Ginkgo Biloba

Latin name: *Ginkgo biloba L.*

Active ingredient: Flavonoids and terpenic lactones.

Mechanism of action: It has been suggested that some of the symptoms of FM may be due to the excessive presence of oxygen-derived free radicals. These are known to trigger pain and inflammation and impair muscle function. Ginkgo biloba extract has antioxidant properties, which may explain some of its benefits (78).

How to use: For 1 cup of tea, add 1 teaspoon of leaves to water and brew for 5-10 minutes. It becomes drinkable after brewing. If it is to be used as ready-made herbal tea, a glass of hot water should be added to the herbal tea and it should be consumed after brewing for 5 minutes.

When it is brought into capsule or tablet form as an extract, 1-2 capsules are consumed per day in determined doses. When consumed as tea, it is recommended to drink 1-2 cups a day.

7.3.1.6. Saffron

Latin name: *Crocus Sativus L.*

Active ingredient: Crocin, picrocrocin, safranal.

Mechanism of action: Saffron shows similar efficacy to SSRIs and TCAs in treating depression, with a good safety profile as reported in previous studies.

Scientific studies: In a double-blinded study, Shakiba et al. (79) compared the effectiveness of Crocus Sativus (saffron) with duloxetine in patients with FM, in which groups received 15 mg *Crocus Sativus* or 30 mg duloxetine for a total of 8 weeks, starting with one capsule per day in the first week and two capsules per day after the second week. As a result of a randomized control study, *Crocus sativus* showed comparable effectiveness in the treatment of FM (79).

In the study conducted by Barmaki et al. (80) to evaluate the effectiveness and safety of a herbal treatment compared to the current therapeutic regimen in patients with FM, all conventional treatment was continued for a 6-month follow-up. As a result, a therapeutic effect was detected on fatigue, emotion and social life, and disease-related depression in patients with FM receiving conventional treatment, according to the Fib-19-01 evaluation (80).

7.3.1.7. Devil's Claw

Latin name: *Harpagophytum procumbens*

Active ingredient: Harpagofitum iridoid glycosides, phytosterols, aromatic acids and flavonoids

Mechanism of action: This herb is a well-known traditional treatment for joint pain and has recently gained attention for its role in relieving muscle pain.

Scientific study: Scientific research has also found that Devil's claw has favorable benefits compared to NSAIDs, in fact, it is a better alternative treatment (81). In a trial designed to test this herb's ability to relieve muscle pain, low doses of devil's claw showed improvement in pain over a four-week period.

Devil's claw has no significant side effects, but it may interact with warfarin. High doses of devil's claw may cause gastric disorders. It is not recommended for pregnant or breastfeeding women (82).

7.3.1.8. Grape Seed Extract

Latin Name: *Vitis vinifera*

Active ingredient: Proanthocyanidin and resveratrol.

Grape seed extract (GSE), rich in polyphenol groups, is a natural plant derivative that is often produced as a waste byproduct in the winemaking process. The oil of wine grape seeds contains natural anti-inflammatory compounds such as powerful antioxidants, procyanidins, anthocyanins, and gallic acid. Neuroprotective, antioxidant and anti-inflammatory effects have been reported in in vitro and animal studies (83).

Mechanism of action: Anti-inflammatory.

GSE can be consumed in liquid form, as an addition to the nutrition program, through tablets or capsules.

Scientific studies: Fujishita et al. (84) showed that Koshu grapes (rich in higher polyphenol and procyanidin oligomer) reduced H_2O_2-induced neuronal cell death by upregulating IL-6, COX-2, and IL-1a in astrocytes with oxidative stress states, suggesting the neuroprotective effect of GSE.

Regarding FM-like symptoms in animals, Mun et al. (85) reported that oligomeric proanthocyanidin complex (OPC) administration had anti-hyperalgesic effects in an acidic saline animal model mimicking FM due to the antioxidant and anti-inflammatory properties of OPC. In the same animal study, they found that the expression of ASIC3, an ion-sensing channel located in the central and peripheral nervous systems, was reduced in the M1 and M2 brains of hyperalgesic animals (85).

In a double-blind, randomized, crossover study comparing three doses of anthocyanidins and placebo, anthocyanins were found to have small but significant results at a dose of 80 mg/day in patients diagnosed as having moderate to severe FM for three months. Patients showed significant decreases in their sleep disturbances and fatigue levels from the first month to the last month of treatment. The recommended daily anthocyanin dose has been increased from 40 mg/day to 80 mg/day due to the optimal benefits seen at this dosage level. Although the results are encouraging, further research is needed due to the small number of experiments (n=12) (86).

7.3.1.9. White Willow Bark

Latin name: *Salix alba*

Active ingredient: Salicin

Mechanism of action: Anti-inflammatory

Willow bark extract is one of the first examples of modern drug development from herbal medicine. It is derived from the willow tree, also known as salix, and is often standardized with salicin; but may also contain other salicylates as well as flavonoids and polyphenols. It has been used for thousands of years for its antipyretic, analgesic and anti-inflammatory effects. The active substances of willow bark extract inhibit COX-2-mediated prostaglandin E2 release and the release of IL-1β and tumor necrosis factor-α (87). This herb acts by reducing inflammation in the body. Salicin, the active ingredient in this herb, lowers fever and reduces inflammation, which in turn relieves pain. It reduces the level of prostaglandins.

Scientific studies: In the open, multicenter observational study of Beer and Wegener (88) with reference treatment; 90 patients received standardized willow bark extract preparation, 41 received standard treatment, and 8 patients received a combination of the two. The tablet containing 60 mg salicin was given twice a day. At the end of the 6-week treatment and follow-up period, it was stated that willow bark extract was at a level comparable to standard treatments without side effects (88).

How to use: It is recommended to add 3 cups of water to a tablespoon of dried white willow bark, boil it well, then squeeze 5 drops of lemon and consume it as a cup of tea during the day.

Expected side effects and conditions when it should not be used: Willow bark is not recommended for people using anticoagulant therapy and may cause gastric disorders in high doses. White willow bark should not be used by people who are allergic to Aspirin.

7.3.1.10. Corydalis

Latin name: *Corydalis alpestris*

Active ingredient: Corydalis, dehydrocorybulbine (DHCB)

Mechanism of action: Corydalis family is a medicinal plant widely used in Chinese herbal medicine. It is mostly used to treat pain and often in combination with other herbs.

Recently, corydalis has attracted attention as a possible treatment for FM due to DHCB compound found in the root of the plant. DHCB is an alkaloid believed to have non-opioid analgesic properties. Synthetic DHCB is used to demonstrate that it is effective in relieving thermally and chemically induced acute pain and persistent tonic pain of inflammatory origin. It is effective at doses that do not induce sedation and produces an antinociceptive response similar to that obtained with high doses of morphine. Additionally, DHCB is effective in relieving neuropathic pain caused by injury. Since the antinociceptive effects of DHCB have been demonstrated in both the acute and inflammatory phases of the formalin assay, its activity may result from direct effects on the central nervous system (89-91).

How to use: Corydalis is commercially available as a whole plant or in granule, tincture and capsule form.

7.3.1.11. Ginseng

Latin name: *Panax ginseng Meyer*

Active ingredient: Ginsenoside

Mechanism of action: Panax ginseng is a plant that has been used in Eastern medicine for years. The molecular and cellular mechanisms of functioning of ginsenosides, the active component of ginseng, include modulation of neurotransmitter function in both peripheral and central systems, inhibition of inflammatory cytokine expression, modulation of ion channel activity in spinal cord neurons, regulation of the TLR4/NF-κB signal transduction axis, and anti-inflammatory effects.

Scientific studies: To evaluate the effectiveness of Panax ginseng extract (100 mg/day) against amitriptyline (25 mg/day) and placebo, patients were evaluated for 12 weeks in a randomized/double-blind study conducted on 38 patients diagnosed as having FM.

Compared with baseline, decrease in pain in terms of VAS score ($p<0.0001$), improvement in fatigue ($p<0.0001$) and improvement in sleep quality ($p<0.001$) were found in the Ginseng group. Ginseng reduced the number of tender points and improved patients' quality of life (using the FM Impact Questionnaire - FIQ) (92).

7.3.1.12. Cannabis

Latin name: *Cannabis indica, Cannabis sativa and Cannabis ruderalis*

Active ingredient: Tetrahydrocannabinol

Mechanism of action: It is an ancient substance that has been used since ancient times to treat various painful conditions. Recent evidence suggests that cannabis may be an effective treatment for FM. Cannabis interacts with the central nervous system through endocannabinoid receptors and signaling molecules and produces analgesic and psychoactive effects.

Scientific studies: In recent years, cannabis and its derivatives have become the focus of attention in the treatment of FM and other rheumatic diseases. Evidence for the use of cannabis in FM is limited; Preliminary clinical data support the molecular basis for the analgesic effects of cannabinoids. However, as cannabis and cannabinoid products become increasingly legal and accessible, more data is being collected regarding its use in patients (93).

7.3.2. Vitamins

Studies have determined that patients with FM are often prescribed nutritional supplements and dietary changes after the disease is diagnosed. Multivitamins and mineral supplements are commonly used.

7.3.2.1. Vitamin C (Ascorbic Acid)

Vitamin C, which has antioxidant properties, can help prevent cell damage by reducing oxidative stress. This may contribute to the relief of FM symptoms.

7.3.2.2. Vitamin B12

Some patients with FM may be deficient in vitamin B, especially B12 level. Positive effects of B12/folic acid supplementation have been reported for patients with FM. In the study of patients with FM who reported themselves as "very advanced", higher and more frequent doses were given over a long period of time and it was determined that the patients' complaints improved.

7.3.2.3. Vitamin D

Low levels of vitamin D have been detected in patients with FM, which also inhibits Mg absorption. Studies on the muscles of patients with vitamin D deficiency have shown a decrease in adenosine triphosphate levels, similar to patients with FM, causing acute pain.

One study showed that vitamin D deficiency was also associated with depression and anxiety in FM. It was reported that vitamin D supplementation could improve the quality of life in patients with FM.

7.3.2.4. Vitamin E (Tocopherol)

It can reduce oxidative stress by protecting cell membranes and providing antioxidant activity. This may help reduce pain and inflammation in patients with FM (94-96).

7.3.3. Antioxidants

In addition to vitamins such as vitamin c (ascorbic acid), vitamin E (tocopherol), herbal products containing resveratrol and elliagic acid have known antioxidant properties. Antioxidants are potential treatments that help relieve symptoms of FM by reducing oxidative stress and inflammation. Glutathione is the most powerful antioxidant the body can produce on its own. Glutathione helps reduce oxidative stress and support the immune system. Selenium is necessary for the production of antioxidant enzymes and supports the function of the immune system. It relieves FM symptoms by reducing oxidative stress. Alpha-Lipoic Acid reduces oxidative stress by reducing intercellular oxidative damage and increasing antioxidant capacity. Additionally, it reduces insulin resistance and relieves neuropathic pain.

7.3.3.1. Coenzyme Q10 (Ubiquinone)

One of the commonly used supplements for FM is coenzyme Q10 (CoQ10). This coenzyme, which has antioxidant properties, increases energy production and reduces oxidative stress. It increases energy levels in patients with FM.

The use of coenzyme Q10 supplements has become widespread after many studies observed CoQ10 deficiency in patients with FM.

One study evaluated four studies on the use of CoQ10 supplements (300 mg/day). Three of these four studies were controlled and one was a clinical study. Findings from controlled studies reported that after CoQ10 treatment alone, patients with FM showed significant improvement in clinical symptoms demonstrated through the FIQ and VAS ($p<0.01$) (97,98).

7.3.4. Combined Products

7.3.4.1. Turmeric and Boswellia serrata

Turmeric and boswellia are well-studied anti-inflammatory compounds that are gaining popularity and are used as adjuncts and also alternatives to traditional treatments for musculoskeletal pain. Curcumin and boswellic acids, the active components of turmeric rhizomes and Boswellia serrata gum resin, are known to inhibit the nuclear factor κB signaling pathway, which is directly involved in inflammatory processes.

Dietary supplements containing Curcuma longa and Boswellia serrata are considered effective compounds in reducing pain in osteoarthritis in the short term.

It was shown that a single-center, active-controlled, open-label pilot study involving 232 participants used turmeric-boswellia formulation (1000 mg daily for 7 days) for acute musculoskeletal pain in the resting position, with results showing pain relief similar to paracetamol (inactivation of neurons/anti-inflammatory effect).

Turmeric and boswellia both have anti-inflammatory properties, but their mechanisms of action are different. Turmeric works by blocking the production of pro-inflammatory cytokines and enzymes; Boswellia shows its anti-inflammatory effect by modulating the immune system and reducing the sensitivity of pain receptors (99-101).

7.3.4.2. Ginkgo Biloba and Q10

It has been suggested that some of the symptoms of FM may be due to the excessive presence of oxygen-derived free radicals. These are known to trigger pain and inflammation and impair muscle function. Both coenzyme Q10 and Ginkgo biloba extract have antioxidant properties, which may explain some of the benefits. Coenzyme Q10 may also improve muscle function and Ginkgo biloba extract may improve vascular function.

The subjective effects of coenzyme Q10 and Ginkgo biloba extract were measured in an open, uncontrolled study in volunteer subjects diagnosed as having FM. The subjects were given oral doses of 200 mg coenzyme Q10 and 200 mg Ginkgo biloba extract per day for 84 days, and their quality of life was monitored at 0, 4, 8 and 12 week intervals.

A gradual improvement in quality of life scores was observed over the study period, with scores at the end showing a significant difference from baseline scores (64% felt better, only 9% felt worse) (78).

7.3.4.3. Turmeric and Pomegranate Peel extract (CurcuNar®)

The anti-inflammatory activity of turmeric has been proven in many studies. Polyphenolic compounds, curcumin, demethoxy curcumin and bisdemethoxy curcumin, collectively known as curcuminoids, are the main components responsible for the biological effects of turmeric. Curcumin is considered the key chemical component contributing to the observed anti-inflammatory activity. In addition to being the best known antioxidants, ellagic acid and pumigalacin found in pomegranate peel also have a synergistic effect with Curcumin in FM due to their anti-inflammatory and neuroprotective properties. Additionally, the ethanolic extract of pomegranate peel and leaves has been shown to inhibit acetylcholine esterase in many neuronal tissues (102-104).

7.3.5. Probiotics

In recent years, altered gut microbiota has been associated with FM, suggesting that altering the gut microbiota (e.g., through probiotics) may be an effective therapeutic treatment. Probiotics are naturally found in fermented foods. These foods include homemade natural yoghurts without additives, pickles, kefir, pickled olives, apple cider vinegar, kombucha tea, and cheese. In addition, various fiber-rich vegetables, nuts, seeds, beans and whole grains also contribute to the protection of intestinal flora.

7.4. New Treatments and Research

Advances in neurobiological research aim to uncover the complex mechanisms in FM. Imaging studies such as fMRI and PET have provided insight into the central nervous system abnormalities associated with FM.

Borsook et al. (105) have changed our perception of chronic pain. From a somatosensory system-focused approach, it has been established that emotional, cognitive, and regulatory brain areas as well as degenerative processes are involved and contribute to the development and persistence of pain symptoms. However, it has revealed associated features such as anxiety, depression and cognitive changes (105).

Genetic research continues to identify potential genetic markers that may be associated with susceptibility to FM. This can facilitate diagnosis, assess disease severity, and guide treatment decisions. There are currently no definitive biomarkers for FM, but research is advancing to define objective measures that may increase diagnostic accuracy (106).

Glutamate, a neurotransmitter, plays a role in pain signaling. Investigational treatments targeting glutamate receptors, such as NMDA receptors, are being investigated to modulate pain perception in FM. These include promising drugs such as ketamine (107).

Increasing evidence links the gut microbiome to a variety of health conditions, including FM (108). Research suggests that results can be obtained through fecal microbiota transplantation (109). Integration of digital health tools, wearable devices, and telemedicine may improve the monitoring and management of FM. These technologies can provide remote symptom monitoring, real-time feedback, and improve access to healthcare resources (110).

8. Conclusion

In conclusion, FM, characterized by widespread musculoskeletal pain and associated symptoms, has a complex and variable clinical picture. FM, which was defined as

a rheumatic disorder in the past and is now understood to have completely different characteristics, appears to require a lot of research in both diagnosis and treatment modalities. Advances in research have elucidated the complex nature of FM, which includes genetic, neurological, immunological, and psychosocial factors. FM severely impairs the quality of life. It affects daily activities, sleep patterns, cognitive function and social interactions.

Diagnosis has now evolved into a more comprehensive assessment that emphasizes the extent of pain and associated symptoms rather than relying solely on tender points. Risk factors such as gender predominance, genetic predisposition, and comorbid conditions still pose questions that need to be investigated. Treatment requires approaches that are specific to the individual and prioritize the symptom. Methods such as pharmacological treatments, non-pharmacological methods, lifestyle changes, alternative and complementary treatments, diet regulation, phytotherapy, use of vitamins and supplements can be used alone or in combination. The inadequacy of pharmacological treatments has made it necessary to turn to other methods. In this field, phytotherapy seems to be a good alternative to be combined with pharmacological treatments. In phytotherapy, combined herbal products or combinations of herbal products and vitamins appear to be more effective. FM is a syndrome that has been forgotten recently and requires a holistic approach to the patient. However, holistic treatment methods that address physical, emotional and social dimensions can be successful and increase the individual's quality of life.

Ethics

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Erratum

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The mistake was made inadvertently by the author.

The second sentence under the "Methods" of the "ABSTRACT" section on page 432 of the relevant article has been revised.

Incorrect sentence: The data were collected with Personal Information Form, the Coronavirus disease-19 (COVID-19) Related Psychological Distress scale (CORPD) and the Patient Assessment of Chronic Illness Care (PACIC) between April **05** and September 30, 2021.

Corrected sentence: The data were collected with Personal Information Form, the Coronavirus disease-19 (COVID-19) Related Psychological Distress scale (CORPD) and the Patient Assessment of Chronic Illness Care (PACIC) between April **15** and September 30, 2021.

In the Turkish version of the relevant article, the second sentence under the "Yöntemler (Methods)" of the "ÖZ (ABSTRACT)" section on page 432 has been revised.

Incorrect sentence: Veriler **05** Nisan-30 Eylül 2021 tarihleri arasında Kişisel Bilgi Formu, Koronavirüs hastalığı-19 (COVID-19) ile İlişkili Psikolojik Sıkıntı Ölçeği ve Kronik Hastalık Bakımını Değerlendirme Ölçeği ile toplandı.

Corrected sentence: Veriler **15** Nisan-30 Eylül 2021 tarihleri arasında Kişisel Bilgi Formu, Koronavirüs hastalığı-19 (COVID-19) ile İlişkili Psikolojik Sıkıntı Ölçeği ve Kronik Hastalık Bakımını Değerlendirme Ölçeği ile toplandı.

The responsible author's email in the "Address for Correspondence" section of the relevant article on page 432 has been edited.

Incorrect e-mail: kubraincirkus@trakya.edu.tr

Corrected e-mail: k.incirkus@iku.edu.tr

The first sentence under the "Data Collection" heading on page 434 of the relevant article has been changed.

Incorrect sentence: The data were collected online via a web-based survey software

(Google Forms) between April **05** and September 30.

Corrected sentence: The data were collected online via a web-based survey software

(Google Forms) between April **15** and September 30.