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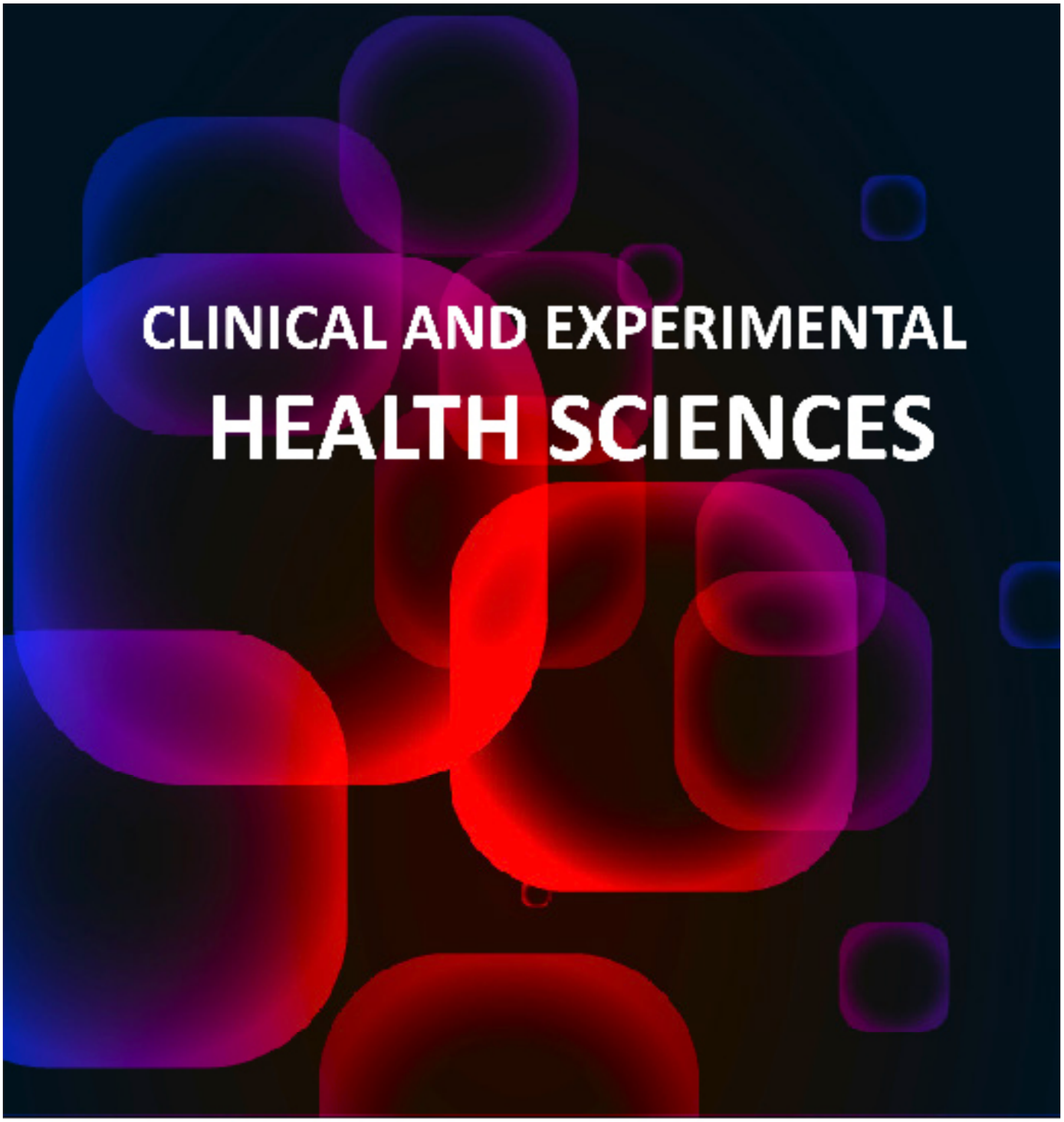

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Clinical and Experimental Health Sciences

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The aim of the journal is to contribute to the literature by publishing clinical and experimental research articles, case reports, letters to the editor, and and editorial comments prepared in accordance with the ethical guidelines in all disciplines of health sciences. The target audience of the journal includes specialists and medical professionals working in all disciplines of health sciences.

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Clinical and Experimental Health Sciences

Contents

ORIGINAL ARTICLES

Investigation of the Expression Level of Autophagy Proteins in Infertile Men 463
Nazlı Can, Kemal Ergin

Are Sinus Lifting Videos on YouTubeTM Useful for Patients? 473
Ömer Ekici, Oğulcan Akkurt

The Relationship Between Pregnancy Stress and Maternal Attachment, Postpartum Depression, and Breastfeeding Self-Efficacy: A Descriptive and Prospective Study 481
Bihter Akın, Süreyya Kılıç, Sema Dereli Yılmaz

Is HPV16 E7 Oncoprotein Labile if it is Unliganded? 489
Özlem Cesur Günay, Nicola J. Stonehouse

Is Youtube™ A Reliable Source On Invisalign First® in Mixed Dentition? 495
Elif Ece Kalaoğlu, Gülay Dumanlı Gök

Post Challenge Effects of Ozg-38.61.3 Gamma Irradiated SARS-CoV-2 Vaccine on Organ Protection in Transgenic Mouse Model 504
Mustafa Öztatlıcı, Derya Dilek Kançağı, Hülya Öztatlıcı, Raife Dilek Turan, Gözde Sır Karakuş, Bulut Yurtsever, Didem Çakırsoy, Selen Abanuz, Cihan Taştan, Samed Özer, Gamze Tümentemur, Murat Kasap, Gürler Akpınar, Büşra Şen Halıcıoğlu, Sevda Demir, Dilek Telci, Fikrettin Şahin, Mehmet İbrahim Tuğlu, Ercüment Ovalı

Rational Drug Use and E-Health Literacy Levels of Inpatients of Internal Medicine Services 517
Derya Çınar, Demet Ayar

Evaluation of the Effects of Different Irrigation Solutions on MTA and Dentin Microhardness 524
Gökay Büyükçolpan, İdil Özden, Hesna Sazak Öveçoğlu

The Effects of Immunocytochemistry on Diagnostic Accuracy in Thyroid Fine Needle Aspiration Cytology: A Retrospective Study 531
Ali Mızrak, Cansu Benli Işık, Esen Gül Uzuner, Saime Gül Barut

Evaluation of The Gap Volume Following Temporomandibular Joint Ankylosis Surgery: A Preliminary Study 538
Canseda Avağ, Emre Tosun, Hakan Hıfzı Tüz

Contents

Breast Self-Examination Practices and Influencing Factors among Healthcare Professionals Consisting of Nurses and Midwives 545
Simge Arık Girgin, Nevin Çıtak Bilgin

Investigation of the Effects of Mouthwashes on the Microhardness and Color Stability of Bulk-fill Composite Resins... 553
Sultan Aktuğ Karademir, Samet Atasoy, Serdar Akarsu, Eda Karaaslan

Attitudes Towards Breastfeeding and Related Factors Among Mothers with Infants Aged 0-6 Months 561
Selin Nur Altun, Dilek Öcalan

Determinants of Sexual Dysfunction in Allergic Rhinitis According to the Unpleasant Symptoms Theory: A Comparative Study 568
İlknur Özkan, Murat Kar, Seçil Taylan

Evaluation of the Impact of Post-Earthquake Trauma Levels on Children's Nutritional Processes in Parents Living in Earthquake Zones 577
Yeliz Serin, Duygu Leylek, Nurgül Arslan

The Effects of Mini-Implant Assisted Rapid Palatal Expansion on Intrabony Canals in The Anterior Maxilla 584
Emre Köse, Özge Ünlüoğlu, Yazgı Ay Ünüvar

Evaluation of the Burden of Caregiving of Individuals Caring for Cancer Patients 591
Hasan Küçükkendirci, Esra Hacılar, Şeyma Betül Yüksek, Tahir Kemal Şahin, Lütfi Saltuk Demir, Yasemin Durduran, Mehmet Uyar

Can Systemic Inflammation Be Predicted in Cervical Discopathy Patients Using Hemogram Data? 597
Deniz Bulut, Hüseyin Aydoğmuş

Prevalence of Hypomineralized Second Primary Molars and Associated Factors in Preschool Children in Trabzon, Türkiye: A cross-sectional study 602
Elif Nahide Taşçı, Nagehan Yılmaz, Merve Nur Öztürk, Hüsna Selin Takaoğlu, Tamer Tüzüner

Validity and Reliability Study of The Turkish Version of The Child Primary Care Adherence Scale 612
Betül Aktaş, Gülçin Uyanık, Dinçer Atila, Medine Yılmaz

The Effects of Different Post Space Conditioning Procedures and Different Endodontic Sealers on the Push-Out Bond Strengths of Fiber Posts 620
Leyla Benan Ayrancı, Ahmet Serkan Küçükekenci, Fatih Sarı, Ahmet Çetinkaya

The Inhibitory Effects of Resveratrol on Tumor Growth and Metastasis in Esophageal Adenocarcinoma 629
Saime Batırel, Bengü Çetinkaya, Ayşe Mine Yılmaz Göler

Turkish Validity and Reliability Study of the Negative Menstrual Attitudes Assessment Scale: A methodological study 636
Zeliha Özşahin, Sinem Güven Santur, Yeşim Aksoy Derya

Validity and Reliability Study of Postpartum Specific Anxiety Scale Short Form Into Turkish 647
Hafize Dağ Tüzmen, Kamile Altuntuğ

Contents

Comparative Molecular Docking Analysis of Carbapenem Antibiotics With NDM-1 and OXA-23 B-Lactamases: Insights Into Binding Mechanisms 654
Emrah Sarıyer, Gamze Ağırbaş

Assessment of Dietary Behaviors and Influencing Factors Among Patients with Heart Failure: A Cross-Sectional Study 661
Duygu Mataracı Değirmenci, Yasemin Kalkan Uğurlu, Zehra Tuğçe Bayrak, Aleyna Aksu

Does Physical Activity Level, Anthropometric Measurements, and Nutritional Status Affect Stool Type in Adult Women? 674
Özlem Baran, Muhammed Enes Kartal, Aybüke Coşkun, Cemaliye Süt Kurt, Birgül Dağ, Nursena Nahya Servi, Gökcen Doğan, Sevinç Yücecan, Ayhan Dağ

The Effect of Text Messages and Education on Cancer Screening on Women aged 30-65 682
Hilal Kutman, Hasibe Kadıoğlu

The Role of The Tissue-Level Yap–Taz Pathway in Glioma Development 690
Sinan Fındık , Ebru Arslan , Pınar Ata , Yaşar Bayri , Adnan Dağçınar

Effect of Abdominal Massage on Abdominal Distension, Pain, Comfort, and Patient Satisfaction After Colonoscopy: A Randomized Controlled Trial 695
Dilek Öztürk, Aysel Gürkan

The Effect of Immunotherapy on Macro-Micronutrient Intake and Its Relationship with Anthropometric Measurements in Patients with Non-Small Cell Lung Cancer 705
Dilek Doğan, Suphiye Mine Yurttagül, Timuçin Çil

CASE REPORT

Diastema Closure Following Detection and Removal of Residual Resins After Orthodontic Treatment: Case Report with 1-year follow-up 713
Bengü Doğu Kaya, Pınar Yılmaz Atalı, Deniz Meltem Sever, Dilek Tağtekin

Direct Composite Restoration of Anterior Diastemas with Multidisciplinary Approach 718
Simge Meşeli, Sergen Ekenel, Bora Korkut, Dilek Tağtekin

Clinical and Experimental Health Sciences

Investigation of the Expression Level of Autophagy Proteins in Infertile Men

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ABSTRACT

Objective: Infertility nowadays affects 10–15% of couples in their reproductive age. Autophagy is a physiologic process responsible for the breakdown of cytoplasmic structures and organelles, and proteins that have lost functionality. In this study, it was aimed to compare the levels of autophagy expression in the spermatozoa of men who have infertility for different reasons.

Methods: A total of 120 men were included in the study, and four groups were designed: men who have no infertility disorder and are healthy according to semen parameters (Group 1, control group), patients diagnosed with Grade 2 varicocele (Group 2), with orchitis-epididymitis (Group 3), and with unexplained infertility (Group 4). Autophagy proteins (LC3A/B, Beclin-1, p62, ATG5, ATG12, and ATG16) were examined by western blot analysis. LC3A/B and Beclin-1 were also studied by immunohistochemistry. In addition, each patient group specimen was investigated ultrastructurally by Transmission Electron Microscopy.

Results: Sperm parameters of the infertility groups decreased compared with the control group. Immunohistochemically, all autophagy proteins were increased in patient groups compared to the control group. Western blot analysis and ultrastructural studies also confirmed these data.

Conclusion: These results showed that varicocele, orchitis-epididymitis, and unexplained infertility may cause impairment in the autophagy mechanism. The explanation of this mechanism can lead to the development of new treatment options.

Keywords: Autophagy, Infertility, Spermatozoon, Immunohistochemistry

1. INTRODUCTION

The World Health Organization (WHO) defines infertility as a couple's inability to become pregnant despite engaging in unprotected sexual intercourse for one (1). Male-induced infertility is 20-30% of reproductive-age couples (2). While the most common cause of male infertility is idiopathic, varicocele comes second, followed by infections and other causes (3). Normal male fertility is through sufficient and mobile sperm production. The balance of the microenvironment plays an essential role in ensuring that spermatogenesis (4;5). Human spermatozoa have the molecular machinery necessary for autophagy activation. This includes the mechanism of maintaining vitality and motility in human sperm physiology (6;7). Autophagy is a mechanism that causes fragmentation of the structures in the lysosome due to the sac's merging with the lysosome. It is carried out by organelles that are impaired or unable to function and macro – and micro-molecules that are wrapped in a sack and sent to the lysosomes (8; 9). Autophagy is also a fundamental cell death mechanism, controlled by multiple proteins (10;11). There are at least 30 proteins, including Atg proteins. 3PI3K, mTOR, AMP-active protein kinase (AMPK) and Beclin-1, related to the regulation of autophagosome formation and activation of autophagy (12; 13). The elimination of damaged or impaired proteins takes place via autophagy. Also, many environmental factors can trigger the autophagy mechanism in cells (14;15). Many diseases can cause male infertility. One of them is idiopathic infertility, the rate of which has increased considerably. The molecular mechanism underlying the disruption in sperm parameters is still not fully understood. The aim of this study is to investigate and compare the autophagy protein levels in sperm samples of male patients who have infertility for different reasons.

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2. METHODS

Ethical approval was obtained from the Human Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (28.11.2016, no. 07/V and 29.06.2020, no. 02/V).

2.1. Study Design and Preparation of Samples

Patients between 18-45 years (n = 120), who attended the Andrology Laboratory from the Urology Clinic of Muğla Sıtkı Koçman University Practice and Research Hospital between 01 January 2017 – 01 January 2018, were included in this study. Ethical approval was obtained from the Human Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (28.11.2016, no. 07/V). Semen samples were taken from the patients after three days of sexual abstinence. Each semen sample was homogenized and then prepared for morphological examination. The patients were divided into four groups:

Group 1 (n = 30); healthy men without infertility disorder (Control group),

Group 2 (n = 30); Patients diagnosed with Grade 2 varicocele without other infertility diseases and whose sperm parameters are below the reference value according to WHO sperm parameters (Varicocele group),

Group 3 (n = 30); which consisted of patients diagnosed with orchitis-epididymitis who had no other infertility-causing diseases, with sperm parameters below the reference value according to WHO semen parameters (Infection group),

Group 4 (n = 30); comprising patients with sperm parameters below the reference value according to WHO semen parameters and who were diagnosed with unexplained infertility (Unexplained infertility group).

Smokers, alcohol users, patients with urogenital system operations or systemic disorders affecting reproduction were excluded for all groups. Samples were divided equally into three Falcon tubes for immunohistochemistry, Western blot, and ultrastructural analysis, and then centrifuged at 1000 rpm for 10 minutes.

Samples were examined under a light microscope (Nikon, 20´ magnification) by a Makler counting chamber. Total sperm count, sperm count per milliliter, percentage of total sperm count, and sperm viability were evaluated. Spermac dye (FertiPro Spermac Stain # FB18S04) was used following the staining protocol. Samples were examined under a light microscope (Nikon, 100x magnification) for morphological evaluation, and 100 cells were evaluated in the area. Head, neck, and tail anomalies were observed and recorded using the percentage value according to Kruger's strict criteria (16).

2.2. Immunohistochemistry

Samples were treated with proteinase K and then incubated with primary antibodies; LC3A/B (D3U4C) XP (Cell Signaling #12741), Beclin 1 (Cell Signaling, CBOW0115051), SQSTM1/p62 D5L7G (Cell Signaling #88588) anti-h/m/rATG5 (R&D System Minneapolis, CEDH011712A), ATG12 (Cell Signaling #2010S), ATG16L1 D6D5 (Cell Signaling #8089S) and secondary antibody conjugate. After repeated washes, samples were stained with DAP (Vector Laboratorie s#SK-4100)+ Harris hematoxylin-eosin (Thermo Scientific™# 6765007) dye. Finally, the slides were covered with Entellan mounting medium and evaluated under a light microscope. Results were calculated using the H-score (H-score = 1 × [%of cells stained by weak (+) positive] + 2 × [% of cells stained with medium (++) positivity] + 3 × [strong (+++) % of positively stained cells]) (17; 18).

2.3. Transmission Electron Microscopy (TEM)

The pellets were embedded in agar and fixed in 2.5% phosphate-buffered glutaraldehyde (Sigma-Aldrich #G5882) at 4 °C for 48 hours. Each sample was placed then in small glass vials for tissue tracking, Sorenson phosphate buffer was added, and held for 3 x 20 minutes. PBS-OsO_4 (osmium tetroxide, TAAB®: Electron Microscopy Sciences#19150) mixture was added to the vials and kept in the dark for 60 minutes. After the dehydration process, the tissues were incubated in a combination of Araldite-propylene oxide (1:1) and Araldite-benzyldimethylamine (BDMA) (1:1), (Ataman chemicals#103833) respectively. Then, semi-thin sections were taken from the Araldite, stained with toluidine blue and finally, thin cross-sections were prepared from the samples. These were enhanced by lead citrate and uranyl acetate and then evaluated by a transmission electron microscopy (TEM; Carl Zeiss Libra 1200) (17).

2.4. Western Blot Analysis

Proteins of semen samples were isolated via a protein extraction kit. Gels were used according to the weight of the proteins (kilodalton). Each well was sequentially loaded with 20 µg/ml of protein. They were transferred then to the nitrocellulose membranes, which were blocked with 5% milk powder at room temperature. Membranes were incubated with LC3A/B (D3U4C) XP (Cell Signaling #12741), Beclin 1 (Cell Signaling, CBOW #0115051) and beta-actin primary antibodies diluted with milk powder overnight at 4°C.

After washing, membranes were incubated with the secondary antibody (anti-rabbit) at room temperature for 60 minutes (19). Chemiluminescent detection was performed using a chemiluminescence ECL kit (Santa Cruz Biotechnology#Sc2004) and an imaging system. Finally, the density of the bands was evaluated with the Image J program (NIH).

2.5. Intrauterine Insemination (IUI):

Pregnancy, abortion, and live birth rates were determined after intrauterine insemination (IUI) in the spouses of patients who received infertility treatment. The information

of these couples was obtained from the Karmed database of Muğla Sıtkı Koçman University Training and Research Hospital (2016-2020). Individuals who continue infertility treatment in an outer center or whose infertility treatment is stopped were excluded from the study.

2.6. Statistical Analysis

A post-hoc power analysis was conducted on the data obtained in the study (G-power software), and Statistical Package for Social Sciences Program (SPSS Inc., Chicago, IL, USA, v.19.0) was used for statistical analysis. After a standard distribution analysis for each sperm parameter, the Kruskal-Wallis test was used for data that was not suitable for normal distribution, and the Mann – Whitney U test was used for binary comparisons. One-way analysis of variance (ANOVA) was performed for data suitable for normal distribution, and the H score results. P values under .05 ($p<.05$) were considered statistically significant.

3. RESULTS

3.1. Spermiogram and Kruger Morphology

It has been found that the average age was 32 for all groups. Spermiogram results which were obtained from patients are shown in Table 1.

Sperms were examined according to Kruger Criteria as healthy, amorphous head, large head, small head, long head, double head, neck/middle defects, tail defects, cytoplasmic droplet, head defect (head without neck and tail) and results are given as % value in Table 2.

Table 1. *Spermiogram Results*

Group / Sperm Parameters	Control Med. (Min-Max)	Varicocele Med. (Min-Max)	Infection Med. (Min-Max)	Unexplained Med. (Min-Max)
Volume (ml)	3 (2-5)	3 (2-7)	3 (2-6)	3 (2-6)
Viscosity	0 (0-0)	0 (0-3)	0 (0-3)	0 (0-3)
Sperm/ml	$65.5x10^{6*}$ ($40x10^{6}$-$100x10^{6}$)	$5x10^{6*}$ ($1x10^{5}$-$10x10^{6}$)	$8x10^{6*}$ ($1x10^{5}$-$15x10^{6}$)	$7.5\ x10^{6*}$ ($1x10^{5}$-$14x10^{6}$)
Total Sperm	$177.5\ x10^{6*}$ ($88x10^{6}$-$340x10^{6}$)	$20x10^{6*}$ ($4x10^{5}$-$42x10^{6}$)	$17x10^{6*}$ ($4x10^{5}$-$60x10^{6}$)	$22x10^{6*}$ ($1.5x10^{5}$-$104x10^{6}$)
Total Movement %	69 (69-76)*	30 (0-40)*	30 (0-40)*	30 (0-40)*
Total Progressive Movement%				
+4 %	30 (15-45)	0 (0-18)	0 (0-19)	1 (0-15)
+3 %	40 (30-50)	30 (0-40)	25 (0-39)	29 (0-37)
+2 %	5 (0-10)	6 (0-10)	5 (0-15)	7,5 (0-20)
+1 %	30 (20-40)*	60 (50-95)*	65 (50-100)*	60 (40-90)*
Leukocyte %	$1x10^{6*}$ ($1x10^{6}$-$1x10^{6}$)	$1x10^{6**}$ ($1x10^{6}$-$2x10^{6}$)	$3x10^{6*,**,***}$ ($2x10^{6}$-$20x10^{6}$)	$1x10^{6***}$ ($1x10^{6}$-$2x10^{6}$)

Statistical significance between the control groups and patient groups (p=.000), ** Statistical significance between the Infection group and the Varicocele group (p=.000), * Statistical significance between the Infection group and the Unexplained infertility group (p=.000)*

Table 2. *Kruger morphology results*

Group / Sperm Parameters	Control Med. (Min-Max)	Varicocele Med. (Min-Max)	Infection Med. (Min-Max)	Unexplained Med. (Min-Max)
Healthy	17 (5-36)*	1 (1-3)*	1 (1-3)*	1 (1-3)*
Amorphous head	19 (8-40)*	34 (18-64)*,**	20 (10-60)*,**,***	30 (2-60)*,***
Large head	5 (0-20)*	6 (0-10)*,+	8 (0-10)*,++	9,5 (0-34)*,+;++
Small head	4 (0-18)	5(0-20)	5 (1-20)	6,5 (0-18)
Long head	5 (0-20)	5 (0-21)	5 (0-17)	5 (0-18)
Double head	6 (1-20)*	5 (0-40)	4,5 (0-10)	2,5 (0-10)*
Neck/middle defects	15 (0-40)*	20 (0-40)*,**	38 (10-50)*,**,***	19,5 (0-39)*,***
Tail defects	10 (5-30)	10 (0-20)	10 (0-30)	10 (0-40)
Cytoplasmic droplet	9 (0-20)	5 (0-20)	6 (0-19)	5 (0-20)
Head defect	0 (0-20)	0 (0-20)	0 (0-19)	0 (0-13)

Statistical significance between the control group and patient groups (p<.05), ** The statistical significance between the Infection group and the Varicocele group, ***Statistical significance between the Infection group and the Unexplained infertility group, + Statistical significance between the Unexplained infertility group and the Varicocele group, ** Statistical significance between the Unexplained infertility group and the Varicocele group (p<.05)*

3.2. Intrauterine Insemination (IUI)

Pregnancy, abortion and live birth rates after intrauterine insemination are given in Figureure 1. (Figure. 1)

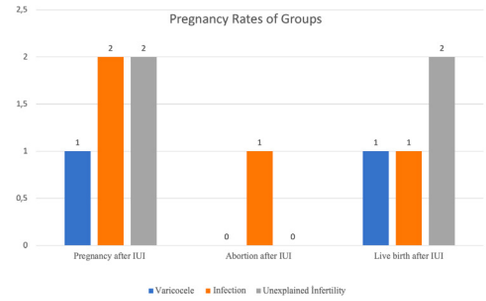

Figure 1. *Pregnancy, abortion, and live birth rates after IUI (intrauterine insemination)*

3.3. Immunohistochemical Analysis

The effect size, calculated on H score values obtained with the LC3A/B protein, was higher than 0.40 according to the ANOVA test. In the G-power program, the post-hoc power analysis value, considered as the highest limit of 0.40 under the effect size, was determined to be 0.96.

The control group showed negative/weak staining for LC3A/B, whereas the patient groups had increased positive staining (Figure. 2 A-D). The H-score values of the control group were significantly lower than the patient groups (p = .000) (Figure. 2 E). The increased LC3A/B expression observed in patient groups may reflect enhanced autophagosome membrane elongation, an essential step in active autophagy.

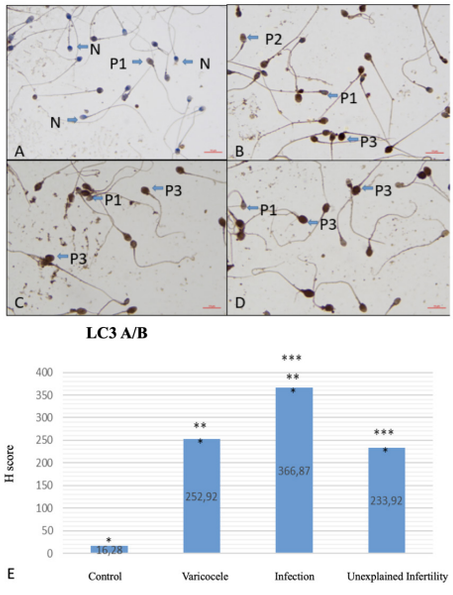

Figure 2. *LC3 A/B staining and H-scores. A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification (N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for LC3 A/B (*Significance between the control group and patient groups, p=.000, **Significance between the infection group and the varicocele group, p=.000, ***Significance between the infection group and the unexplained infertility group, p=.000).*

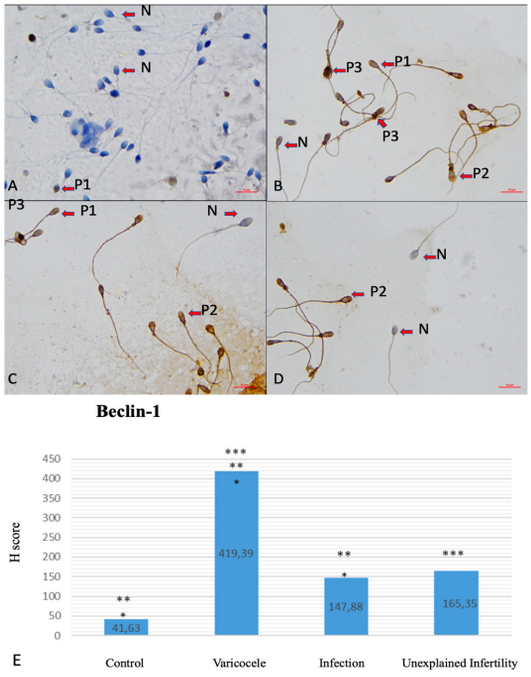

Figure 3. *Beclin-1 staining and H-scores. A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification (N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for Beclin-1 (*Significance between the control group and patient groups, p=0.000, **Significance between the varicocele group and the infection group, p=.000, ***Significance between the varicocele group and the unexplained infertility group, p=.000).*

The control group had negative/weak staining for Beclin-1, whereas the other three groups had increased positive staining. The most intense staining was in the varicocele group (Figure. 3 A-D). The H-scores of the control group were significantly lower than the patient groups (p = .000), and higher in the varicocele group compared with the infection and unexplained infertility groups (p =.000) (Figure. 3 E). Beclin-1, a key initiator of autophagy, was more strongly expressed in the varicocele group, suggesting early-stage activation of autophagic signaling.

Negative/weak staining for P62 was shown in the control group, and increased positive staining was seen in the patient groups. The most intense staining was observed in the infection group (Figure. 4, A-D). The H-scores of the control group were significantly lower than in the patient groups (p = .000). The H-score values of the infection group were higher compared to the varicocele and unexplained infertility groups (p = .000). The unexplained infertility group had higher H-scores compared with the varicocele group (p = .028) (Figure. 4 E). Elevated p62 expression, especially in the infection group, may indicate impaired autophagic degradation, as p62 accumulates when autophagic flux is disrupted.

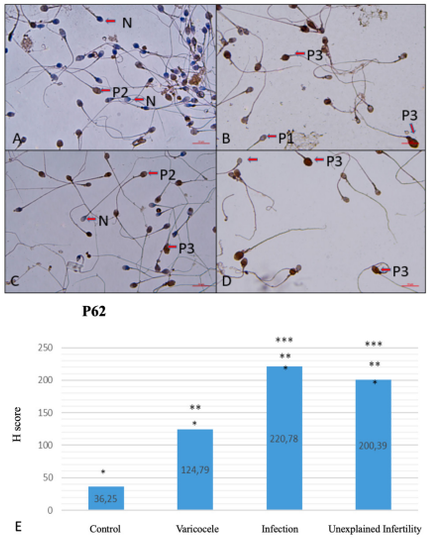

Figure 4. *P62 staining and H-scores. A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification (N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for P62 (*Significance between the control group and patient groups, p=.000, **Significance between the infection, varicocele and unexplained infertility group, p=.000, ***Significance between the infection group and the unexplained infertility group, p=.028).*

Negative/weak staining for ATG-5 was shown in the control group, and increased positive staining was seen in the other three groups. The most intense staining was observed in the unexplained infertility group (Figure. 5 A-D). The H-scores of the control group was significantly lower than of the patient groups (p= .000). The unexplained infertility group had a higher H-score than the varicocele group (p = .000), and the varicocele group had a higher H-score than the infection group (p = .028) (Figure. 5 E).

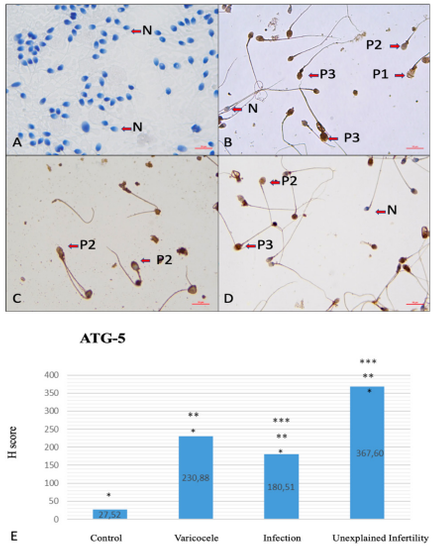

Figure 5. *ATG-5 staining and H-scores A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for ATG-5 (*Significance between the control group and patient groups, p=.000, **Significance between the varicocele, infection and unexplained infertility group, p=.000, *** Significance between the infection group and the unexplained infertility group, p=.028).*

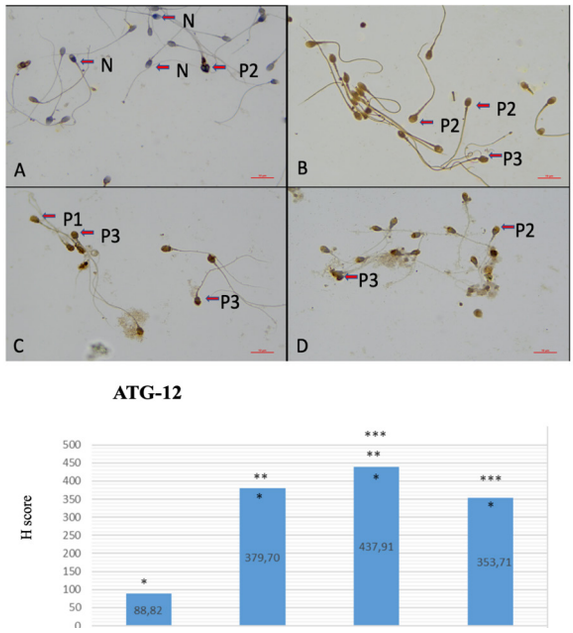

Figure 6. *ATG-12 staining and H-scores A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for ATG-12 (*Significance between the control group and patient groups, p=0.000, **Significance between the varicocele and infection group, p=.004, ***Significance between the infection and unexplained infertility group, p=.000).*

Negative/weak staining for ATG-12 was shown in the control group, and increased positive staining was seen in the patient groups. The most intense staining was observed in the infection group (Figure. 6, A-D). The H-scores of the control group were significantly lower than in the patient groups (p= .000). The H-score of the infection group was higher than the varicocele group (p = .004) and unexplained infertility group (p = 0.000) (Figure. 6 E).

Negative/weak staining for ATG-16 was shown in the control group, and increased positive staining was seen in the patient groups (Figure. 7 A-D). The H-scores of the control group were significantly lower than in the patient groups (p= .000). The H-scores of the varicocele group was higher than the infection group (p = .001) and higher in the unexplained infertility group compared to the infection group (p = .000) (Figure. 7 E). Among these proteins, ATG5 functions in the early stages of autophagy by forming a covalent conjugate with ATG12, which is essential for autophagic membrane formation. The detection of the highest levels of ATG5 and ATG16 both of which play a critical role in the elongation of the autophagosomal membrane in the unexplained infertility group suggests that a compensatory autophagic response may have been activated in this group in response to increased cellular stress.

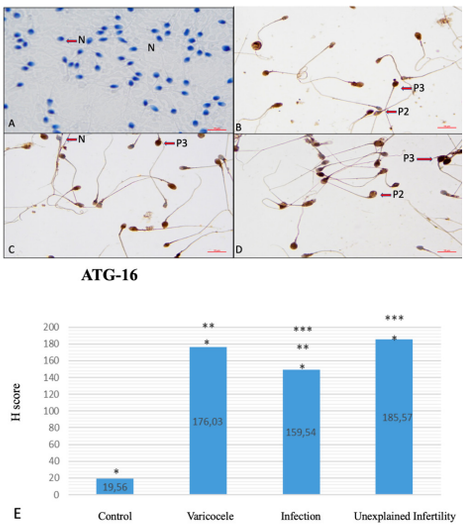

Figure 7. *ATG-16 staining and H-scores A. Control Group, B. Varicocele Group, C. Infection Group, D. Unexplained Infertility Group, x100 magnification (N: negative staining, P1: light staining, P2: medium staining, P3: intense staining), E. H-score values for ATG-16 (*Significance between the control group and patient groups, p=.000, **Significance between the varicocele and infection group, p=.001, ***Significance between the infection and unexplained infertility group, p=.000).*

3.4. Western Blot Analysis

LC3A/B expression level was higher in the patient group as compared with the control groups. Evaluation of band density showed that LC3A/B expression in the patient groups was statistically higher than in the control group (p<.05) (Figure. 8 and Figure. 9)

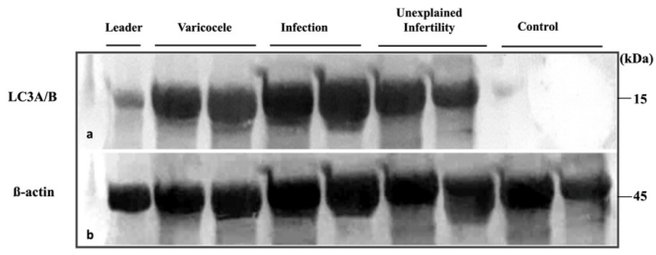

Figure 8. *a. LC3A/B blot bands; leader, varicocele, infection, unexplained infertility, control, b. ß-actin bands; leader, varicocele, infection, unexplained infertility, control*

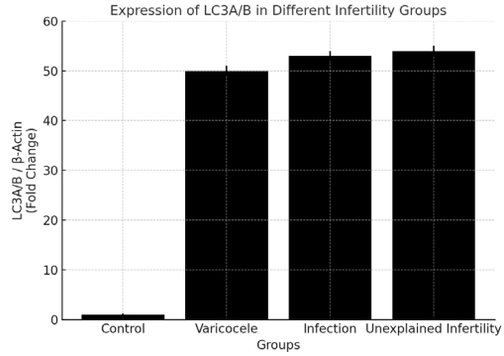

Figure 9. *Fold changes observed after the ratio of LC3 A/B to beta-actin*

Beclin-1 expression level was higher in the patient group as compared with the control groups. Evaluation of band density showed that Beclin-1 expression in the patient groups was statistically higher than in the control group (p<.05) (Figure.10 and Figure. 11) LC3A/B is a key autophagy marker involved in the elongation and maturation of the autophagosomal membrane, and particularly the increase in its LC3-II form indicates the initiation of autophagic activity. Beclin-1 is part of the core autophagy initiation complex and regulates autophagosome nucleation through its interaction with class III phosphatidylinositol 3-kinase (PI3K). The concomitant upregulation of these two proteins suggests that the autophagy process is activated at its early stages and that cells may be mounting an autophagic response to physiological stress conditions. Our findings indicate that autophagy may be involved in the pathophysiological processes underlying male infertility and that the autophagic response appears to be particularly prominent in conditions such as varicocele and unexplained infertility.

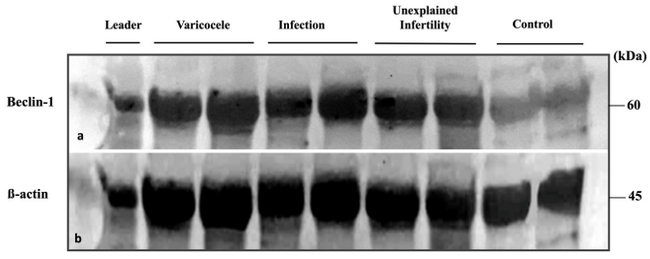

Figure 10. a*. Beclin-1 blot bands; leader, varicocele, infection, unexplained infertility, control,* ***b.*** *ß-actin bands; leader, varicocele, infection, unexplained infertility, control.*

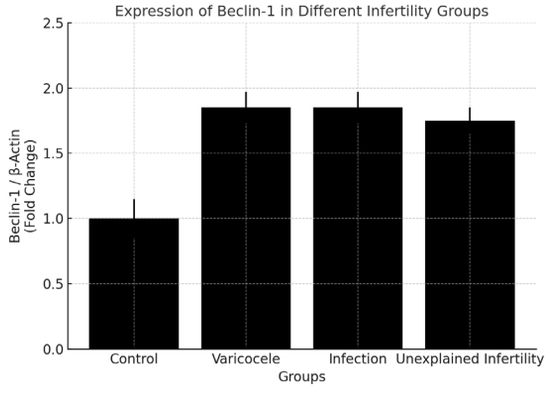

Figure 11*-Fold changes observed after the ratio of Beclin-1 to beta-actin*

3.5. Transmission Electron Microscopy

Normal morphology was seen in the healthy sperm of the control group (Figure.12 A). On the other hand, morphological structure deterioration was observed in the varicocele, infection, and unexplained infertility groups (Figure.12 B-D). Head, acrosome, neck, and middle part integrity were impaired in most spermatozoa. Also, increased autophagic vesicles in the patient groups were observed intensely in the head and neck region. (Figure.12

B-D). These ultrastructural alterations may reflect cellular stress-induced autophagic activation in the spermatozoa of infertile patients. The presence of double-membrane vesicle-like structures, particularly in the head and neck regions, is suggestive of autophagosomes, which play a central role in the sequestration and degradation of damaged organelles and cytoplasmic components. The accumulation of such vesicles in the patient groups may indicate an attempt by sperm cells to eliminate dysfunctional structures and preserve cellular homeostasis. However, the simultaneous observation of prominent structural defects suggests that autophagic clearance may be insufficient or incomplete, potentially contributing to impaired sperm function in these infertility conditions.

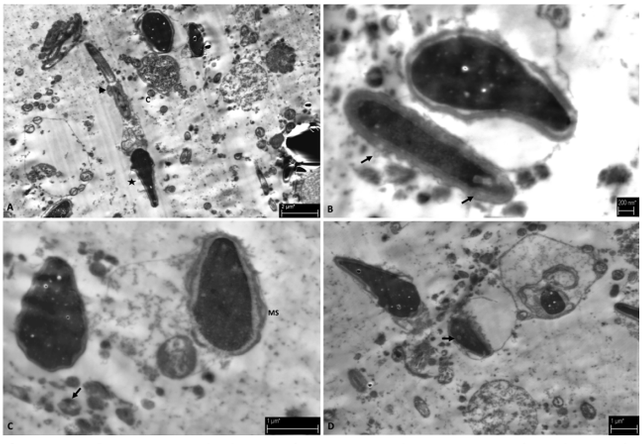

Figure 12. *Transmission electron microscopy showing the ultrastructural characteristics of the sperm in all groups. The healthy spermatozoon of the control group (A), increased autophagosomal vesicles in the varicocele group (B), infection group (C), and unexplained infertility group (D) (arrow: autophagosomal vesicle, arrowhead: mitochondria, asterisk: head, C: centriole, MS: multilamellar structure).*

4. DISCUSSION

Diseases with abnormal spermatogenesis, like varicocele and orchitis-epididymitis, are associated with a deterioration in the cell death mechanism involved protecting of spermatogenic cells. This disruption leads to breaks in the endoplasmic connections with Sertoli cells and causes advanced male infertility, including oligospermia, azoospermia, asthenospermia, and cryptorchism (20; 21). Recent studies have demonstrated that autophagy mechanisms play active roles not only during testicular development but also in the functional maturation stages of ejaculated spermatozoa. In the study conducted by Aparicio et al. (2023) on stallion spermatozoa, it was shown that the LC3 protein is converted from LC3I to LC3II during sperm capacitation and becomes colocalized with acrosomal membranes during the acrosome reaction. Additionally, autophagy-related proteins such as Beclin-1 and ATG16 were reported to localize in both the sperm head and tail regions, suggesting potential involvement in processes such as motility regulation and cytoplasmic residue clearance. These findings indicate that the autophagy system not only serves to eliminate intracellular damage but may also contribute to the maintenance of sperm physiology and the support of fertilization capacity. Similarly, in our study, significantly increased expression levels of LC3A/B, Beclin-1, and ATG16 were detected in the varicocele and unexplained infertility groups, which may reflect both a testicular stress response and a functional autophagic adaptation involved in the regulation of mature sperm functions (22).

Varicocele results in an increase in temperature in the testes and many pathologies caused by varicocele like hypoxia, oxidative stress, and abnormal sperm function can cause irreversible damage to the spermatogenesis process. In the advanced stages of varicocele, the testicular structure deteriorates, the seminiferous epithelial layer becomes thinner, the seminiferous tubule size decreases, and lumen irregularities occur (12). Recent literature highlights oxidative stress and inflammation as central mechanisms in the pathophysiology of varicocele-induced male infertility. Wang et al. (2025) emphasized that scrotal hyperthermia, testicular hypoxia, and cadmium accumulation contribute to increased ROS production, disrupting spermatogenesis, sperm motility, and testicular microenvironment homeostasis. Moreover, the overexpression of HIF-1α, IL-6, and TNF-α in testicular tissues has been associated with enhanced germ cell apoptosis and impaired Sertoli and Leydig cell function (23). In line with these findings, our study demonstrated a significant upregulation of autophagy-related markers such as LC3A/B, Beclin-1, ATG5, and ATG16 in the varicocele group, suggesting that autophagy may act as a compensatory response to oxidative and inflammatory stress. According to a study by Zhu et al. Autophagy were firstly observed in the seminiferous epithelia cells in varicocele rats testes. HIF-1α/BNIP3/Beclin1 autophagy signaling pathways play an important role in the activation of autophagy. The damaged organelles and proteins in the seminiferous epithelia cells activate autophagy. Autophagy can timely remove these damaged substrates to protect cells from secondary lesion. They hypothesize that autophagy may protects seminiferous epithelia cells from apoptosis at the beginning of varicocele, and apoptosis may inhibit autophagy pathways in later stages (24). In this study, the sperm parameters of patients with Grade 2 varicocele were examined according to WHO 2010 criteria, and a significant decrease in sperm count was observed in the patient groups compared to the control group. Likewise, the percentage of sperm with progressive movement and normal morphology was significantly lower than the control group as compared with the other patient groups. Also, the expression of autophagy markers was significantly increased in the patient groups. Beclin-1 expression was significantly higher in the varicocele group compared with the other patient groups. It was thought that the reason for this was that the patients with Grade 2 varicoceles might have damaged sperm, especially the acrosome, and head components, after an increase in the temperature of the testicles.

There is a close relationship between infection in the male reproductive system organs and impairment of spermatogenesis, decrease in sperm production, and

disorders in mature sperm function. However, there seem to be unexplained events in the relationship between infections and male infertility. Agarwal et al. reported that the cell integrity and function of Sertoli cells after the testicular infection was preserved, but in cells of the spermatogenic series, deteriorations have been found (25). Autophagy is involved in the cellular defense against microbial infections. Autophagosomes may directly uptake and degrade invading viruses intracellularly. These results suggest that autophagy restricts infectious agents in these cell types. Wu at al. showed that male germ cell and macrophages abundantly expressed beclin-1 and LC3, thereby indicating that these cells are well equipped with an autophagic machinery (26). Accordingly, in our study, a decrease in semen parameters was observed in the infection group in this study. However, there was a significant increase in LC3A/B and Beclin-1 expression in this group. The highest level of LC3A/B among all groups was found in the infection group. In recent years, experimental studies have increasingly demonstrated the protective role of autophagy in Sertoli cells during spermatogenesis. In a study conducted by Xiong et al. (2023), Sertoli cell-specific deletion of the Atg5 gene was associated with significant reductions in sperm count, motility, and the expression of meiotic proteins such as STRA8 and SYCP3 following cadmium (Cd) exposure. The same study also revealed that Atg5 deficiency suppressed intratesticular retinoic acid levels and the expression of RA-synthesizing enzymes ALDH1A1 and ALDH1A2 through downregulation of the transcription factor WT1. Moreover, overexpression of WT1 in Sertoli cells reversed Cd-induced suppression of ALDH1A1, highlighting the essential role of Atg5-mediated autophagic responses in protecting spermatogenesis from environmental toxins. In line with these findings, the increased expression of ATG5 observed in the varicocele and infection groups in our study may reflect an adaptive autophagic response to cellular stress and may contribute to the protection of germ cells against apoptosis (27).

Although the exact etiology of unexplained male infertility is unknown, it is attributed to many environmental factors like exposure to certain chemicals (e.g., heavy metals, pesticides), electromagnetic radiation, smoking, alcohol use, chronic stress, and infections in the male reproductive system (28). Most of these factors eventually cause oxidative stress. Numerous free radicals present a pathological response that can lead to decreased sperm count and motility and the development of abnormal sperm morphology. In this study, decreased sperm count and motility were observed in the group with unexplained male infertility compared to the control group. Also, the numbers of sperm with typical morphology were significantly decreased in this group.

Reactive oxygen species (ROS) are involved in the mechanism through the ATG4 protein, which plays a crucial role in autophagosome formation. ATG4 protein plays a role in forming LC3 protein in suitable and mature form. ATG4 regulation with ROS occurs through oxidation of the cysteine amino acid in the catalytic domain of this protein. In this way, autophagosome formation is suppressed by inactivated ATG4 (29). ROS are signal molecules that regulate cell death or survival at various levels in connection with autophagy. Autophagy causes a disruption in catalase, which is an important enzyme that protects the cell from damage by ROS. Catalase degradation and ROS accumulation due to autophagy have been shown to contribute to cell death. In the present study, expressions of the autophagy markers increased in the unexplained male infertility group compared to the control group. When the patient groups were compared, it was observed that the expressions of ATG5 and ATG16 increased, especially in the unexplained infertility group. These findings suggest that oxidative stress may induce autophagic activation through these core autophagy-related proteins. Uribe et al. (2022) reported that, in human spermatozoa exposed to oxidative stress, the levels of ATG5 and ATG16, along with other autophagy markers such as LC3-II and pAMPK, were elevated, indicating the activation of a cytoprotective autophagic response. Moreover, in their study, inhibition of autophagy with chloroquine resulted in a marked decrease in sperm quality and mitochondrial function, as well as an increase in apoptotic markers (30). These results support the hypothesis that autophagy functions as a survival mechanism under stress conditions. In this context, the increased expression of ATG5 and ATG16 observed in the unexplained infertility group in our study may reflect a compensatory cellular defense mechanism aimed at preserving sperm function in response to subclinical oxidative damage. The potential protective roles of ATG5 and ATG16 in maintaining sperm function suggest that these proteins may serve as valuable biomarker candidates for elucidating the underlying pathophysiology of multifactorial conditions such as unexplained male infertility. The expression levels of autophagy markers are important indicators for evaluating the initiation and effectiveness of the autophagic response. In our study, significant increases in the levels of LC3A/B, Beclin-1, ATG5, and ATG16 were observed in the patient groups. These increases suggest that cells activate autophagy, particularly in response to oxidative stress and structural damage. However, the concurrent increase in p62 levels indicates a disruption in the degradation phase of autophagy, suggesting that autophagic flux could not be completed. These findings show similarities with a study conducted using a cisplatin-induced testicular injury model. In that study, following cisplatin administration, autophagy markers (Beclin-1, LC3B, ATG5, ATG7) were significantly decreased, accompanied by p62 accumulation and mTOR upregulation, indicating that autophagy was suppressed. However, with thymoquinone (TQ) treatment, these parameters returned to normal and autophagic flux was restored. In our study, the increased levels of autophagy-initiating proteins indicate activation of autophagy at the cellular level; however, when considered together with the accumulation of p62, it suggests that the process may not have been sufficiently completed at the lysosomal degradation stage(31). Recent advanced imaging studies have demonstrated that the p62 protein accumulates not only within the intracellular compartment but also in the intercellular regions of epithelial cells. In particular, a volume electron microscopy-based study conducted by Xia Li et al.

(2025) identified novel structures called intercellular organelle reservoirs (IORs) located between epididymal epithelial cells, where autophagy-related markers such as p62, LC3, and LAMP1 were found to be highly concentrated. In our study, the increased expression of p62 observed in patient groups may similarly indicate a disruption in autophagic degradation and an impairment in autophagic flux. Therefore, p62 may serve not only as a marker for evaluating the efficiency of the autophagic process but also as a valuable biomarker for assessing the preservation of the functional integrity of sperm cells (32).

Also, in this study, the sperm samples of each group were examined ultrastructurally. It was observed that in the samples of the control group, the integrity of the sperm was preserved, and the morphological structure was typical. Most sperm of the varicocele group, had an amorphous head structure. Especially in the part of the head, saccular structures considered as autophagosomal vesicles were observed. Similar structures and images were detected in the sperms of infertility and unexplained infertility groups. Double-membrane autophagosomal vesicle-like structures were observed particularly in the head and neck regions of spermatozoa in the varicocele and unexplained infertility groups. These ultrastructural findings suggest that autophagic activity may be morphologically activated. Similarly, in a study conducted using a TBC1D20-deficient mouse model, electron microscopy revealed the accumulation of large double-membrane autophagosomes due to impaired lysosomal fusion, accompanied by significant structural alterations in testicular tissue and defective acrosome formation as a result of disrupted autophagic flux. In this context, the structural abnormalities observed in our study may also be associated with the accumulation of autophagic intermediates caused by incomplete progression of the lysosomal degradation phase (33).

5. CONCLUSION

In conclusion, our study demonstrates that autophagy plays a significant role as a molecular regulatory mechanism in different etiologies of male infertility. The significant increase in the expression levels of autophagy-related proteins such as LC3A/B, Beclin-1, ATG5, and ATG16 suggests that the autophagic process is activated in sperm cells and functions as a protective response under cellular stress. Moreover, the observation of double-membrane autophagic vesicles in electron microscopy findings supports the morphological evidence of this molecular response. These findings indicate that autophagy may serve as a potential target mechanism in maintaining sperm structural integrity and functionality, and that autophagy-related markers should be considered in the diagnostic and therapeutic approaches to male infertility.

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Ethics Committee Approval: *This study was approved by Ethics Committee of Muğla Sıtkı Koçman University Medicine Faculty, (approval date November 28th, 2016 and number07/V.)*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea:NC
Design of the study:NC
Acquisition of data for the study:NC,KE
Analysis of data for the study:NC,KE
Interpretation of data for the study:NC
Drafting the manuscript:NC,KE
Revising it critically for important intellectual content:KE
Final approval of the version to be published:NC,KE

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Clinical and Experimental Health Sciences

Are Sinus Lifting Videos on YouTube™ Useful for Patients?

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ABSTRACT

Objective: Patients now frequently use YouTube™ to find information on health-related topics. The aim of this study is to evaluate the quality and adequacy of YouTube™ content related to sinus lift surgery for patients.

Methods: In this cross-sectional study, the first 150 results of the search using the keyword 'sinus lift' on YouTube™ were examined. By applying the inclusion criteria, 72 videos were evaluated and the content of these videos was analyzed by two independent researchers. While the content levels of the videos were evaluated with the Total Content Score (TCS), the content quality was evaluated with the Video Information and Quality Index (VIQI), modified-DISCERN and Global Quality Scale (GQS) methods.

Results: According to the criteria determined in this study, 18.1% of the videos were found to be of low quality, 63.8% of them were of medium quality, and 18.1% were of good quality. While procedure, material and indication categories were the most mentioned topics in the videos, complications, and post-operative findings were seen to be the least mentioned topics in the videos. There was a statistically significant positive correlation between the total content score and video duration, number of likes, number of dislikes, number of comments, VIQI, and DISCERN scores.

Conclusions: The content of the sinus lift-related YouTube™ videos reviewed was of moderate quality. Specialists who perform sinus lift procedures should be aware of the information on the Internet and direct their patients to the most accurate and up-to-date sites.

Keywords: Maxillary sinus floor augmentation, videorecording, Internet, patient education.

1. INTRODUCTION

Tooth loss is a common problem and hence dental implants have become a widely adopted practice in modern dentistry (1). The posterior maxilla poses various complications for implant dentistry. Pneumatization of the maxillary sinus is one such issue (2). The sinus lift procedure involves elevating the sinus and applying various bone grafts to place dental implants in these areas, where the maxillary sinus is displaced towards the crest in the posterior toothless areas of the upper jaw. It is applied with two methods: closed (crestal approach) and open (lateral window technique). Complications such as Schneider membrane perforation, bleeding, implants being pushed into the sinus, and infection may occur during or after sinus floor elevation (3).

The Internet's visually rich content, simple use, and accessibility make it a useful resource for sharing and obtaining health-related information (4). YouTube is one of the most commonly used platforms for free video sharing, ranking after Google and Facebook. Over 65,000 new videos are uploaded daily, and more than 100 million videos are viewed each day on the platform (5). It has been reported that an individual spends an average of at least 15 minutes per day on this video-sharing platform (6,7). The extensive visual content in the videos and the easy accessibility of the site encourage patients to share their experiences, which has made YouTube™ popular for information search (5). In 2007, the initial study evaluating the content of health-related videos on YouTube™ was carried out, with a focus on vaccination (8). Numerous studies have been performed to date in the literature to evaluate the quality of YouTube™ videos regarding various diseases, operations, and treatment procedures in the field of health (9,10).

A sinus lifting procedure is performed in most dental implant patients with insufficient posterior maxillary crest height (11). YouTube™ has become a preferred online resource where individuals seek information on various medical topics, including sinus lift procedures. These videos, which

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are uploaded without any peer review or scientific filtering, have the possibility of misinforming patients (12). Previous studies within healthcare have shown the quality of information available online to be generally of poor quality (13,14). Regarding oral and maxillofacial surgery (OMFS), there are studies in which the contents prepared on topics such as dental implants (4), oral cancers (15), impacted tooth extractions (16), orthognathic surgery (17), TMJ arthrocentesis (18), and bruxism (19) are evaluated in terms of quality and usefulness. The purpose of this study was to evaluate the information content, quality, and reliability of sinus-lifting videos on YouTube™.

2. METHODS

To achieve the research objective, we designed and implemented a cross-sectional study. The study population comprised all YouTube videos about sinus lifting accessible on October 14, 2023, between 9:00 AM and 5:00 PM. The study was granted an ethics committee exemption as it utilized only publicly available internet data.

2.1. YouTube™ Search

Google Trends was used to determine the most common search terms for a sinus lift. It was found that "sinus lift" was the most often used phrase among "sinus augmentation," "sinus lift," "sinus lift surgery," and "sinus lift for implant" in this application (Figure 1) (Google Trends, 2023). 'Worldwide', 'last 5 years', and 'YouTube™ searches' filters were used in the search parameters.

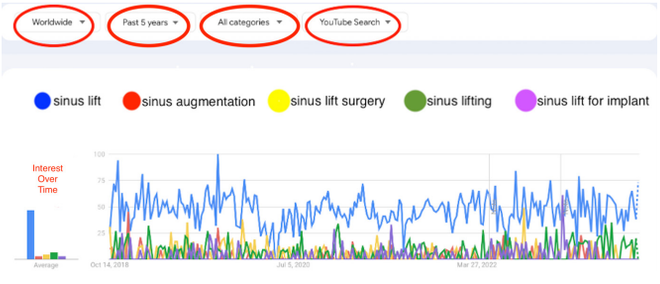

Figure 1. *Comparative search of Google Trends for 'sinus lift', 'sinus augmentation', 'sinus lift surgery', 'sinus lifting' and 'sinus lift for implant' topics, using 'worldwide' and 'past 5 years' filters.*

To avoid possible false and misleading results due to search history, a new YouTube™ account was created before videos were reviewed. Videos were watched on the YouTube™ platform using the Google Chrome browser without activating incognito mode. The default "sort by relevance" filter was applied when conducting YouTube searches.

Previous research in the literature shows that most YouTube™ users often search for the first 60-200 videos that appear in the search results and do not continue searching after the fifth page (20,21). The first 150 videos that were searched for the term "sinus lift" were evaluated for our study. The search was conducted once, and since the ranking of search results may vary depending on factors such as the day, time, etc., a playlist containing the first 150 videos displayed at the specified time was created. The URL of each video was then recorded in an Excel file.

2.2. Selection of Videos

Only videos with English content, acceptable image quality, and informative content about sinus lift were included in the study. Other than English, which are copies of each other, related to treatments other than the sinus lift procedure and irrelevant to the subject, videos with poor audio and video quality, without any narrator (audio or subtitles), where the number of likes and dislikes cannot be displayed, without comments, uploaded for advertising purposes only, and longer than 30 minutes were not included in the study. Since the number of likes, dislikes, and comments are important in terms of viewer engagement and video preference, videos that cannot be viewed or do not allow comments were excluded, similar to studies in the literature (22,23). In addition, this selection criterion allowed the calculation of the interaction index for all videos.

2.3. Analysis of Videos

Two senior oral surgeons (ÖE&OA) trained and calibrated analyzed the videos evaluated as a result of the inclusion criteria and made content reviews and scoring without being aware of each other to preserve the objectivity of the study. Additionally, before finishing their review, the researchers did not have access to the number of comments, likes, and dislikes to allow objective evaluation. When there was disagreement among researchers regarding the scoring of a video, they discussed the literature. Inter-rater reliability was evaluated using the Intraclass Correlation Coefficient (ICC), where values closer to 1 signify a higher level of agreement among raters.

2.4. Data Saved in Videos

The duration, the number of views, comments, likes, and dislikes, the days since upload, the number of subscribers to the channel, the country of origin, and the source of the upload (Health professional, healthcare website, etc), the reason of exclusion if the video is to be excluded from the study, the narrator of the video (Dentist, voice-over, subtitles, etc), the audience it aims to address (physicians and/or students, patients, etc) were recorded for each video. The following formulas were used to calculate viewer interaction and viewing rate: Interaction index = (likes – dislikes / total views) * 100% and viewing rate = (views/ days since upload)*100 %.

2.5. Evaluation of Video Content (Total Content Score/ Usefulness Score)

Total content score (TCS) was created by adapting similar studies in the literature (4,10,18,24,25). The TCS consisted of the following 9 parameters: definition, indication, surgical procedure, etiology, radiography, materials used, complications, postoperative findings, and video animation. For each criterion met, the videos were divided into three groups according to their TCS: low/poor (0-3 points), average (4-6 points), and excellent/adequate (7-9 points).

2.6. Reliability Analysis (Modified DISCERN Questionnaire – MD)

DISCERN is a tool used to evaluate the reliability of online health information (13). The form consists of five questions about the following topics: evaluation of content ambiguity, bias/balance, clarity, reliability, and provision of additional information sources for video analysis. This method was described by Singh et al and was later modified for use with YouTube™ (modified reliability questionnaire) and became a tool used to evaluate the quality of online health information (13,26). A score of 1 is given according to the degree to which each criterion was met, and each video is scored between 0-5.

2.7. Quality analysis (Global Quality Scale – GQS)

The GQS is a five-item Likert scale used to evaluate the quality and information flow of a video in relation to how helpful it is to patients. Each video is scored from 1 to 5 depending on its quality (1 = very poor quality; 5 = excellent quality). Bernard et al developed it to evaluate the accuracy of information regarding inflammatory bowel disease (27).

2.8. Video Informatıon and Quality Index (VIQI)

The overall content and quality of the videos were analyzed using the VIQI scale. In this scale, each of the various video features (4 criteria) is scored from 1 to 5 using a Likert scale. Five points indicate excellent quality, whereas one point indicates lower quality. Information flow, information accuracy, quality (including criteria for images, animation, community conversations, video subtitles, and report summaries), and accuracy (degree of consistency between video title and content) are among the video features that are evaluated (28). Each video has a total VIQI score between 1 and 20.

2.9. Statistical analysis

The statistical analysis of the data was conducted using the IBM SPSS Statistics version 27 package application. Analysis results were presented as mean ± standard deviation for quantitative data, and frequency and percentage for categorical data. To determine whether the data were normally distributed, the Shapiro-Wilk normality test was performed. The One-way ANOVA, post Hoc Tukey HSD was utilized to analyze normally distributed data between groups. To examine correlations among parameters, Spearman correlation analysis was used. Inter-rater reliability was evaluated using the ICC. The statistical significance level for all data was set at $p< .05$.

3. RESULTS

The first 150 videos resulting from the search term 'sinus lift' were examined and 78 videos were not included in the study because of applying exclusion criteria (Figure 2). Of the 72 videos evaluated in our study, it was observed that the lateral window technique (open sinus lift) was mentioned in 43.1%, the crestal technique (closed sinus lift) was mentioned in 36.1%, and both techniques were mentioned in 20.8%. When the examined videos were classified according to the upload source, it was seen that 44.4% of the videos were uploaded by individual healthcare professionals, 25% by healthcare companies, 20.8% by healthcare websites and 9.7% by healthcare institutions (hospitals/polyclinics). When the videos were classified according to the audience group they addressed, it was seen that 62.5% targeted physicians and students, 25% targeted patients, and 11.1% targeted the public. 1.4% of the videos were intended to share patients' own experiences. The upload dates of the videos included in the study range from 2008 to 2023, with the vast majority determined to have been uploaded in 2021.

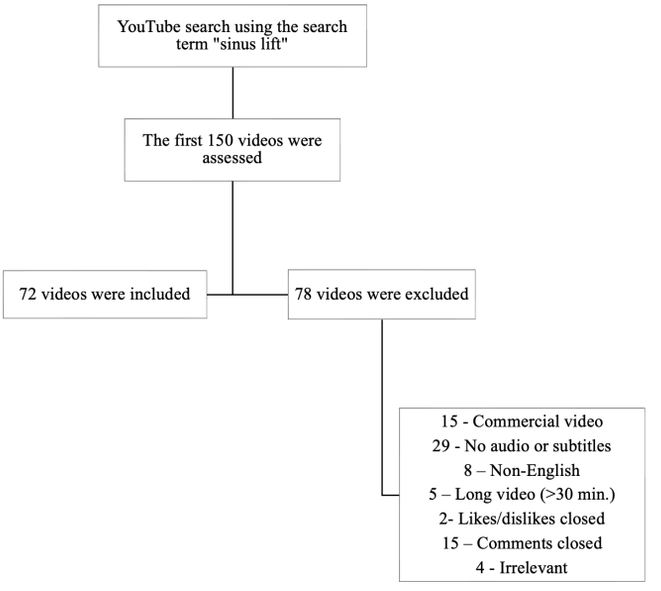

Figure 2. *Pathway of the analyze.*

Most of the videos were from the USA (37.5%), South Korea (13.9%), India (9.7%) and the UK (8.3%), respectively (Figure 3). Demographic data regarding the videos examined were given in Table 1. In total, 18.1% of the videos were of low quality, 63.8% were of medium quality, and 18.1% were of good quality. The videos received the most TCS points from procedure (97.2%) and indication (90.3%) information, respectively, and the least from post-operative information

(33.3%) and complications information (11.1%) criteria (Figure 4). Although not included as a criterion for the usefulness score, it was observed that none of the videos addressed the topic of cost.

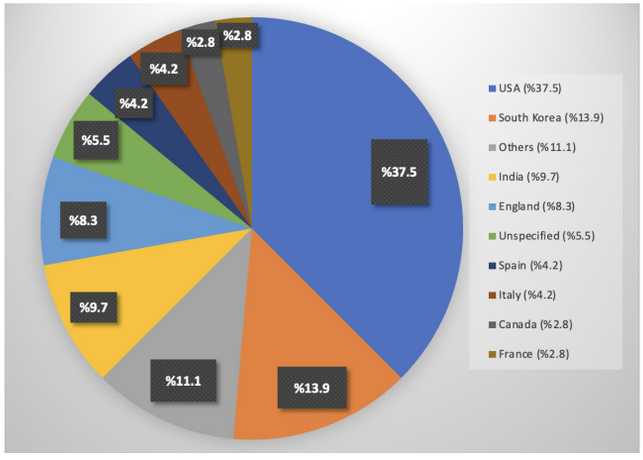

Figure 3. *Country of origin.*

Table 1. *Descriptive Statistics*

Demographics	Mean	SD	Median	Min	Max
Video Length (seconds)	358.6	343.4	222.5	15.0	1541.0
No. of views	28245.0	56492.8	6588.0	136.0	307733.0
No. of likes	204.8	379.7	59.0	.0	2200.0
No. of dislikes	10.2	23.2	2.0	.0	129.0
Interaction index	0.9	0.8	0.7	.0	4.4
Viewing rate	1608.9	3440.7	473.1	13.7	24280.5
No. of comments	11.9	23.9	4.0	.0	130.0
Days since upload	1997.8	1337.1	1965.5	127.0	5707.0
Subscriber count of the uploader	76583.0	289223.1	4960.0	16.0	1930000.0
TCS	4.9	1.6	5.0	1.0	8.0
GQS	2.9	0.9	3.0	1.0	5.0
DISCERN	2.6	1.0	3.0	0.0	5.0
VIQI	13.3	2.7	14.0	5.0	18.0

TCS: Total Content Score; GQS: Global Quality Scale; VIQI: Video Information and Quality Index; SD, Standard Deviation; Min: Minimum; Max: Maximum.

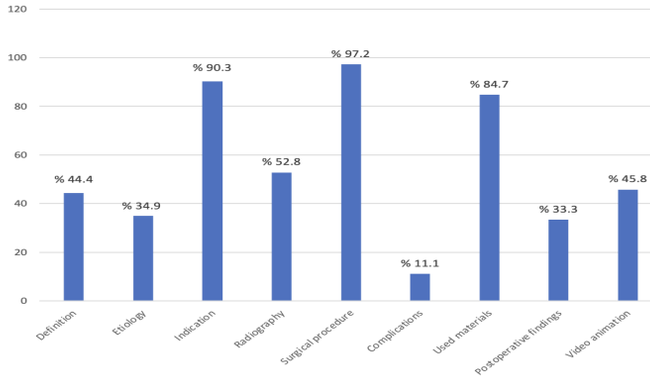

Figure 4. *Rates of sinus lift videos passing the usefulness score criteria.*

Descriptive statistics of video demographic data was given in Table 1. This table also includes the average TCS, GQS, DISCERN and VIQI scores of all videos (Table 1). The duration of the video ($p<.001$), number of views ($p<.05$), viewing rate($p<.01$), number of likes ($p<.01$), number of dislikes ($p<.05$), and number of comments ($p<.001$) of excellent videos were found to be statistically significantly higher than those of poor-quality videos. Additionally, it was found that videos with high-quality material had statistically significantly higher DISCERN, GQS, and VIQI scores than those with low-quality content ($p<.001$) (Table 2). TCS and the duration of the video ($r=.450$, $p<.01$) the number of views ($r=.288$, $p<.05$) the number of likes ($r=.363$, $p<.01$) the number of dislikes ($r=.269$, $p<.05$) and the number of comments ($r=.390$, $p<.01$) showed a statistically significant positive correlation. There was a statistically significant positive correlation between VIQI and video duration ($r=.362$, $p<.05$), number of likes ($r=.300$, $p<.05$), and number of dislikes ($r=.234$, $p<.05$). Also, a statistically significant positive correlation was found between TCS and VIQI ($r=.706$, $p<.001$), DISCERN ($r=.620$, $p<.001$) and GQS values ($r=.746$, $p<.001$) (Table 3). An acceptable level of consistency was observed between each researcher for the DISCERN (ICC = 0.875), VIQI (ICC = 0.975), TCS (ICC = 0.910), and GQS scores (ICC = 0.872) (Table 4).

Table 2. *Comparison of YouTube™ video variables according to total content scores (TCS)*

Variables	Total Content Score			
	Poor (n=13)	Moderate (n=46)	Excellent (n=13)	
	Mean±SD	Mean±SD	Mean±SD	*p-value* [a]
Video Length (seconds)	264.9±194.8	283.0±266.7	719.6±469.4	.000***
No. of views	7533.6±7714.5	26001.3±52503.0	56895.4±84337.3	.020*
No. of likes	66.3±121.3	168.4±317.5	471.9±597.5	.001**
No. of dislikes	4.0±6.5	8.1±20.0	23.4±37.3	.012*
Interaction index	0.7±0.6	0.9±0.8	1.1±0.6	.087
Viewing rate	334.2±352.3	1742.4±3950.3	2411.1±2979.2	.005**
No. of comments	6.3±13.6	7.3±13.7	33.4±43.5	.000***
Days since upload	2331.0±1250.3	1887.4±1387.9	2055.2±1273.5	.559
Subscriber count of the uploader	5650.0±8746.0	111488.0±358118.4	24006.3±28353.1	.122
DISCERN	1.7±1.2	2.6±0.8	3.6±0.6	.000***
GQS	2.0±0.8	2.8±0.6	4.1±0.8	.000***
VIQI	10.9±3.1	13.1±2.1	16.4±1.2	.000***

*TCS: Total Content Score; GQS: Global Quality Scale; VIQI: Video Information and Quality Index; SD; Standard Deviation. *: p<.05; **: p<.01; ***:p<.001;*
[a]*: One-way ANOVA and Tukey's post hoc tests*

Table 3. *Correlation matrix between scores for TCS, DISCERN, GQS, VIQI and Youtube™ demographics.*

		TCS	DISCERN	GQS	VIQI
TCS	r	1	0.620	0.746	0.706
	p[ǂ]		.000***	.000***	.000***
Video Length	r	0.450	0.173	0.219	0.362
	p[ǂ]	.001**	.147	.065	.002**
No. of views	r	0.288	0.070	0.114	0.203
	p[ǂ]	.014*	.558	.341	.087
No. of likes	r	0.363	0.188	0.170	0.300
	p[ǂ]	.002**	.113	.153	.010*
No. of dislikes	r	0.269	0.110	0.090	0.234
	p[ǂ]	.022*	.359	.450	.048*
Interaction index	r	0.143	0.197	0.142	0.124
	p[ǂ]	.232	.097	.235	.298
Viewing rate	r	0.231	0.082	0.132	0.220
	p[ǂ]	.051	.496	.271	.064
No. of comments	r	0.390	0.237	0.193	0.286
	p[ǂ]	.001**	.045*	.105	.015*

*TCS: Total Content Score; GQS: Global Quality Scale; VIQI: Video Information and Quality Index; r: Correlation Coefficient; p: Statistical Value; *: Correlation is significant at the 0.05 level (2-tailed); **: Correlation is significant at the 0.01 level (2-tailed). ***: Correlation is significant at the 0.001 level (2-tailed) ǂ: Spearman correlation analysis*

Table 4. *TCS, DISCERN, VIQI and GQS scores*

	Data	Researcher 1	Researcher 2
TCS	Mean	4.9	4.2
	SD ICC[ǂ]=.910	1.6	1.5
DISCERN	Mean	2.6	2.3
	SD	1.0	1.1
	ICC[ǂ]=.875		
VIQI	Mean	13.3	12.4
	SD	2.7	2.6
	ICC[ǂ]=.975		
GQS	Mean	2.9	2.6
	SD	0.9	0.9
	ICC[ǂ]=.872		

TCS: Total Content Score; GQS: Global Quality Scale; VIQI: Video Information and Quality Index, ICC: Intraclass Correlation Coefficient; SD; Standard Deviation, ǂ: Intraclass correlation coefficient

4. DISCUSSION

With the growing popularity of social media and the internet in today's information age, access to information using YouTube™ has increased significantly (13,29). On YouTube™, users can share videos for free without peer review, and it is thought that videos uploaded without any scientific filtering may mislead patients (12). This study evaluated the quality and reliability of sinus lift surgery information found in YouTube™ videos. It was observed that the majority of the 72 videos examined according to the determined criteria were generally of medium quality.

In YouTube™ studies published in the literature, it was seen that most of the videos were uploaded from the United States (30,31). Similarly to these results, our study shows that 37.5% of the videos were uploaded by users in the USA. Consistent with the findings of Paksoy and Gaş (32), our study demonstrates that a substantial proportion of the videos (44.4%) is contributed by healthcare professionals. Kovalski et al. also stated that 75% of YouTube™ videos about leukoplakia come from independent users and corporate advertisements (9). According to a study analyzing videos of orthognathic surgery, patients uploaded 77.5% of the videos (21). These variations likely stem from the topic's popularity, audience interests, and content creators' motivations. Broad health topics like leukoplakia attract independent users, while patient-uploaded videos are more common for experience-driven subjects like orthognathic surgery. Conversely, niche topics like sinus lifting, requiring technical expertise, are primarily covered by healthcare professionals.

In this study, the most mentioned topics in the videos were indications and surgical procedures, while complications and post-operative symptoms (swelling, bleeding, etc.) were the least mentioned topics, consistent with previous studies (10,19,32). The reason for the high rates of topics such as indications and surgical procedures may be that healthcare professionals share their own case experiences. When the usability score criteria of the videos (n=13) with weak content were evaluated, it was seen that only one of them gave information about complications that may develop and three of them gave information about post-operative symptoms. Additionally, it has been observed that videos with more animation content have higher usability scores. It has been observed that in these types of videos, the necessary information is shortly conveyed to the patients. It was determined that none of the videos that were analyzed mentioned the issue of cost. Similar to our study, there are other studies that found that the cost issue was addressed least (10,33). We think that this may be because the cost varies depending on the amount of material used (bone graft, etc.) and the brand.

In this study, video durations and content levels were found to be positively correlated. Similar to our study, studies conducted by Li et al on food poisoning, Pons-Fuster et al on diabetes and oral health, Gas et al on botox treatment for bruxism, and Lena and Dindaroglu on lingual orthodontic treatment all found that longer durations and higher information content were associated with high-quality content. They stated that users are more interested in longer videos and that this is because as the video duration increases, areas that are not mutually exclusive are revealed (19,28,34,35). However, Erdem and Sisik (36) could not detect a significant difference between video duration and content levels in their studies on obesity surgery and Ayrancı et al (24) on genioplasty. Additionally, Ayrancı et al found the duration of videos with weak information content to be significantly longer.

Our research revealed a positive correlation between TCS scores and the number of comments (r=.390, p<.01), views (r=.288, p<.05), likes (r=.363, p<.01) and dislikes (r=.269, p<.05) on the videos. On the other hand, Şimşek et al, in their study on teeth whitening, found that there was no significant

difference in the number of dislikes and likes between content levels (37). Ayrancı et al found that the number of dislikes and likes and interaction indexes of videos containing bad information were quite high compared to other videos. According to their analysis, the result showed that people prefer to watch videos with low information value (24).

In our study, neither the VIQI score (r=.124, p>.05) nor the TCS (r=.143, p>.05) showed a positive correlation with interaction rates. Hassona et al did not find a significant difference between the interaction indexes and viewing rates and content levels in their study on oral cancers (15). On the contrary, Şimşek et al reported in their study that interaction indexes and viewing rates varied significantly according to content levels (37). In this study, high-content videos received significantly higher VIQI scores than low-content videos. Lena and Dindaroğlu showed in their study that the interaction index, watching rate, and TCS are positively correlated (28). Considering the results of various studies, it deserves to be noted that the content of videos with high interaction might not be of the same quality. Additionally, in our study, a positive relationship was discovered between VIQI score and video duration, number of likes and number of dislikes. According to research by Ustdal et al rich content videos exhibited higher GQS levels than weak content videos (38). In our study, DISCERN, GQS scores, and TCS were found to positively correlate. It has been shown that a 7-category evaluation tool developed for the content of sinus lift surgery videos with the lateral window technique shows a positive correlation with the content quality of the videos (39).

Of the videos in this study, 18.1% had low quality, 63.8% had medium quality, and 18.1% had good quality overall. In the current literature, there are different results for the quality evaluation of videos examining topics related to dentistry. It has been reported that videos on topics such as head and neck cancers, impacted tooth extractions, orthognathic surgery in the field of OMFS are insufficient in terms of information quality (15–17). However, in studies evaluating videos on Botox injection in wrinkle removal and bruxism treatment, the content of YouTube™ videos was found to be of high quality and it was reported that they could be a useful resource for Patients (19,40). These different outcomes may be due to variations between the subjects analyzed, the evaluation criteria and the parameters used. YouTube™ contains videos that pose various risks, such as the presence of incomplete and uncontrolled/unreferenced information and these videos are used by patients to gain information. Therefore, checking video sources in the field of health and evaluating the contents in terms of information accuracy can be an important initiative to improve the content of YouTube™ videos (15). Kidy et al suggested that informative videos should be published by various national and international professional organizations and that such videos should be made available to the public after going through an evaluation process similar to article submissions (16).

Our study had several limitations. Only videos whose native language was English were evaluated. International studies can shed light on how various cultures may affect the content of YouTube™ videos and how patients from other nations assess the videos' potential health risks. Due to YouTube's™ high level of dynamic nature, a large number of videos are added and removed daily. In our study, the evaluation of the videos was carried out within a single day and provided only a snapshot of the data accessible during a certain period. Depending on the time and day, different results may occur. The exclusion of conference videos with high-quality information content prepared by health professionals or health institutions due to video duration limitations and the exclusion of videos without narrators (including subtitles, voice-over, etc.), even if they were successful surgery videos, may have caused low TCS scores. Similarly, the exclusion of videos with invisible like/dislike counts and disabled comment sections may have affected TCS scores. Despite these limitations, this study provides the first comprehensive assessment in the literature of the content quality and educational value of YouTube videos on sinus lift surgery.

5. CONCLUSION

As a result of our study, it was seen that the quality of sinus lift videos on YouTube™ was moderate and could provide limited benefit to patients. Essential topics such as complications and postoperative findings are under-referred, suggesting that healthcare professionals must address these gaps and contribute to creating higher-quality educational content on online platforms. Before health-related educational videos are uploaded to social platforms, the content quality of these videos should be evaluated with different tools. All efforts should focus on encouraging collaboration between healthcare professionals, academic institutions, and content creators to produce and promote reliable and informative videos. Future research could explore the effectiveness of such collaborations in improving the quality and accessibility of online resources and patient outcomes associated with using these materials.

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Clinical and Experimental Health Sciences

The Relationship Between Pregnancy Stress and Maternal Attachment, Postpartum Depression, and Breastfeeding Self-Efficacy: A Descriptive and Prospective Study

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ABSTRACT

Objective: This study aimed to examine the relationship between pregnancy stress and maternal attachment, postpartum depression risk, and breastfeeding self-efficacy.

Methods: This study was designed as a descriptive and prospective study. A total of 237 pregnant women were included. The Pregnancy Stress Rating Scale (PSRS) was administered at hospital admission for delivery. The Maternal Attachment Scale (MAS), Edinburgh Postpartum Depression Scale (EPDS), and Breastfeeding Self-Efficacy Scale (BSES) were used in the 1st and 3rd months postpartum.

Results: The mean PSRS score was 75.76±28.25, indicating moderate stress. Mean maternal attachment scores were 100.26±7.00 (1st month) and 102.55±5.12 (3rd month), while EPDS scores were 17.05±3.51 and 19.17±2.59, suggesting high depression risk. Breastfeeding self-efficacy decreased from 59.16±14.63 to 52.44±17.69. A positive correlation was found between PSRS and maternal attachment ($r = .32$, $p < .001$), while PSRS was negatively correlated with breastfeeding self-efficacy ($r = -.28$, $p = .002$) and positively with EPDS (1st: $r = .35$; 3rd: $r = .41$; $p < .001$). Maternal attachment and breastfeeding self-efficacy were positively correlated ($r = .39$, $p < .001$), while both were negatively correlated with EPDS scores ($p < .05$).

Conclusions: Pregnancy stress is associated with increased postpartum depression risk, reduced breastfeeding self-efficacy, and higher maternal attachment. These findings highlight the need for early stress screening and psychosocial support during and after pregnancy.

Keywords: Pregnancy; stress; attachment; postpartum depression risk; breastfeeding.

1. INTRODUCTION

In a woman's lifetime, pregnancy represents a pivotal period. Pregnancy, typically regarded as a joyful phase for women, may also signify a critical juncture characterized by shifts in biopsychosocial equilibrium, alterations in familial and occupational roles, the acquisition of additional responsibilities, and the process of adapting to a new maternal identity. (1). Physiological, biological and psychological changes occur during pregnancy (2). Failure to adapt to these changes during pregnancy can cause anxiety and stress in women, resulting in pregnancy anxiety and depression (3). Stress experienced during pregnancy has many negative effects on maternal and infant health, such as preterm birth, abortion, babies small for gestational age, low birth weight, and difficult labor (4-7). In addition inability to effectively cope with the stress experienced during pregnancy may cause delayed maternal attachment, development of postpartum depression, and failure to maintain effective breastfeeding. Maternal attachment is an important process that begins with the conception of a woman, continues after birth, and enables the development of the role of motherhood (8). The mother's attachment to her baby begins with the woman's positive response to her pregnancy and becomes stronger as the woman's belly expands and she feels the baby's movements. Healthy maternal attachment and maternal role are affected by the psychosocial health status of the woman. Some studies indicate a statistically significant positive correlation between the psychosocial health status of pregnant women and maternal attachment, the role of motherhood. Therefore, reducing the stress level of the woman during pregnancy and adapting to this period can increase mother-infant attachment (5,7). Postpartum depression may develop with the addition of postpartum stress factors if mental problems experienced during pregnancy are not detected and

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intervened in the early period (4). Postpartum depression is a mental disorder that can occur at any time in the first year after delivery and is seen in 10% to 15% of women who have given birth (9). The study reports that the risk of developing postpartum depression increases 3.61 times in women who cannot effectively cope with the stress experienced during pregnancy (10). Postpartum depression is not only a problem experienced by the mother in the postpartum period, but it is a condition that should be determined and resolved in the early period due to its many negative effects on the social, mental and emotional development of the baby. The perception of breastfeeding self-efficacy expresses the mother's thoughts about breastfeeding, how much effort she will make to breastfeed her baby, and her emotional readiness (11). In short, the perception of breastfeeding self-efficacy is the competence that the mother feels to be able to breastfeed her baby (12). However, the stress experienced during pregnancy affects breastfeeding self-efficacy and prevents breastfeeding effectively (4,13). A study shows that stress management counseling during pregnancy can increase breastfeeding self-efficacy and ensure effective continuation of breastfeeding (13). No study has been found in the literature to determine the effect of the stress level experienced during pregnancy on maternal attachment, postpartum depression and breastfeeding self-efficacy. The objective of this study was to ascertain the association between the degree of stress encountered during pregnancy and maternal attachment, postpartum depression, and breastfeeding self-efficacy.

2. METHODS

2.1. Design

This descriptive and prospective study was conducted in Turkey between April and December 2021.

2.2. Sample

The population of the study consisted of all pregnant women. The sample of the study consisted of pregnant women who applied to the antenatal clinic of a public hospital in the Central Anatolia Region for antenatal follow-up between the specified dates, met the inclusion criteria, and agreed to participate after being informed about the study. The sample size of the study was calculated using the G*Power 3.1.9.2 program with a 95% power level, based on the mean score (59.49±8.46) of the Breastfeeding Self-Efficacy Scale reported in the study by Yenal and colleagues. As a result, it was determined that 237 participants would be sufficient.

The study included women aged 18 and older who were literate in Turkish, were at 28 weeks of gestation or beyond, had not previously given birth, and had no diagnosed psychiatric disorders. Women who developed any complications during pregnancy were not included in the study.

2.3 Assessment Tools

Pregnancy Stress Rating Scale (PSRS): The scale utilized in this study was originally developed by Chen (2015) and subsequently validated for Turkish usage, with reliability assessment conducted by Akın and Erbil in 2022 (15,16). Pregnancy-related stress is assessed in the following five sub-dimensions: "Stress arising from the labor process and the search for a safe process in terms of mother and baby in childbirth", "Stress arising from baby care and changing family relationships", "Stress arising from defining the role of motherhood", "Stress arising from seeking social support", and "Changing physical appearance and stress caused by function." The highest score of 144 can be obtained from the PSRS. The scale is designed to evaluate the stress levels of pregnant women and is generally applicable from the 12th week of pregnancy onward. Cronbach's alpha coefficient of Turkish version of total Pregnancy Stress Rating Scale was .92 (16). The Cronbach's alpha value was found to be .95 in this study.

Maternal Attachment Scale (MAS): MAS was designed to assess maternal love attachment by Mary E. Muller in 1994 (17). Turkish validity and reliability study was conducted by Kavlak and Şirin (18). The Cronbach Alpha internal consistency reliability of the Maternal Attachment Scale was found (to be) .77 in mothers with a 1-month-old baby and .82 in mothers with a 4-month-old baby. It was concluded that the internal consistency reliability of the scale was high. Findings regarding the test-retest and split-half reliability of the scale also support the reliability of the scale. In this study, the Cronbach's alpha value was found to be .96 in the 1st month and .97 in the 3rd month.

Edinburgh Postpartum Depression Scale (EPDS): EPDS was defined by Cox et al. in 1987 (19). In the validation study conducted in Turkey by Engindeniz et al. in 1997, sensitivity and specificity were determined as 84% and 88%, and the cut-off score was shown to be 12–13 for identifying women at risk of postpartum depression (20). In this study, the Cronbach's alpha value was found to be .67 in the 1st month and .69 in the 3rd month.

Breastfeeding Self-Efficacy Scale (BSES): BSES was initially developed by Dennis and Faux (1999) as a 33-item scale (21). This scale assesses evaluates mothers' perceived competence in breastfeeding. Breastfeeding Self-Efficacy Scale-Short Form comprises of 14 items assessing breastfeeding self-efficacy. The scale yields a minimum score of 14 and a maximum score of 70, with higher scores indicating greater breastfeeding self-efficacy. Alush-Tokat and Okumuş (2010) conducted the Turkish adaptation of the scale, reporting a Cronbach's alpha value of .86, signifying high reliability (22). In this study, the Cronbach's alpha value was found to be .97 in the 1st month and .98 in the 3rd month.

2.4. Data Collection

PSRS was administered to women who were at 28 weeks of gestation or beyond and presented to the antenatal outpatient clinic for routine prenatal care. According to the original validation study, the PSRS is appropriate for use during the second and third trimesters of pregnancy. MAS, EPDS, and

BSES were filled in the first and third months after delivery by the researcher via telephone interviews by contacting each participant individually. During the phone calls, the researcher read each question aloud, recorded the participant's responses in real time, and ensured that all items were answered completely and accurately (Figure 1). Since one pregnant woman gave birth prematurely before the first-month follow-up, seven women did not answer the phone, and five women refused to respond to the questionnaire in the third month, 13 women were excepted from the study, resulting in a final sample size of 237 participants upon termination of the study

2.5. Ethics

In order to conduct the study, ethical approval was obtained from the Non-Interventional Research Ethics Committee of the Faculty of Health Sciences at Selçuk University (Decision No: 2021/581). Institutional permission was also obtained from the hospital where the study was conducted, and written informed consent was obtained from the women who participated in the study. All stages of the research were carried out in accordance with the principles of the Declaration of Helsinki.

2.6. Research Questions

1. What is the level of stress among pregnant women?
2. What is the level of maternal attachment in women?
3. What is the prevalence of postpartum depression in women?
4. What is the level of breastfeeding self-efficacy in women?

2.7. Data Analysis

Mean, standard deviation, median, numbers and percentages were used in the evaluation of the data. The Kolmogorov-Smirnov test was employed to assess whether the data exhibited a normal distribution. Comparisons of pregnant women in terms of stress during pregnancy, maternal attachment, depression level and breastfeeding self-efficacy according to their sociodemographic and obstetric characteristics were made with Mann Whitney U test and Kruskall Wallis test. The correlation between pregnancy stress, maternal attachment, postpartum depression, and breastfeeding self-efficacy was evaluated using the Pearson correlation test. In addition, multiple regression analyses were conducted for pregnant women's PSRS, EPDS, and BSES scores. The statistical significance level of $p < .05$ was adopted.

3. RESULTS

In the study, the mean age of the participating women was 22.97±3.08, and the mean (gestational week) week of gestation when they were included in the study was 31.97±5.94. The mean PSRS score of the women was 75.76±28.25. Given that the maximum achievable score on the scale is 144, it indicates that women experience moderate levels of stress during pregnancy.

Table 1. *Mean total scores of women's PSRS, MAS, EPDS, and BSES.*

Variables	Mean ±SD (min – max)
Pregnancy Stress Rating Scale	75.77±28.25 (8-144)
Maternal Attachment (1st Month)	100.26 ±7.00 (66-104)
Maternal Attachment (3rd Month)	102.55±5.12 (61-104)
Edinburgh Depression Scale (1st Month)	17.05±3.51 (0 – 29)
Edinburgh Depression Scale (3rd Month)	19.17±2.59 (0 – 24)
Breastfeeding Self-Efficacy (1st month)	59.16±14.63 (14-70)
Breastfeeding Self-Efficacy (3rd month)	52.44±17.69 (17-70)

PSRS: Pregnancy Stress Rating Scale; MAS: Maternal Attachment Scale; EPDS: Edinburgh Postpartum Depression Scale; BSES: Breastfeeding Self-Efficacy Scale; SD: Standard Deviation

The mean MAS score of the women in the first month after delivery was found to be 100.26 ±7.00, and 102.55±5.12 in the third month. The maximum achievable score on the scale being 104 suggests that the maternal attachment level among women is high. While the mean EPDS score was 17.05±3.51 in the 1st month, it increased to 19.17±2.59 in the 3rd month. The cut-off point of EPDS is 12-13, and it has been found that women are at risk for depression in the 1st and 3rd months, and this risk increases even more in the 3rd month. The opposite is found in breastfeeding self-efficacy. While the mean BSES score was 59.16±14.63 in the 1st month, it decreased to 52.44±17.69 in the 3rd month (Table 1). There was no relationship between women's age, education, family type, number of pregnancies, getting information about birth and the scale mean score. While the stress level of the unemployed women was higher during pregnancy than the employed the depression levels of the employed women were found to be higher in the first month postpartum compared to the unemployed women. Moreover, it was observed that the breastfeeding self-efficacy levels during the first month postpartum were significantly lower among women who underwent cesarean section compared to those who had a vaginal delivery ($p < .05$) (Table 2).

Table 3 displays the association between the PSRS scores and the MAS, EPDS, and BSES. A positive correlation was found between the 2nd, 3rd, 5th sub-dimension of thePSRS and the mean total score of the PSRS and the level of maternal attachment in the 1st month. It is observed that as stress increases during pregnancy, the level of attachment increases in the 1st month postpartum. There is a negative correlation between the PSRS 4th sub-dimension and the depression level in the 1st month postpartum. As the stress due to seeking social support increases, the depression level in the 1st month decreases. There is also a positive correlation between PSRS 2nd sub-dimension and maternal attachment level in 1st month postpartum. As the stress arising from baby care and changing family relationships increases, the level of maternal attachment in the 1st month also increases. Another important result is that there is a positive correlation between the 2nd, 3rd, and 4th sub-dimensions of PSRS and breastfeeding self-efficacy in the 3rd month ($p<.05$).

When the relationship between maternal attachment and breastfeeding self-efficacy and depression levels was examined, a positive and significant relationship was found between breastfeeding self-efficacy and maternal attachment levels in

the 1st and 3rd months. This shows that as breastfeeding self-efficacy increases, maternal attachment level also increases. A negative correlation was found between breastfeeding self-efficacy level and depression level in the 1st month. As the breastfeeding self-efficacy level of women increases, their depression levels decrease (p<.05). A significant negative correlation was identified between maternal attachment and depression levels in both the 1st and 3rd months postpartum. As depression levels increased, maternal attachment levels were observed to decrease (*p*<.05) (Table 4).

Table 2. *The relationship between sociodemographic and obstetric characteristics of pregnants and PSRS, MAS, EPDS and BSES.*

Characteristics	n (%)	PSRS Mean ±SD (Median)	MAS (1st Month) Mean ±SD (Median)	MAS (3rd Month) Mean ±SD (Median)	EPDS (1st Month) Mean ±SD (Median)	EPDS (3rd Month) Mean ±SD (Median)	BSES (1st month) Mean ±SD (Median)	BSES (3rd month) Mean ±SD (Median)
Age								
≤19	31(13.1)	66.0±29.64 (70)	99.29±10.01 (104)	101.87±7.81 (104)	16.54±2.73 (17)	19.22±3.12 (21)	57.87±16.25 (64)	49.22±22.23 (64)
20-30	201(84.8)	77.80±27.65 (80)	100.00±6.50 (104)	102.63±4.66 (104)	17.10±3.58 (18)	19.13±2.52 (20)	59.72±16.25 (66)	52.81±17.05 (63)
≥31	5(2.1)	54.60±27.76 (54)	103.40±1.34 (104)	103.40±0.54 (103)	18.00±5.14 (19)	20.40±1.94 (21)	44.8018.70 (40)	57.60±10.03 (63)
KW*; p		**6.388; .04**	1.992; .36	1.752; .41	2.793; .33	0.266; .30	3.955; .13	0.495; .78
Education								
Primary	69 (29.1)	60.60±22.41 (59)	100.69±6.94 (104)	102.78±4.31 (104)	14.40±3.50 (15)	19.40±2.45 (21)	61.02±12.19 (66)	53.18±16.45 (63)
High school	96(40.5)	67.00±14.00 (67)	100.13±7.60 (104)	102.37±5.69 (104)	18.33±3.21 (17)	19.10±2.53 (20)	59.18±14.42 (60)	52.00±18.07 (64)
University	72(30.4)	79.83±38.31 (71)	100.01±6.24 (103)	102.56±5.10 (104)	17.50±3.33 (19)	19.04±2.81 (20)	57.34±16.87 (67)	51.73±18.49 (63)
KW*; p		3.009; .22	3342; .18	2698; .26	0.614; .73	2117; .34	0.158; .92	0.581; .74
Family type								
Nuclear family	207 (87.3)	69.27±32.28 (61)	101.36±7.18 (104)	102.43±5.44 (104)	16.09±3.85 (16)	19.23±2.54 (20)	48.18±19.37 (51)	52.41±17.89 (64)
Extended family	30 (12.7)	73.66±12.70 (81)	102.00±3.46 (104)	103.36±1.69 (104)	18.33±1.15 (19)	18.76±2.89 (20)	58.33±10.11 (53)	52.66±16.53 (61)
MWU; p**		3103.50; .99	2551; .07	2846.50; .32	2855.50; .41	2942; .53	2569; .12	2931; .62
Number of pregnancies	223 (94.1)	76.11±28.24 (78)	100.18±7.03 (104)	102.51±5.25 (104)	17.08±3.52 (18)	19.19±2.55 (20)	59.71±14.27 (66)	52.39±17.87 (64)
1	14 (5.9)	76.09±29.19 (73)	100.90±7.23 (104)	103.00±2.40 (104)	16.18±3.76 (17)	18.54±3.63 (20)	51.63±18.02 (53)	52.90±15.33 (53)
≥2								
MWU; p**		1293.50; .28	1319.50; .28	1506.50; .77	1432.50; .60	1463.50; .68	1079.50; .05	1549; .96
Working status								
Not working	215 (90.7)	77.09±28.41 (79)	100.23±6.98 (104)	102.51±5.33 (104)	16.89±3.48 (17)	19.11±2.56 (20)	59.18±14.51 (66)	52.53±17.77 (64)
Working	22 (9.3)	62.77±23.37 (64)	100.50±7.27 (104)	103.00±2.37 (104)	18.54±3.59 (19)	20.08±1.67 (21)	58.95±16.20 (69)	55.00±15.98 (64)
MWU; p**		**1693; .02**	2221; .60	2196; .89	**1567; .00**	1880; .17	2069.50; .32	2274; .61
Notify about birth	105 (44.3)	70.12±33.53 (64)	102.62±2.32 (104)	102.62±5.02 (104)	16.50±3.16 (16)	19.43±2.08 (20)	50.50±17.17 (53)	52.88±16.99 (64)
Yes	132 (55.7)	70.33±24.14 (70)	100.00±9.79 (104)	102.49±5.22 (104)	16.66±4.32 (18)	18.96±2.92 (20)	50.16±20.67 (51)	52.09±18.28 (63)
No								
MWU; p**		6029; .08	6098; .07	6851; .84	6176.50; .14	6715.50; .67	6736.50; .70	6678; .61
Type of birth								
Normal delivery	216 (91.1)	75.94±28.44 (77)	100.12±7.13 (104)	102.5±5.32 (104)	17.13±3.52 (18)	19.16±2.58 (20)	60.25±13.64 (66)	53.01±17.47 (64)
Cesarean section	21 (8.9)	73.95±26.76 (67)	101.66±5.39 (104)	103.09±2.25 (104)	16.19±3.44 (17)	19.28±2.75 (20)	47.95±19.54 (52)	46.57±19.27 (52)
MWU; p**		2.424; .60	2100; .33	2250; .92	2070; .22	2300; .97	**1620; .00**	1800; .08

**Kruskall wallis test (KW); ** Mann Whitney U Test (MWU)*

Table 3. *Correlation coefficients of PSRS sub-dimension and total scores, and MAS, EPDS, and BSES.*

Variables		MAS (1st Month)	MAS (3rd Month)	EPDS (1st Month)	EPDS (3rd Month)	BSES (1st Month)	BSES (3rd Month)
PSRS Subscale 1 Labor & Safety Stress	**r,p**	0.069	0.084	-0.010	-0.004	0.066	0.076
PSRS Subscale 2 Baby Care & Family	**r,p**	0.202**	0.164*	-0.018	0.056	0.126	0.169**
PSRS Subscale 3 Motherhood Role	**r,p**	0.202**	0.125	-0.084	0.007	0.088	0.130*
PSRS Subscale 4 Social Support	**r,p**	0.099	0.092	-0.125	-0.028	0.082	0.135*
PSRS Subscale 5 Body Image & Function	**r,p**	0.143*	0.086	-0.057	-0.046	0.007	-0.020
PSRS Total Score	**r,p**	0.178**	0.139*	-0.062	0.003	0.094	0.124

Pearson Correlation Analysis was used.

**P<.05; **P<.01; ***P<.001*

Table 4. *Correlation between MAS, EPDS and BSES.*

Variables	MAS (1st Month)	MAS (3rd Month)	EPDS (1st Month)	EPDS (3rd Month)	BSES (1st Month)	BSES (3rd Month)
	r,p	r,p	r,p	r,p	r,p	r,p
MAS (1st Month)	1	0.623***	-0.139*	0.312***	0.341***	0.278***
MAS (3rd Month)		1	-0.138*	0.466***	0.227***	0.239***
EPDS (1st Month)			1	0.595***	0.148*	0.025
EPDS (3rd Month)				1	0.103	0.105
BSES (1st Month)					1	0.674***
BSES (3rd Month)						1

Pearson Correlation Analysis was used.
**P<.05; **P<.01; ***P<.001*

Table 5. *Multiple regression analysis of pregnant women's PSRS, EPDS, and BSES.*

	β	t	p	%95 CI	
Model 1: Variables Affecting Pregnancy Stress Levels (PSRS)					
Age	0.561	0.933	**<.001**	0.540	3.782
Type of birth	-2.274	-0.341	.77	-0.315	3.923
R=0.063, R^2=0.004, Durbin-Watson=1.505, df (234), F=0.463 (p=.63)					
Model 2: Variables Affecting Postpartum Depression Risk (EPDS – 1st Month)					
Working status	0.496	0.302	**.10**	1.639	0.103
R=0.106, R^2=0.011, Durbin-Watson=2.109, df (235), F=2.687 (**p=.10**)					
Model 3: Variables Affecting Breastfeeding Self-Efficacy (BSES – 1st Month)					
Type of birth	-3.286	3.317	**.25**	-4.005	0.000
R=0.253, R^2=0.064, Durbin-Watson=1.872, df (3,229), F=16.043 (**p<.001**)					

CI: Confidence Interval, Note: p values less than .05 are shown in bold in the table.

According to the multiple regression analysis, in Model 1, which examined factors affecting pregnancy stress levels (PSRS), only maternal age was found to have a statistically significant effect (β=0.561, p<.001). This finding suggests that as maternal age increases, the level of pregnancy-related stress may also increase. In Model 2, the relationship between postpartum depression risk (EPDS) in the first month and working status was assessed, but no statistically significant relationship was found (β=0.496, p=.10). Model 3 investigated the effect of type of birth on breastfeeding self-efficacy (BSES), and a statistically significant relationship was found (β=-3.286, p<.001). The negative β coefficient indicates that women who delivered by cesarean section had lower breastfeeding self-efficacy scores compared to those who had vaginal births. These results demonstrate that the type of birth has a significant impact on breastfeeding self-efficacy, while the examined demographic variables alone have limited explanatory power in predicting pregnancy stress and postpartum depression levels (Table 5).

4. DISCUSSION

The findings obtained from this study provide information about the level of stress experienced by women during pregnancy and its relationship with maternal attachment, risk of postpartum depression, and breastfeeding self-efficacy levels in the first and third months after delivery. It was concluded that the stress level experienced by pregnant women was moderate (Table 1). Other studies conducted in Turkey also report moderate to high levels of pregnancy stress (4, 23, 24). When examining the relationship between women's sociodemographic characteristics and stress status, unemployed women were found to experience higher levels of stress compared to employed women (Table 2). Koyucu et al. (2020) found no relationship between sociodemographic characteristics and pregnancy stress (24). Conversely, another study reported that women with unintended pregnancies and multiple children had higher stress levels (23). The moderate stress level in our study may be related to the majority of participants having a high school or higher education level, planned pregnancies, and active access to healthcare services before delivery.

Maternal attachment levels were found to be high in the study, consistent with other literature (8,25,26). No significant relationship was found between maternal attachment and sociodemographic characteristics, whereas Bilgin and Alpar (2018) reported associations with education level, employment status, and income perception (8). This discrepancy might be due to the early postpartum timing of the assessment in our study, when emotional intensity from childbirth may overshadow individual differences.

The risk of postpartum depression was observed to be higher in the first month and increased further by the third month. Employed women had higher depression risk scores than unemployed women in the first month, which may relate to challenges such as taking leave, quitting, or returning to work (27). Extending postpartum leave up to six months has been reported to reduce depressive symptoms (28). Although postpartum leave allows mothers to spend time with their babies, returning to work can provoke anxiety and adjustment difficulties, contributing to higher depression risk among employed women.

Another significant finding was the decrease in breastfeeding self-efficacy at the third month compared to the first. This may be influenced by social and cultural factors. In Turkey, family and relative support is strong in the early postpartum weeks but declines by the third month. Working mothers returning to their jobs may face challenges with milk expression and limited time with their infants, negatively impacting self-efficacy. Cultural pressures and traditional knowledge gaps may also encourage early cessation of breastfeeding. Therefore, sustainable support for breastfeeding self-efficacy is essential not only in the early postpartum period but extending through and beyond the third month. Additionally, women who gave birth by cesarean section had lower breastfeeding self-efficacy at one month compared to those with vaginal births. The literature shows inconsistent findings

on delivery mode and breastfeeding self-efficacy (29–31). Cesarean births often involve more pain, which can hinder infant care and bonding (32), likely explaining lower self-efficacy.

The study findings indicate that as pregnancy stress increases, breastfeeding self-efficacy and maternal attachment also increase, while risk of postpartum depression decreases (Table 3). This suggests that the stress experienced by pregnant women may be adaptive rather than solely negative. Moderate stress can enhance maternal responsibility and caregiving behaviors. However, excessive stress-as reported in the literatüre-has detrimental effects on maternal and infant health. Therefore, maintaining stress within certain limits may be beneficial, but excessive stress remains a risk. Some studies report increased attachment with maternal happiness, but no direct link was found between prenatal stress and maternal attachment (33, 34). Niegel et al. found that trauma symptoms from prior births increased attachment in subsequent pregnancies (35). Another study showed that prenatal trauma symptoms did not predict postpartum depression risk (35). Although high prenatal stress negatively impacts health (34), the moderate stress level found here may reflect maternal motivation to care well for the baby, thereby positively influencing breastfeeding, attachment, and depression risk.

A positive correlation between breastfeeding self-efficacy and maternal attachment was observed (Table 4), consistent with findings from Turkey (36). The relationship between breastfeeding and depression risk revealed that higher depression risk correlates with lower breastfeeding self-efficacy. Literature supports that postpartum depressive symptoms reduce breastfeeding self-efficacy (31, 37). An important finding is that increased depression risk correlates with decreased maternal attachment. Negative associations between postpartum depression risk and maternal attachment have been reported (38, 39). Based on these data and the literature, postpartum depression risk is considered a significant health problem adversely affecting breastfeeding self-efficacy and maternal attachment. Additionally, regression analysis showed that maternal age affected pregnancy stress levels, while mode of delivery significantly influenced breastfeeding self-efficacy (Table 5). These findings suggest the importance of considering both psychosocial and obstetric factors when supporting maternal mental health and breastfeeding outcomes.

Limitations

This study's limitations include the absence of pre-delivery evaluations of maternal depression and attachment levels. Furthermore, maternal stress and depression levels were assessed solely through self-report scales. Additionally, the generalizability of the findings is limited to Turkey and may not extend to other countries.

5. CONCLUSION

This study demonstrated that stress experienced during pregnancy was significantly associated with maternal attachment, breastfeeding self-efficacy, and postpartum depression levels. Findings indicate that women experienced moderate levels of stress during pregnancy and exhibited high levels of maternal attachment in both the first and third months postpartum. However, the risk of postpartum depression was high in both the first and third months, with a notable increase observed in the third month. Breastfeeding self-efficacy was found to decrease over time and was significantly lower among women who underwent a caesarean section compared to those who had a vaginal delivery.

Significant positive correlations were identified between specific subdimensions of pregnancy-related stress and maternal attachment levels in the first postpartum month. Stress related to seeking social support was found to be negatively correlated with depression levels in the first postpartum month. In addition, stress associated with infant care and family relationships during pregnancy was positively correlated with maternal attachment levels. In the third postpartum month, positive correlations were found between breastfeeding self-efficacy and specific subdimensions of pregnancy-related stress. Breastfeeding self-efficacy was positively correlated with maternal attachment and negatively correlated with depression levels at both the first and third postpartum months. The multiple regression analysis revealed that maternal age had a statistically significant impact on pregnancy-related stress levels, whereas mode of delivery was a significant predictor of breastfeeding self-efficacy. The findings suggest that the mode of delivery and psychosocial stress experienced during pregnancy may influence key variables related to maternal and infant health in the postpartum period, highlighting the need for comprehensive support programs for women throughout pregnancy and the postpartum period.

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Clinical and Experimental Health Sciences

Is HPV16 E7 Oncoprotein Labile if it is Unliganded?

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ABSTRACT

Objective: Human papillomavirus (HPV) infection is a major global health concern due to its association with several types of cancer, particularly cervical cancer. Among the various viral proteins produced by HPV, E6 and E7 oncoproteins have been identified as key players in the oncogenic transformation of infected cells. HPV E7 oncoprotein primarily targets the retinoblastoma protein (pRb) and related family members, retinoblastoma-like protein 2 (p130) and retinoblastoma-like protein 1 (p107) and disrupts their association with E2F transcription factors, releasing the E2F for transcriptional activation of genes required for cell cycle progression. E7 is an unstable protein with a half-life of less than 2 hours. We aimed to investigate whether the stability of the E7 protein would change when it has a binding partner such as pRb, p130 and p107.

Methods: We transfected HEK293T cells with either Green Fluorescent Protein tagged E7 (GFP-E7) construct alone or together with one of the pRb (RB1), p130 (RBL2) or p107 (RBL1) constructs and observed the expression of GFP-E7 under a fluorescent microscope. We have also analysed the expression levels of E7, pRb, p130 and p107 by western blotting using specific antibodies.

Results: We showed that E7 expression was more prominent in human embryonic kidney 293T (HEK293T) cells where one of the cellular target proteins was co-expressed.

Conclusion: HPV E7 oncoprotein is an indispensable component for the pathogenesis of cervical cancer, and understanding the molecular mechanisms of how E7 maintains its stability in the cell may enable the development of targeted treatment approaches.

Keywords: Human papilloma viruses, uterine cervical neoplasms, protein stability, papillomavirus E7 proteins, green fluorescent proteins

1. INTRODUCTION

HPV is a highly prevalent sexually transmitted infection that can cause various health problems, including genital warts and certain types of cancer such as cervical, anal, vaginal, vulvar, penile, and oropharyngeal cancers. International Agency for Research on Cancer of World Health Organization (IARC-WHO) has indicated that cervical cancer is the fourth most common type of cancer in women and the third leading cause of death globally (1). Of the many HPV types, HPV16 and 18 are known causative agents of cancer, with several other HPV types likely. Although it has been almost 18 years since the vaccine (which targets types 16 and 18) was first introduced to the market, according to reports published by the WHO, only some 60% of its members included the HPV vaccine in their national vaccination programmes until 2022 (2). Besides, considering that 80% of sexually active individuals will acquire at least one type of HPV at some point in their lives, it is predicted that the incidence of HPV will increase significantly, particularly in underdeveloped countries. Although the presence of the virus does not indicate that infected individuals will develop cancer since the infection is usually cleared by the host immune system, persistent infection may lead to high-grade cervical intraepithelial neoplasia (CIN) and subsequently to cervical cancer, and as a result, it may grow into a global health burden due to the expected high morbidity and mortality rates of HPV-associated cancers (3).

HPV DNA has been detected in more than 90% of malignant cervical lesions; in particular high-risk HPV 16 and 18 have been determined to be closely associated with the development of cancer (4). Among the various viral proteins produced by HPV, E6 and E7 are the major oncogenes that have been identified as key players in the oncogenic transformation of infected cells. HPV E6 oncoprotein is known for its ability to promote the degradation of the tumour suppressor protein, p53. The HPV E7 oncoprotein primarily targets the retinoblastoma protein (pRb) and related family members, p107 and p130

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mainly through the cullin-2 ubiquitin ligase complex and protein tyrosine phosphatase non-receptor type 14 (PTPN14) through association with the p600 ubiquitin ligase (5,6). E7 binds to the pocket domain of these proteins, leading to the disruption of their association with E2F transcription factors and the release of E2F for transcriptional activation of genes required for cell cycle progression. By overriding the cell cycle control mechanisms regulated by the pRb-E2F pathway, HPV E7 promotes uncontrolled cell proliferation and inhibits cellular senescence and apoptosis, which are crucial protective mechanisms against cancer development.

E6 and E7 oncoproteins collaborate to immortalise human keratinocytes and to stimulate cellular transformation, leading to malignancy (7). While human keratinocytes continue to grow unlimitedly in the presence of both E6 and E7, transformation of human keratinocytes cannot occur in the absence of E7 (7). Therefore, expressions of both E6 and E7 are required to sustain the malignant phenotype. Moreover, in a recent study, E6 and E7 from several HPV types were shown by flow cytometry-based fluorescence resonance energy transfer (FRET) assays and *in vitro* analytical ultracentrifugation to directly interact with each other (8).

E7 is a short polypeptide containing approximately 100 amino acid residues and is highly conserved amongst many HPV types, which includes three conserved regions termed CR1, CR2, and CR3 (9). While CR1 and CR2 are supposedly disordered, CR3 involves two CxxC zinc-binding motifs separated by 29 or 30 amino acids and is responsible for the dimerization of E7 (10-12). CR1 and CR2 are vital for the induction of cellular transformation, with CR2 contains an LxCxE motif to which pRb family proteins can bind (13,14). It is a short-lived protein with a half-life of less than 2 hours due to its rapid turnover rate in cells (15,16). Its degradation in cervical cancer cells is carried out via the ubiquitin-proteasome pathway (17). The first 11 amino acid residues from the E7 amino (N) – terminus act as an important signal for ubiquitination as well as mediating the interaction with other cellular proteins. Removing the N-terminal residues or adding an N-terminal but not a carboxyl (C)-terminal tag stabilizes E7 oncoprotein in cells, indicating that E7 is N-terminally ubiquitinated to direct proteasomal degradation (17).

In this study, our aim was to evaluate GFP-E7 protein expression levels when E7 protein was expressed alone in HEK293T cells or with one of its binding partners (pRb, p107 or p130), and E7 expression levels were found to be higher when E7 and one of its interacting partners were co-expressed.

2. METHODS

2.1. Cell culture and transfection of cells: HEK293T cells were maintained in DMEM (Lonza, Switzerland) supplemented with 10 % foetal bovine serum (FBS), 100 units/ml penicillin, 0.1 mg/ ml streptomycin and 1 % L-glutamine in T-25 flasks. Cell passages were performed every 3-4 days before they became 80-90 % confluent. Cells were detached from the surface of the flask by using Trypsin-EDTA solution and collected in appropriate tubes. Flasks were placed in the horizontal position in the humidified incubator (37°C; 5 % CO_2). Images were captured by a fluorescence microscope. For polyethylenimine (PEI) transfection, 2-3 µg of plasmids (pcDNA3-E7-EGFP, pcDNA4MaxMycRb, pcDNA1-HA-p130 and pCMV-HA-p107) was diluted in 200 µl of serum-free media (DMEM, Lonza, Switzerland) and mixed by vortexing. 10 µl of PEI (1 µg / µl) reagent was added to plasmid suspension and incubated at room temperature for 10-15 minutes. PEI-plasmid mixture was applied onto HEK293T cells.HEK293T cells were either transfected with one of the *GFP-E7*, *pRb*, *p130* or *p107* constructs alone or co-transfected with *GFP-E7 + pRb*, *GFP-E7 + p107* or *GFP-E7 + p130* plasmids.Cells were incubated in the humidified CO_2 incubator at 37°C for 24 hours before the experiment.

2.2. Preparation of protein samples: Cells were collected in 1,5 ml eppendorf tubes and cell suspension was centrifuged in the cold centrifuge at 4°C at 13,200 rpm for 2 minutes. Cell pellet was re-suspended in appropriate volume of RIPA buffer containing Ethylenediaminetetraacetic acid (EDTA) free protease inhibitor tablet (Roche, Switzerland), DNase (5 µg/ ml) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 10 mM $MgCl_2$, and incubated on ice for 30 minutes, vortexing every 10 minutes. Total protein sample was mixed with 2 x SDS-loading buffer and boiled to denature at 95 °C for 5 minutes. Protein samples were loaded in the SDS-acrylamide gel for separation after 1 min centrifugation at 13,200 rpm.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting: 10%, 12 % or 15 % acrylamide gels with 6 % acrylamide stacking layers were prepared for the analysis of protein samples. After placing the gels in tetra cell electrophoresis tank (Bio-Rad, Hercules, California, USA) in 1 x SDS-PAGE running buffer, each sample was loaded into wells along with Pageruler™ prestained protein ladder (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Electrophoresis was performed at 200 V for 1 hour. Following the separation of proteins by SDS-PAGE according to their sizes, gels were placed in XCELL™ Blot Module western blotting tanks (Invitrogen, Waltham, Massachusetts, USA) and protein was transferred onto nitrocellulose membranes (Whatman, Little Chalfont, Buckinghamshire, UK) at 30V and 4°C for 2-2.5 hours. Nitrocellulose membrane was washed in 15 ml of 1 x TBST buffer and blocked in 15 ml of blocking buffer (5 % non-fat dried milk dissolved in 1 x TBST) for 0.5-1 hour at room temperature. Appropriate primary antibody (anti-pRb, anti E7, anti-HA or anti-GAPDH with working dilutions of 1/2000, 1/1000, 1/2000 and 1/6000 respectively) was then added to the blocking buffer and incubated overnight on a shaking platform in the cold room. Membranes were washed with 1 x TBS-T 4 times each for 10 minutes. The membranes were then probed with anti-mouse or anti-rabbit peroxidase conjugated secondary antibodies (Sigma, Burlington, Massachusetts, USA) (1/2000) for 1 hour at room temperature with shaking. When incubation time is over, membranes were washed in 1

x TBS-T 4 times each for 10 minutes, then drained of excess TBS-T buffer and incubated in chemiluminescence reagent (ECL) for 1 min at room temperature followed by exposing to X-ray film (Hyperfilm ECL, Amersham, Buckinghamshire, UK).

3. RESULTS

We evaluated the level of E7 expression when it was expressed alone or co-expressed with one of its binding partners e.g. pRb, p130 and p107 tumour suppressor proteins using plasmid transfections. First, HEK293T cells were transfected with one of the *GFP-E7*, *pRb*, *p130* or *p107* constructs alone and then co-transfected with both *GFP-E7 and pRb, p107 or p130* plasmids. Cells were allowed to express proteins for 24 hours before imaging for GFP-E7 expression under a fluorescence microscope and protein extraction. Extracted protein was analysed by SDS-PAGE and western blotting using anti-E7 (for GFP-E7), anti-pRb, anti-HA (for p130 and p107) and anti-GAPDH (housekeeping control) antibodies as primary and anti-mouse and anti-rabbit as secondary antibodies. It is clear from Figure 1 that when GFP-E7 was co-expressed with any of the interacting partners (pRb, p130 and p107), more GFP signal was detectable, showing that GFP-E7 was stabilized through binding to one of its partners. It was also observed that GFP-E7 was mainly localizing in the nucleus of HEK293T cells. Western blotting was also performed to quantitate the difference in the GFP-E7 expression levels (Figure 2). Membranes were developed with anti-pRb, anti-HA and anti-E7 antibodies. Protein samples obtained from *GFP-E7* transfected cells showed much lower GFP-E7 expression levels than those from co-transfected with either *pRb, p130 or p107*.

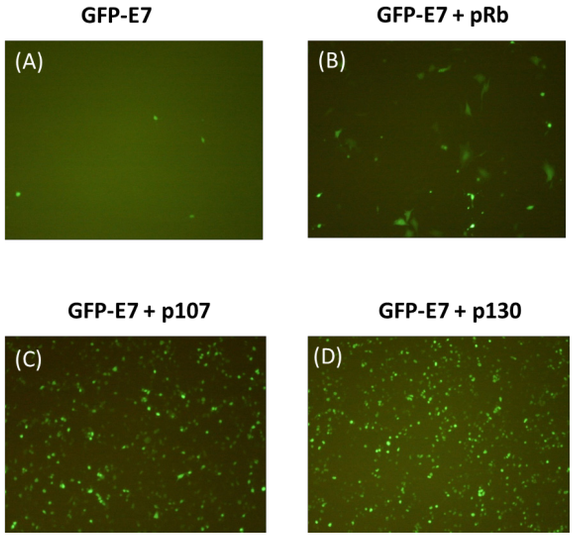

Figure 1. *Co-expression of E7 and one of its binding partners changes the E7 oncoprotein levels in HEK293T cells. HEK 293T cells were either transfected with 2 µg of GFP-E7 construct alone (A) or co-transfected with 2 µg of GFP-E7/1 µg of myc-pRb (B), 2 µg of GFP-E7/4 µg of HA-p107 (C) and 2 µg of GFP-E7/0.5 µg of HA-p130 (D) using PEI transfection reagent. Cells were allowed to co-express proteins for 24 hours and images were captured by a fluorescence microscope to show the difference of GFP-E7 expression when expressed with a binding partner.*

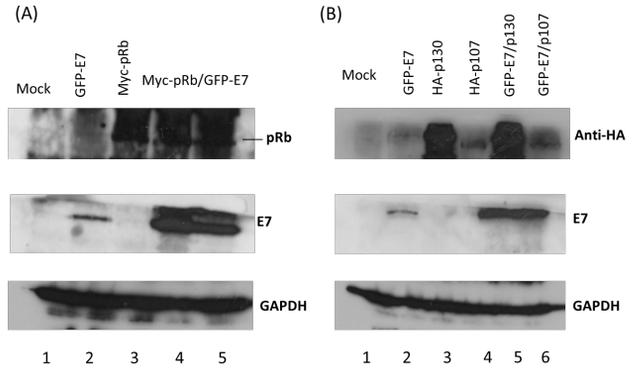

Figure 2. *The effect of co-expression of GFP-E7 and either pRb or its related proteins (p130 and p107) on the levels of E7 oncoprotein in HEK293T cells. A) HEK 293T cells were not transfected with any of the plasmid constructs (Lane 1) or transfected with 2 µg of GFP-E7 (Lane 2) or 1 µg myc-pRb (Lane 3) alone or co-transfected with 2 µg GFP-E7/ 1 µg myc-pRb (Lanes 4 and 5). Cells were allowed to co-express proteins for 24 hours to show the difference of GFP-E7 expression levels when it is expressed with a binding partner. B) HEK 293T cells were transfected with 2 µg of GFP-E7 alone (Lane 2), 0.5 µg of HA-p130 alone (Lane 3) or 4 µg of HA-p107 alone (Lane 4) or co-transfected with 2 µg of GFP-E7/ 0.5 µg of HA-p130 (Lane 5) and 2 µg of GFP-E7/ 4 µg of HA-p107 (Lane 6) and incubated for 24 hours for the expressions of proteins. Cells were collected, lysed, and prepared for SDS-PAGE electrophoresis as described previously. Western blotting was performed, and membranes were incubated in primary (anti – pRb, anti-HA, anti-E7 and anti-GAPDH) and secondary antibodies (anti-mouse or anti-rabbit).*

4. DISCUSSION

Cervical cancer is among the most common female cancers, and causative agents responsible for most cases are HPVs, in particular high-risk HPV types 16 and 18. HPV genome integrates to the host genome, which is one of the critical steps in the transformation of infected epithelial cells. HPV genome encodes oncoproteins including E6 and E7 to trigger cell proliferation and to inhibit apoptosis (18). E7 regulates transcription of genes required for transition to S-phase in HPV-transformed epithelial cells, and continuous expression of E7 is highly important to maintain a malignant phenotype in cancerous cells. E6 and E7 have been recognized as unstable proteins and can be degraded by the ubiquitin-proteasome system (19). Therefore, it is of vital importance to understand the molecular mechanisms underlying how this oncoprotein maintains its stability in cells. (20).

HPV E6 and E7 oncoproteins employ the ubiquitin ligase E6AP to target p53 and cullin-2 ubiquitin ligase complex Cullin2 and PTPN14 through association with the p600 ubiquitin ligase to target pRb, p130, and p107, respectively for degradation, leading to escape from apoptosis and progress through the cell cycle. Interestingly, a recent paper using GST pulldown and coimmunoprecipitation assays have indicated a potential association between E7 (independent from E6) and E6AP although multiple proteomic analyses were unable to find a direct interaction between them (6). The same study suggested that together with E6, E7 could also be stabilized by interacting with E6AP in cells derived

from cervical cancer. The findings obtained from RT-qPCR, proteasome inhibition assays and determination of the half-life of E7 protein demonstrated that when E6AP was depleted (e.g. through silencing with siRNAs), E7 expression was found to be dramatically decreased and half-life reduced by almost 50% in cervical cancer cells. In agreement with these results, exogenous overexpression of E6AP rescued the E7 expression levels.

Because the E6 and E7 oncoproteins are inherently expressed together, it is not easy to determine the potential effects of each when expressed separately in physiological systems. Therefore, exogenous expression of each oncoprotein via a plasmid construct may be important in studying their effects individually in cells. Thus, we transfected HEK293T cells with several plasmid constructs to study E7 stability. The stability of E7 protein can be analysed by determining its half-life using inhibitors like cycloheximide, understanding how it is degraded (using proteasome inhibitors such as MG132), and identifying factors (e.g., binding proteins or post-translational modifications) that influence its stability. Here, we tried to use the role of protein-protein interactions to understand E7 stability. For this purpose, we compared E7 levels in the presence and absence of its target proteins (pRb and its related proteins). Our Western blot and fluorescent imaging results using GFP-E7 showed that exogenously expressed GFP-E7 was not as stable when GFP-E7 were co-expressed with one of its binding partners (pRb, p130 and p107). Similarly, another study used a recombinant EGFP-tagged adenovirus fused with HPV16 E7 to infect HEK293T cells and an HPV-negative cervical cancer C33A cells showed a stable expression of E7 oncoprotein in those cells (21).

E7 is an unstable protein, and its degradation occurs through the ubiquitin-proteasome pathway both *in vitro* and in cells. Addition of an N-terminus tag such as myc-tag increases the stability of E7 while reducing its transforming capabilities, possibly disturbing its interaction with other cellular target proteins. E7 exist mostly as stable dimers under physiological conditions, which is required for its cellular actions. E7 carboxyl terminus is more structured than its N-terminus and consists of a zinc-binding domain that includes two Cys-X-X-Cys motifs that play a crucial role in its dimerization (22,23). A recent study using analytical ultracentrifuge analysis have revealed that the E7 protein, which is fluorescently labelled with enhanced yellow fluorescent protein EYFP at the N-terminus, also forms a dimer (8). Similarly, in our experiments we expressed E7 with N-terminal fusion of GFP in cells alone or together with pRb-related proteins.

Our study showed that the expression of E7 was much more evident, if GFP-E7 and one of its binding partners are co-expressed in cells, possibly due to further stabilization by interactions with its cellular substrates such as pRb, p130 and p107. Upon binding to pRb and its related proteins, E7 recruits cellular ubiquitin ligase complexes, leading to their ubiquitination and subsequent proteasomal degradation. When pRb and its family members are degraded, the E7 protein itself is not affected. In fact, the degradation of its target protein e.g. pRb may leave E7 in a “free” state, potentially stabilizing E7 levels or leading to an accumulation of E7 in the cell. Green fluorescent signal from *p130* and *p107* transfected HEK293T cells was more noticeable which indicates that E7 expression was much higher when GFP-E7 is co-expressed with p130 or p107 rather than pRb. This may be because E7 is more effective at inactivating pRb but better stabilised when bound to p130 or p107. Or very simply, there may be differences in the transfection efficiencies of the plasmids with which we transfect the cells such as the *pRb* plasmid being less effective.Besides, when E7 binds to one of its cellular targets, it may possibly be protected from proteasomal degradation, causing an increase in its half-life. Adenovirus E3 ubiquitin ligase generated by the viral E4orf6 and E1B55K protein stabilizes adenovirus E1A protein and, increases its half-life both in both virus-infected cells and plasmid-transfected cells, resulting in the accumulation of E1A products (24). By using E1A mutants, this stabilization was suggested to be due to the interaction of E4orf6 with the N-terminus of E1A protein that is responsible for the association with proteasomes, leading to the inhibition of E1A protein degradation, and thus an increase in the half-life of E1A product. Here, in this study E7 was detected by fluorescent imaging both in the cytoplasm and predominantly in the nucleus of HEK293T cells transfected with *GFP-E7* constructs. Although the main localization of HPV16 E7 is the cell cytoplasm and cell nucleus (16,25,26), it has previously been shown that E7 can also exist in the cell membrane of HPV-transformed cells (27). HPV16 E7 has been reported to involve a nuclear export signal that allows it to shuttle between the nucleus and cytoplasm (26). In another study, it was shown by cell fractionation assays that E7 was predominantly cytoplasmic in CaSki cells (28). This seems an unexpected result considering that E7 is supposed to transactivate various transcription factors (such as E2F-1 and Fos) by binding to pRb-related proteins in the nucleus, which is essential for cellular transformation. However, E7 was also demonstrated to interact with various cytoplasmic proteins such as actin and M2 pyruvate kinase (29,30). In CaSki cells treated with a proteasome inhibitor, MG132, a great deal of E7 was observed to accumulate in the nucleus compared to the cytoplasm, suggesting that nuclear E7 may be more unstable than its cytoplasmic counterpart (28). Therefore, we may be seeing E7 expression very densely accumulated in the nuclear region because of the stabilization of E7 through binding to pRb, or its related proteins overexpressed following plasmid transfections. Moreover, HPV E7 oncoprotein has been shown to interact with a variety of other cellular proteins involved in chromatin remodelling, transcriptional regulation, and DNA repair processes, thereby contributing to the dysregulation of gene expression and genomic stability in HPV-infected cells. Therefore, the stability of E7 in the cell varies depending on its relationship with its many target proteins.

5. CONCLUSION

HPV E7 oncoprotein (together with E6) play a critical role in the pathogenesis of cervical cancer by disrupting key cellular

pathways involved in cell cycle regulation, genomic stability, and tumour suppression. In our study, we tried to show that the stability of E7 when expressed on its own is low if cellular target proteins are not present in cells. Understanding the molecular mechanisms by which E6 and E7 maintain their stability and increase their half-lives in cells and contribute to oncogenesis is essential for the development of targeted therapeutic strategies aimed at blocking the oncogenic activities of these viral proteins.

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Design of the study: OCG
Acquisition of data for the study: OCG
Analysis of data for the study: OCG, NJS
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Drafting the manuscript: OCG, NJS
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Clinical and Experimental Health Sciences

Is Youtube™ A Reliable Source On Invisalign First® in Mixed Dentition?

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ABSTRACT

Objective: Clear aligner systems, particularly Invisalign First®, have become an increasingly popular treatment option for children during the mixed dentition stage. With the growing reliance of patients and caregivers on digital platforms such as YouTube™ for health-related information, concerns about the accuracy and educational value of this content are becoming increasingly significant. This study aimed to evaluate the quality, usefulness, and reliability of YouTube™ videos discussing Invisalign First® for children.

Methods:A systematic search was conducted on YouTube™ using the keywords “invisalign first” and “clear aligners and mixed dentition”. For each term, the first 185 English-language videos were collected, generating a total of 370 videos. Videos were included if they were uploaded between 22 May 2018 and 23 September 2023, in English, and provided information relevant to clear aligner use in children with mixed dentition. After applying exclusion criteria, 74 videos were selected for final evaluation.

Each video was assessed independently by two experienced clinicians—a pediatric dentist and an orthodontist, each with 13 years of professional experience in orthodontic care for children in the mixed dentition. Videos were evaluated using the Global Quality Scale (GQS) and a 12-item usefulness score designed to measure the comprehensiveness and relevance of the educational content.

Data collected from each video included length (in minutes), number of views, likes, dislikes, and days since upload. From these variables, the interaction index and viewing rate were calculated. The videos were categorized into four source groups:(1) Dental companies/clinics (2) Dentists/Orthodontists, (3) Laypersons, (4) Others. The intended target audience was classified as: (1) professional, (2) layperson, or (3) both. The Shapiro–Wilk test was used to test the normality of numerical data. Non-normally distributed variables were analyzed using the Spearman rank correlation coefficient. Group comparisons were conducted using the Mann–Whitney U test, chi-square test, and Pearson correlation coefficient. A p-value of <.05 was considered statistically significant.

Results: Of the 74 videos analyzed, 35.1% were uploaded by dental clinics, 31.1% by dentists/orthodontists, 21.6% by laypersons, and the remainder by others. The majority of videos (62%) targeted laypersons, while 24% targeted both professionals and lay audiences.The average video length was 4.2 minutes (SD = 1.6). The average number of views per video was 12340, ranging from 580 to 156000 views. The mean interaction index was 1.8, and the viewing rate was 2.3. 44% of videos were classified as poor quality based on GQS. 30% were considered generally poor quality. Only a small fraction (14%) were rated as good or excellent. Based on the usefulness score, most videos were rated as slightly useful or moderately useful. Videos rarely included balanced discussions of risks, limitations, or alternative treatments. A strong positive correlation was observed between usefulness and GQS ($r = 0.817$, $p = 0.000$), suggesting that higher-quality videos also tended to offer more useful educational content. The inter – and intra-examiner reliability of scoring was high, demonstrating excellent agreement between the evaluators. This indicates that the evaluation criteria were applied consistently and that the scoring method was robust. There are several limitations to this study. First, there is currently no standardized, validated tool for objectively assessing video-based educational content, making the evaluation process inherently subjective despite the use of structured scoring systems. Second, video selection was based on YouTube’s™ default relevance ranking, which may be influenced by algorithms, advertisements, or user personalization settings. Lastly, YouTube™ content is dynamic, and video availability or ranking may change over time, potentially affecting reproducibility.

Conclusion: YouTube™ videos discussing Invisalign® First for children in the mixed dentition stage are generally of low quality and limited usefulness. Although many videos mention the benefits of clear aligner treatment, few provide comprehensive, balanced, or professionally verified information. Given the increasing reliance on social media platforms for healthcare information, it is crucial to develop strategies for guiding the public toward reliable, evidence-based content. Furthermore, establishing validated, quantitative tools for evaluating video content would greatly improve future research in this area.

Keywords: Orthodontic appliances (removable), Invisalign First**,** pediatric dentistry, orthodontics**,** social media**,** consumer health information

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1. INTRODUCTION

Postponement of orthodontic intervention until the end of all permanent teeth eruption is an old practice considering contemporary approaches advocate for orthodontists to assess children by the age of 7 to identify potential issues that could benefit from early treatment (1,2). However, the suitability of early treatment is a debated topic, while some studies highlight its high effectiveness, others question its utility (3). In spite of potential extension of total treatment duration due to the need for a second phase, many professionals prefer early treatment, believing it produces superior results compared to the conventional single-phase approach in adolescence (4). Early intervention aims to prevent or minimize the progression to full-blown malocclusion later in life and eliminates factors that could disrupt the regular development of dental arches (5). Research indicate that early-phase-orthodontic treatment not only enhances psychosocial development in children experiencing persistent teasing at school due to "buck or crowding teeth", but also improves masticatory function. Also, dental trauma studies reveal that individuals with overjet exceeding 3 mm face a risk of injury more than twice as high as those with a normal overjet (6). Therefore, for many reasons children with orthodontic malocclusion are considered suitable candidates for early-phase treatment (7).

As being in adult orthodontics, the growing demand for orthodontic appliances that offer enhanced aesthetics and comfort compared to traditional ones, the Invisalign First® system, introduced in 2018, has revolutionized for children and teenagers in mixed dentition, addressing issues such as arch expansion, crowding, spacing, and other malocclusions (6,8,9). Compared to traditional appliances, Invisalign First® provides improved manageability for both clinicians and patients, eliminate food restrictions, reduce the risk of appliance damage due to weekly change, minimize the need for emergency appointments, simplify oral hygiene routines and enhance patient comfort (10,11). However, clear aligner therapy has notable limitations, particularly in achieving complex tooth movements and managing minimum anchorage cases, where conventional appliances remain indispensable (12). Also, due to its removable nature, noncompliance with wearing the aligners remains a significant drawback (13,14). This becomes more significant when considering that age span of orthodontic patients usually lies in the period of adolescence, a transitional period that may give birth to difficulties for patients, their guardians, and orthodontist.

Literature consistently shows that patients, especially prospective ones, tend to prefer more aesthetic appliances, largely influenced by visual appeal and perceived comfort (15). In the context of Invisalign First®, parents perceptions are further shaped by digital media, emphasizes aesthetics and comfort for promotional purposes (16). The rapid growth of social media has transformed the way audiences and professionals' access and share information about health. YouTube™ is one of them which is the largest video-sharing platform, offering easily accessible, long-form content suitable for health education. Unlike Instagram™ or TikTok™, which favor brief, entertainment-focused posts, YouTube™ allows for more detailed explanations, making it a preferred source for patients and caregivers seeking orthodontic information (17–19). However, the quality and reliability of information on YouTube™ videos remain uncertain (20). Since the content on this open platform is not peer-assessed, it is essential to critically evaluate the available data for determination of pre-orthodontic education value. Previous studies evaluating the quality and content of social media videos on orthodontic clear aligners (21–24) have consistently concluded that the information provided is largely inadequate. Cesur et al. (24) investigated only the videos uploaded by an orthodontist and found that videos about orthodontic aligner treatment have average reliability and quality but largely insufficient content. Although a few studies have evaluated YouTube™ videos on clear aligner, there is currently a lack of research specifically Invisalign First® treatment. Therefore, the present study aims to evaluate the quality and educational value of YouTube™ videos that discuss orthodontic clear aligner systems for children in mixed dentition. The null hypothesis (H_o) posits that there is no significant quality or educational value in YouTube™ videos discussing Invisalign First® treatment during the mixed dentition phase. The corresponding alternative hypothesis (H_1) is that YouTube™ videos on Invisalign First® treatment during the mixed dentition phase provide significant quality and educational value. By conducting an evaluation of these videos, valuable insights into the usefulness and reliability of YouTube™ as a source of information for patients, parents, and healthcare professionals in the field of orthodontics and pediatric dentistry has been provided.

2. METHODS

2.1. Study Design

This study adopted a cross-sectional design to assess the informational content available on YouTube™ concerning the use of Invisalign FirstÒ in managing mixed dentition. The review of existing literature revealed only two prior studies focusing on YouTube™ videos related to clear aligner systems and Invisalign – one 140 videos (23) and the other 85 videos (21) – highlighting a significant gap in current knowledge. To ensure unbiased results, all browsing data, cookies and prior search history were cleared before initiating the search.

On June 9, 2023, a systematic Youtube™ search was conducted using the keywords "invisalign first" and "clear aligners and mixed dentition." The term "clear aligners in mixed dentition" was selected based on terminology derived from the Medical Subject Headings (MeSH) database to ensure the use of standardized biomedical vocabulary. "Invisalign" was included due to its status as the most widely recognized brand name for clear aligner therapy, frequently referenced by both clinicians and patients. This combination

of brand-specific and clinically relevant terms was chosen to reflect real-world search behavior while maintaining methodological rigor.

Following the approach of previous studies that analyzed between 60 and 200 videos (23), the first 185 English-language videos for each search term were selected, resulting in a total of 370 videos. These videos were compiled into a dedicated playlist and subjected to comprehensive content analysis, providing a broad and representative overview of the information currently available to the public.

2.2. Data Collection

An initial assessment was conducted to filter out non-English language content, videos lacking sound or headings, duplicate videos, commercials, videos with insufficient information, irrelevant videos, and videos longer than fifteen minutes. Videos between 05/22/2018 and 23/09/23 in the English language that provided information about clear aligner systems used in mixed dentition were included in the study. Data were collected for each video, including video title, source, target audience, duration, upload dates, number of views, likes, dislikes, and comments. Descriptive statistics were employed to summarize the characteristics of the included videos. Viewing rates and interaction rates were calculated as follow:

$$Viewing\ rate = \left(\frac{(\ Number\ of\ views\ )}{(\ Number\ of\ days\ since\ upload\ )}\right) \times 100$$

$$Interaction\ rate = \left(\frac{(\ Number\ of\ likes\ ) - (\ Number\ of\ dislikes\ )}{(\ Number\ of\ views\ )}\right) \times 100$$

Figure 1. *Formulas for viewing rate and interaction rate 4*

Additionally, mean scores were calculated. Video sources were categorized into four groups: dental companies/clinics, dentists/orthodontists, laypersons, and others. Target audiences were categorized as: professional, lay person and both.

2.3. Assessment of Video Quality and Usefulness

A pediatric dentist and an orthodontist, each with 13 years of clinical experience in managing mixed dentition cases, independently evaluated the videos for their quality and educational usefulness. Each video was also rated using a 12-point usefulness score system, assessing factors including accuracy, clarity, age suitability, daily wear duration, estimated treatment duration, benefits, limitations, potential risks or complications, maintenance, motivation, treatment steps and comparison to other modalities (Table 1). Based on total score, videos were categorized as not useful (0), slightly useful (1-4), moderately useful (5-8), or very useful (9-12). To assess intra-rater reliability, twenty randomly selected videos were re-evaluated after one month.

Table 1*. Usefulness score for evaluation the videos.*

Scoring item	Description	Score
Accuracy	To provide accurate and evidence-based information	1
Clarity	The use of clear language, visuals, and demonstrations	1
Age of Treatment	The appropriate age for clear aligner treatment in children with mixed dentition	1
Adequate Daily Usage Duration	The importance of consistent and appropriate daily usage of clear aligners	1
Treatment Duration	The typical treatment duration (18 months)	1
Benefits	Improved aesthetics, oral health etc.	1
Limitations	Complex treatments, skeletal problems etc	1
Potential Risks or Complications	Dental caries, unpredictable tooth movements, Aligner loss, breakage etc.	1
Maintenance	To properly clean clear aligners and offer advice on daily tooth brushing and oral hygiene practices	1
Motivation for Treatment	Motivation and encouragement for children undergoing clear aligner treatment. Success stories or testimonials etc.	1
Treatment Procedure Details	Comprehensive details about the treatment procedure, including steps involved, adjustments, and any necessary follow-up visits	1
Comparison to Other Treatment Modalities	Comparison to traditional braces or other alternatives.	1

The global quality scale (GQS), a standardized 5-point tool, was used to assess video quality, including aspects such as audiovisual clarity, content accuracy, professionalism, and educational value. GQS scores ranged from 1=very poor quality (poor flow, lack of information, nothing useful for patients), 2=generally poor quality (low level of flow, some information listed but many important topics are missing, limited use to patients), 3=moderate quality (suboptimal flow, some important information discussed adequately, other pieces of information discussed poorly, somewhat useful to patients), 4=good quality (generally good flow, most relevant information listed, some topics not addressed, useful to patients), 5=excellent quality (excellent flow, very useful for patients).

2.4. Statistical Analysis

Normality of numerical data was tested using the Shaphiro Wilk test. Spearman rank correlation coefficient was used to examine relationships between non-normally distributed variables. Intraclass correlation coefficient (ICC) were calculated. SPSS 22.0 Windows version package program was used in the analysis. $p<.05$ was considered statistically significant.

3.RESULTS

Of the 370 videos screened, 296 were excluded for reasons such as exceeding 15 minutes (n=53), unrelated treatment topics (n=64), not specific to children (n=63), non-English content (n=30), music (n=16), silent (n=41), dublicates (n=17), or advertisements (n=12). Among the 74 eligable videos, 44.6% were uploaded by dental clinics, 32.4% by dentists/orthodontists, 12.2% by companies, 8.1% by laypersons, and 2.7% by health-related YouTube™ channels.

The target audience was mostly laypersons (62.2%). Most videos originated from America (60.3%), particularly the USA and Canada (Table 2). The average duration was 2.93 ± 3.02 minutes. The mean views per video were 1712.15 ± 5578 (range: 5-38745). The average number of comments was 1.04 ± 4.25; mean likes were 16.91 ± 63.46. Dislikes were negligible. Mean interaction index was 0.81 ± 1.24, and mean viewing rate was 174.44 ± 466.04. Mean usefulness and GQS scores were 2.34 ± 2.09 and 1.85 ± 1.01, respectively. Nearly half of the videos (44.6%) were slightly useful, 29.7% were moderately useful. Only 1.35% of the videos were highly useful, while 24.32% failed to provide any useful information specific to clear aligners (Figure 1). No moderately or very useful videos were uploaded by the layperson (Table 3).

Table 2. *Demographic distrubution of the included videos.*

		n	%
Source of the upload	Channel	2	2.7
	Clinic	33	44.6
	Dental Company	9	12.2
	Dentist/Orthodontist	24	32.4
	Lay person	6	8.1
Target audience	Professional	12	16.2
	Lay person	44	62.2
	Both	16	21.6
Continent	America	38	60.3
	Europe	11	17.5
	Asia	11	17.5
	Australia	3	4.8

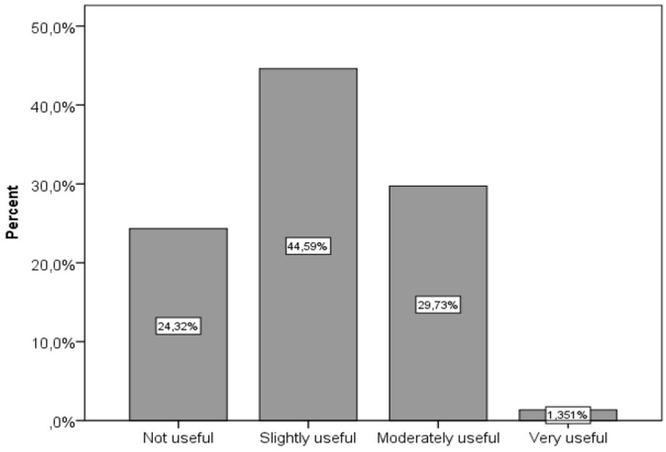

Figure 2. *Percentages of usefulness score*

Table 3. *Demographic distrubution according to usefulness score groups.*

		Usefulness score groups				
		Not useful	Slightly useful	Moderately useful	Very useful	Total
		n(%)	n(%)	n(%)	n(%)	n(%)
Source of the upload	Channel	0 (0)	0 (0)	2 (9.1)	0 (0)	2 (2.7)
	Clinic	9 (50)	15 (45.5)	8 (36.4)	1 (100)	33 (44.6)
	Dental Company	2 (11.1)	5 (15.2)	2 (13.6)	0 (0)	9 (12.2)
	Dentist/orthodontist	5 (27.8)	9 (27.2)	10 (40.9)	0 (0)	24 (32.4)
	Lay person	2 (11.1)	4 (12.1)	0 (0)	0 (0)	6 (8.1)
Target audience	Both	0 (0)	8 (24.2)	8 (36.4)	0 (0)	16 (21.6)
	Lay person	13 (72.2)	20 (60.6)	12 (54.5)	1 (100)	46 (62.2)
	Professional	5 (27.8)	5 (15.2)	2 (9.1)	0 (0)	12 (16.2)
Continent	America	12 (75)	17 (58.6)	9 (52.9)	0 (0)	38 (60.3)
	Europe	3 (18.8)	5 (17.2)	3 (17.6)	0 (0)	11 (17.5)
	Asia	1 (6.3)	6 (20.7)	3 (17.6)	1 (100)	11 (17.5)
	Australia	0 (0)	1 (3.4)	2 (11.8)	0 (0)	3 (4.8)

Invisalign FirstÒ benefits were mentioned in 58% of videos. Nearly 45% conveyed information in a clear and understandable manner. Comperative discussions with other treatment (e.g., braces, removable appliance) were found in 39%, while 35% addressed treatment steps and follow-up procedures. About 31% of the videos provided motivational content, such as success stories or testimonials from satisfied patients or dentists. 28% mentioned appropriate age for Invisalign FirstÒ. 72% lacked evidence-based data, references or citations to scientific studies. 20% gave maintenance guidance, while 18% mentioned treatment limitations. Risks, such as aligner loss or breakage were addressed in 16% of the videos. The expected treatment duration and recommended daily use were discussed in 16% and 14% of the videos, respectively.

Based on GQS, 44% of videos were rated poor, 30% generally poor, 16% moderate, and just (-8% good. A strong positive correlation existed between usefulness and GQS scores (r=0.817, p=0.000). No significant relationship was found between GQS and the number of views (p=0.193), likes (p=0.08) or comments (p=0.09). A weak but positive correlation was observed between GQS and video length (r=0.415, p=0.000), suggesting that longer videos tended to score slightly higher GQS scores, although the correlation was not very strong. Viewing rate (p=0.944) and the interaction index (p=0.060) did not correlate with GQS. However, a moderate positive correlation was found between video length and usefulness score (r=0.548, p=0.000). No other demographic variables were significantly associated with usefulness. Videos with higher views also had more likes (r=0.604, p=0.000)(Table 4).

Inter-rater reliability was high (ICC=0.898, 95% CI:0.889–0.990), as was intra-rater reliability (ICC=0.982, 95% CI:0.967–0.992).

Table 4. *Correlation matrix displaying spearman rank correlation coefficients between scores for usefulness score, GQS and video demographics*

		GQS Score	Usefulness Score	Number Of Views	Number Of Likes	Number Of Comments	Minute	Time From The Day The Video Was Uploaded To The Present	Interaction Rate	Viewing Rate
GQS Score	r	1.000	0.817*	0.193	.088	.196	0.415*	-0.034	-0.008	0.221
	P	.	.000	.102	.457	.098	.000	.774	.944	.060
	N	73	73	73	73	72	73	73	73	73
Usefulness Score	r	0.817*	1.000	0.218	.125	.220	0.548*	0.083	0.003	0.193
	P	.000	.	.061	.289	.062	.000	.485	.978	.100
	N	73	74	74	74	73	74	74	74	74
Number of Views	r	0.193	0.218	1.000	.604*	.459*	0.537*	0.323*	0.332*	0.788*
	P	.102	.061	.	.000	.000	.000	.005	.004	.000
	N	73	74	74	74	73	74	74	74	74
Number of Likes	r	0.088	0.125	0.604*	1.000	0.479*	0.265*	0.282*	0.878*	0.496*
	P	.457	.289	.000	.	.000	.023	.015	.000	.000
	N	73	74	74	74	73	74	74	74	74
Number of Comments	r	0.196	0.220	0.459*	0.479*	1.000	0.434*	0.011	0.327*	0.458*
	P	.098	.062	.000	.000	.	.000	.925	.005	.000
	N	72	73	73	73	73	73	73	73	73
Minute	r	0.415*	0.548*	0.537*	0.265*	0.434*	1.000	0.139	0.184	0.419*
	P	.000	.000	.000	.023	.000	.	.239	.117	.000
	N	73	74	74	74	73	74	74	74	74
Time	r	-0.034	.083	0.323*	0.282*	0.011	0.139	1.000	0.144	-0.231*
	P	.774	.485	.005	.015	.925	.239	.	.222	.047
	N	73	74	74	74	73	74	74	74	74
Interaction Rate	r	-0.008	.003	0.332*	0.878*	0.327*	0.184	0.144	1.000	.261*
	P	.944	.978	.004	.000	.005	.117	.222	.	.025
	N	73	74	74	74	73	74	74	74	74
Viewing Rate	r	0.221	0.193	0.788*	0.496*	0.458*	0.419*	-0.231*	0.261*	1.000
	P	.060	.100	.000	.000	.000	.000	.047	.025	.
	N	73	74	74	74	73	74	74	74	74

**p<.05 significance, r: Spearman rank correlation coefficient*

4. DISCUSSION

The present study identified under-appreciated and missing aspects of Invisalign FirstÒ treatment presentation to audience and provides a snapshot of the educational performance of Invisalign FirstÒ promotion on Youtube™ by being the first study on the matter so far. A 12-point usefulness score system was developed drawing inspiration from the guidelines provided by the American Association of Orthodontics and incorporating insights from previous relevant studies. This scoring system was tailored to assess the perceived usefulness of the videos in providing educational information and guidance on Invisalign FirstÒ. By utilizing this customized approach, it was aimed to capture the effectiveness and relevance of the videos on present specific research context. Several studies have also developed tailor-made usefulness score systems in various fields. These include assessing oral hygiene for children (20), space maintainers (25), early childhood caries (26), denture care (27), and oral cancer (28). These studies highlighted the importance of creating specific scoring systems to evaluate the usefulness of videos within their research areas. Similarly, the present study recognized the need for a specialized scoring system to assess the usefulness of videos on Invisalign FirstÒ treatment. The inter – and intra-examiner reliability values were strong, indicating excellent agreement among scorers. These results demonstrate that the scoring method is highly reliable, with minimal inconsistency both between and within evaluators.

Unlike earlier studies that identified laypersons as the predominant source of clear aligner-related content, the present findings highlight a noticeable shift, with healthcare professionals—particularly dental clinics and orthodontists—emerging as the primary contributors to videos on Invisalign First® (21,23). The difference in uploaders could be attributed to the relatively new nature of Invisalign FirstÒ treatment, prompting healthcare professionals to share information with parents. It is worth noting that Invisalign FirstÒ targets the age group of 6-10 years old, while most YouTube™ uploaders are adults sharing their testimonials, which were not relevant to the present study focusing on mixed dentition. Interestingly, both Duman's and Capan's studies (20,25) also identified healthcare professionals as the primary uploaders of educational videos on YouTube™. This study targeted laypersons, consistent with the findings of previous studies (18,21) rather than specifically targeting healthcare professionals.

In present study, a global quality score (GQS) was utilized to assess the quality of the videos (21,29). Present GQS results indicated that approximately half of the videos were classified as poor quality, with an additional 30% falling under the category of generally poor quality consistently with previously mentioned studies.

The analysis revealed a general lack of highly valuable educational content, with most videos offering only limited usefulness. Despite the majority being uploaded by professionals rather than laypersons, the overall usefulness remained modest. Given the professional backgrounds of most uploaders, higher usefulness scores might be predicted. Also, Cesur et al. (24) investigated only the videos uploaded by an orthodontist and found that videos about orthodontic aligners have average reliability and quality but largely insufficient content. This observations aligns with the conclusions drawn by Knösel et al. (30), suggesting that the predominant purpose of orthodontists' contributions on YouTube™ leans toward advertising rather than purely education.

Notably, the importance of consistently wearing the aligners all day, was inadequately emphasized in the videos. Instances such as aligner loss and breakage, which are common occurrences in this age group received minimal attention. The failure on predicted tooth movements or accelerated root resorption of deciduous teeth, which may necessitate intraoral scanning for revision, were also seldom addressed. One of the most frequently asked questions by families, especially at the start of treatment, is the expected duration of the treatment. Information regarding treatment duration, which plays a crucial role in enhancing patient compliance and motivating families, was likewise insufficiently provided in the videos. Moreover, the videos failed to adequately address the limitations of Invisalign FirstÒ treatment, such as its suitability for skeletal problems that were not suitable for camouflage treatment. Other essential topics that received minimal attention included the maintenance of aligners, which is crucial for oral health, emphasizing proper tooth brushing and daily cleaning of the aligners.

Previous studies evaluating the quality and content of social media videos on orthodontic clear aligners (21–24) have consistently concluded that the information provided is largerly inadequate. Although the present study focused solely on videos about Invisalign FirstÒ in the mixed dentition period, the findings echoed those previous reports – reinforcing the concern that most of the videos fail to deliver sufficient educational content.

In the present study, a significant and strong positive correlation was found between the usefulness and GQS, indicating that videos rated as more useful were also perceived to have higher overall quality. Ustdal and Guney (21) found a moderate correlation between GQS and content score, as well as a correlation between GQS and reliability score.

In the present study, longer videos tended to have higher video quality and educational value. Similarly, Sadry et al. (23) and Cesur et al. (24) found a statistically significant difference in the quality level of information content and video durations. However, Lena and Dindaroglu (31) reported that viewers lost interest in longer videos, with the average length of rich-content videos was 7.47 minutes.

The lack of a significant correlation between video popularity metrics—such as views, likes, comments, viewing rate, and interaction index—and the GQS suggested that viewer engagement did not necessarily reflect the educational

quality of the content. Consistent with previous researches, this discrepancy underscores the risk that widely viewed videos may prioritize entertainment or marketing over accuracy, potentially leading to misinformation among viewers seeking health-related guidance (32)(33).

Invisalign FirstÒ is gaining increasing attention due to its ability to provide maxillary expansion, distalization and teeth aligning in the mixed dentition period, along with its aesthetic advantages over traditional removable appliances. The esthetic appeal of clear aligner is often the most influential factor in patients' decision-making when they are not provided with comprehensive information about alternative appliances (34). However, Marañón-Vásquez et al. (34) demonstrated that when laypersons receive detailed information about available treatment options, their priorities shift from primarily aesthetic and financial concerns to factors such as treatment duration, anticipated outcomes, and quality of life during use. This discrepancy between the marketed image of Invisalign FirstÒ and its actual clinical effectiveness—which heavily depends on patient compliance—highlights the importance of managing patient expectations through accurate and balanced information. Therefore, both the influence of patients in appliance selection and the role of media in shaping these preferences warrant closer examination, particularly in light of the growing marketing presence of companies like InvisalignÒ and the increasing number of dental professionals producing social media content.

Healthcare professionals are constantly utilizing social media like Youtube™ to connect with their target patients and to advance their careers by building learning platforms. Considering educational content of the social media sources play a crucial role in patient education (35), it is essential to discuss the educational aspects rather than advertising content, including the pros and cons of the treatment which may be unfamiliar to parents in greater detail.

This study has several limitations that should be acknowledged. First, there are currently no standardized or validated quantitative tools for evaluating video content. As a result, the analysis relied on subjective scoring methods, which may introduce potential bias despite efforts to ensure evaluator consistency. The customized usefulness scoring system, while structured, remains inherently subjective. Additionally, the sorting of videos was based on YouTube's™ default relevance algorithm, which may be influenced by various factors such as personalization, user behavior, regional preferences, or paid promotions. This algorithm is neither transparent nor static; it can change over time without notice, potentially affecting which videos are displayed for the same search terms at different times or from different devices. As a result, the ranking and selection of videos in this study may not be fully reproducible or representative of all possible content. This evolving and opaque nature of YouTube's™ algorithm introduces a limitation in standardizing video selection criteria across studies, potentially impacting the generalizability and consistency of findings over time.

5. CONCLUSION

Based on the observed lack of reliable and educational content in the analyzed YouTube™ videos, the results support the null hypothesis, confirming that YouTube™ is not a significant source of quality information regarding Invisalign First®. Videos were generally of low quality and limited usefulness, which poses a risk when such content is used as a primary source for clinical decision-making. This underscores the need for more trustworthy and scientifically accurate resources. Dental professionals should be encouraged – by their professional associations and licensing bodies—to produce concise, evidence-based videos that are accessible and informative to the general public. Health authorities and public health organizations could play a critical role by collaborating with experts to create centralized, high-quality video content, or by endorsing existing trustworthy resources through verification systems. Additionally, educational institutions and dental schools can integrate media literacy and digital communication training into their curricula, empowering future clinicians to contribute to responsible health communication online. Regulatory bodies should consider establishing content quality guidelines, digital certification systems, or partnerships with social media platforms to supervise and promote reliable health-related content, thereby helping to mitigate misinformation and improve public health literacy. Finally, the development of a standardized, validated scoring system—such as a dedicated usefulness index—should be prioritized to improve the objective assessment of health-related video content in future research.

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Clinical and Experimental Health Sciences

Post Challenge Effects of Ozg-38.61.3 Gamma Irradiated SARS-CoV-2 Vaccine on Organ Protection in Transgenic Mouse Model

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ABSTRACT

Objective: Coronavirus disease 2019 (COVID-19) is an infectious outbreak caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and virus-related deaths are increasing day by day. For this reason, vaccine studies and their urgent use are of great importance to prevent the pandemic. In this study, multi-organ damages caused by SARS-CoV-2 virus in human – angiotensin-converting enzyme type 2 (ACE2) transgenic mice and the protective effects of OZG-38.61.3 gamma irradiated SARS-CoV-2 vaccine against viral damage were investigated.

Methods: For this purpose, transgenic K18-hACE2 BALB/c mice were randomly allocated into 4 groups, negative control group (NC), positive control group (PC, SARS-CoV-2 infected), and 2 different doses of OZG-38.61.3 vaccine (Challenge 1, dose of 10^{13} and Ch2, 10^{14} viral particle after SARS-CoV-2 infection). After the administrations, lung, heart and kidney tissues were examined by histopathological, immunohistochemical and TUNEL analysis.

Results: Our results showed that the vaccine doses decreased the apoptosis, oxidative stress and inflammation parameters caused by virus in lung, heart, and kidney tissues. It was also found that the vaccine protected the expressions of tight junction proteins in the kidneys.

Conclusion: According to our findings, it is suggested that the OZG-38.61.3 can be an effective and protective vaccine that can be safely used against the SARS-CoV-2 virus.

Keywords: COVID-19, SARS-CoV-2, human ACE2 transgenic mouse, Organ damage, Vaccination

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome 2 RNA virus, called the new type of Coronavirus (SARS-CoV-2), is an infectious disease (1). After the COVID-19 disease was first seen in Wuhan, China, it spread rapidly around the world and was declared a global pandemic by WHO on March 11, 2020.

Taxonomically, SARS-CoV-2 is classified as a member of the SARS-related coronavirus (SARS-CoV) in the betacoronavirus (βCoV) genus of the Coronaviridae family (2). There are three transmembrane proteins in the lipid bilayer: Spike (S) protein, envelope (E) protein and membrane (M) protein (3). Spike proteins present on the virus bind to angiotensin converting enzyme 2 (ACE2) receptors to enter the host cell, and the viral envelope fuses with the cellular membranes. Thus, the viral genome enters the host cell (4). Expression of the ACE2 receptor on the surface of alveolar epithelial type II cells, heart, kidney, intestine and endothelial cells make these cells

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and organs as a target for SARS-CoV-2 (1). The immune system perceives the emitted viral particles as foreign and presents them to natural killer and cytotoxic T cells. Thus, the immune system is activated by the production of high amounts of proinflammatory cytokines and chemokines. As a result of the activation of the immune system more than normal, cytokine storm develops, and multi-organ failure is seen with thrombotic tendency.

Appropriate animal models are needed to elucidate the pathogenesis of the disease and to develop effective vaccines or therapeutics (5). As stated in our previous study, mice are generally preferred in antiviral studies due to their protein similarities and simple genetic structures (6). However, while SARS-CoV-2 can use human, bat, and civet angiotensin-converting enzyme type 2 (ACE2) receptors for entry into target cells, classical experimental mice do not have these receptors. Therefore, human ACE2 (hACE2) receptor-encoding transgenic mice were preferred in this study. In our previous study, the protection and safety of the OZG-38.61.3 vaccine candidate against SARS-CoV-2 infection was demonstrated.

In this study, the effects of OZG-38.61.3 vaccine, which is a candidate for COVID-19 treatment, on intense inflammation and oxidative stress in SARS-CoV-2 infection, as mentioned above, were evaluated. In this study, it was tested how protective OZG-38.61.3 could be in controlling the damage caused by the virus after viral challenge.

2. METHODS

2.1. SARS-CoV-2 isolation-propagation and cell culture

Nasopharyngeal and oropharyngeal cavity samples were obtained from patients who were diagnosed as COVID-19 by Real-Time PCR in Acıbadem Altunizade Hospital, Acıbadem Mehmet Ali Aydınlar University Atakent, and Maslak Hospitals. The study design was approved by the Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (ATADEK-2020/05/41). All techniques had been executed according to the applicable guidelines. The SARS-CoV-2 isolation-propagation and cell culture was reported in our previous report (7)

2.2. Inactivation of SARS-CoV-2

2.2.1. Gamma irradiation and Glutaraldehyde mediated inactivation kinetics

In order to perform the gamma irradiation mediated inactivation kinetics study, 5 different dose ranges between 3-65 kGy were decided (3-7 kGy, 10-25 kGy, 18-35 kGy, 25-45 kGy, 45-65 kGy). After gamma irradiation inactivation at different doses were completed, colorimetric MTT assay was performed to evaluate the viability of Vero cells cocultured with vaccine for 72 hours. For glutaraldehyde inactivation, viruses were inactivated and fixed with 2.5% glutaraldehyde in PBS (0.1 M, pH 7.2) for 2.5 h.

2.2.2. Quantitative RT-PCR to determine viral RNA copy number

Total RNA isolations were performed from gamma and glutaraldehyde inactivated samples using Direct-zol RNA Miniprep Kits (Zymo Research, USA), and concentrations were determined using QUBIT fluorometer with the QUBIT RNA HS Assay (Thermo Fisher Scientific, USA). Quantitative RT-PCR was performed with the QuantiVirus SARS-CoV-2 Test Kit (Diacarta) according to the manufacturer's protocol. The quantitative RT-PCR analysis was analyzed in Roche Lightcycler 96.

2.2.3. Single immunodiffusion assay

This method was modified from single radial immunodiffusion assay (8). The protocol was described in our previous report (6).

2.2.4. Two-dimensional (2D) gel electrophoresis

SARS-CoV-2 (active virus), gamma and glutaraldehyde inactivated samples were concentrated with 3K ultrafiltration spin filters (Amicon Ultra Centrifugal Filters 3K Cat no UFC500396, Millipore) in the BSL-3 laboratory, and focusing buffer containing chaotropic agent (8 M urea, 2% (w/v) CHAPS, 50 mM DTT, % 0.2 3-10 ampholyte, 0.001% bromphenol blue) was added in equal volume (50µL) upon our recommendation. The added buffer inactivated all virus particles in the samples and made the protein content workable. 20 µg of each sample was subjected to 2D gel electrophoresis based on the Bradford measurement method. Prepared samples were soaked in 7 cm pH 3-10 NL IPG strips by passive rehydration method overnight. After rehydration, the proteins were focused at an increased voltage of 25,000V in total. After focusing, the strips were washed with Buffer I (6 M Urea, 0.375 M Tris.HCl pH 8.8, 2% SDS, 20% Glycerol, 2% (w/v) DTT) and II (6 M Urea, 0.375 M Tris.HCl pH 8.8, % 2 SDS, 20% Glycerol, 2.5% (w/v) Iodine Acetamide) for 15 minutes and separated in 12% keratin-free SDS-PAGE gels at 180V current. When the run were completed the gels were fixed and then the protein spots were made visible by silver staining, a sensitive protein staining technique.

2.2.5. Cytokine Bead Array (CBA) as an Immunogenicity Assay

Following the isolation of peripheral mononuclear cells (PBMC) by overlaying blood on FicollPaque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), 18-35 kGy, 25-45 kGy, 45-65 kGy gamma irradiated vaccine samples were incubated with PBMCs at 37°C for 24 hours. To analyze IFNγ level, MACSPLEX Cytokine 12 kit, human (Miltenyi Biotec) was used for the Cytokine bead array following the manufacturer's protocol. And then samples were collected into sample tubes, and flow analysis was done. Flow analysis was performed with the MACSQUANT Analyzer (Miltenyi Biotec).

2.3. Challenge Assay

2.3.1. OZG-38.61.3 inactivated SARS-CoV-2 vaccine production and Transgenic animal study

During virus replication, 90% confluent Vero cells in cell culture flasks with a larger surface area were gradually cultured with virus-containing supernatant. The virus was

concentrated by centrifugation in a special tube with a 100 KDa filter. The concentrated virus was stored at – 80 °C before irradiation. All concentrated viruses obtained are pooled and washed two times with distilled water for diafiltration in a 100 KDa concentrator. The concentrated virus mixture was decided to be inactivated by irradiation at 25 kGy because of kinetic study performed. Transgenic animal study was carried out with respect to the protocol reported in our previous study (6). Transgenic animal study was made by using nonlethal TCID50 dose to observe the protection and especially the effect of antibody dependent enhancement in viral challenge. TCID50 dose of SARS-CoV-2 were calculated and published in our previous study (6,9).

2.3.2. Histopathological Examination, Immunohistochemistry and TUNEL Assay

Lung, heart and kidney tissues were taken immediately after dissection and fixed in 10% and embedded in paraffin. Sections (5 µm thick) were stained with H&E and Masson's trichrome. Ashcroft scoring method was used for histopathological evaluations of pulmonary fibrosis. Immunohistochemical analyses were performed as described previously. The monoclonal antibodies used were Caspase-3, iNOS, IL-6, IL-10, TGF-β1, eNOS, TNF-α, Occludin and Claudin-1 (10,11).

To examine in situ cell apoptosis, the 5-µm thick sections of the lung, heart and kidney tissues were used for TUNEL staining. The TUNEL method was performed according to the manufacturer's (Millipore, 2869035) instructions (12).

2.4. Statistical Analysis

Normally distributed data in bar graphs was tested using student's t-tests for two independent means. The Mann–Whitney U test was employed for comparison between two groups of non-normally distributed data. Each data point represents an independent measurement. Bar plots report the mean and standard deviation of the mean.

3. RESULTS

3.1. The Effectiveness of Gamma Irradiation Inactivation Results

3.1.1. Gamma Irradiation Inactivation Kinetics

As a result of the analyzes made with the product obtained by 5 different dose range between 3-65 kGy of gamma irradiation, it was found that the safe inactivation range for SARS-CoV-2 started above >25 kGy (Supplementary Figure 1A), but when it exceeded 45 kGy, it has been observed that viruses cause a significant decrease in antigenic expression ability, T cell stimulation ability and viral RNA copy number (7). Therefore, it has been confirmed that the ideal dose range is 25 kGy in terms of inactivity and immunogenicity.

Changes in viral RNA copy number were compared between non-inactivated, gamma-inactivated and chemically inactivated viruses, and gamma irradiation inactivation was shown to preserve RNA copy number more effectively than chemical inactivation. It was determined that 7 times higher viral RNA copy number could be preserved with gamma irradiation (Supplementary Figure 1B).

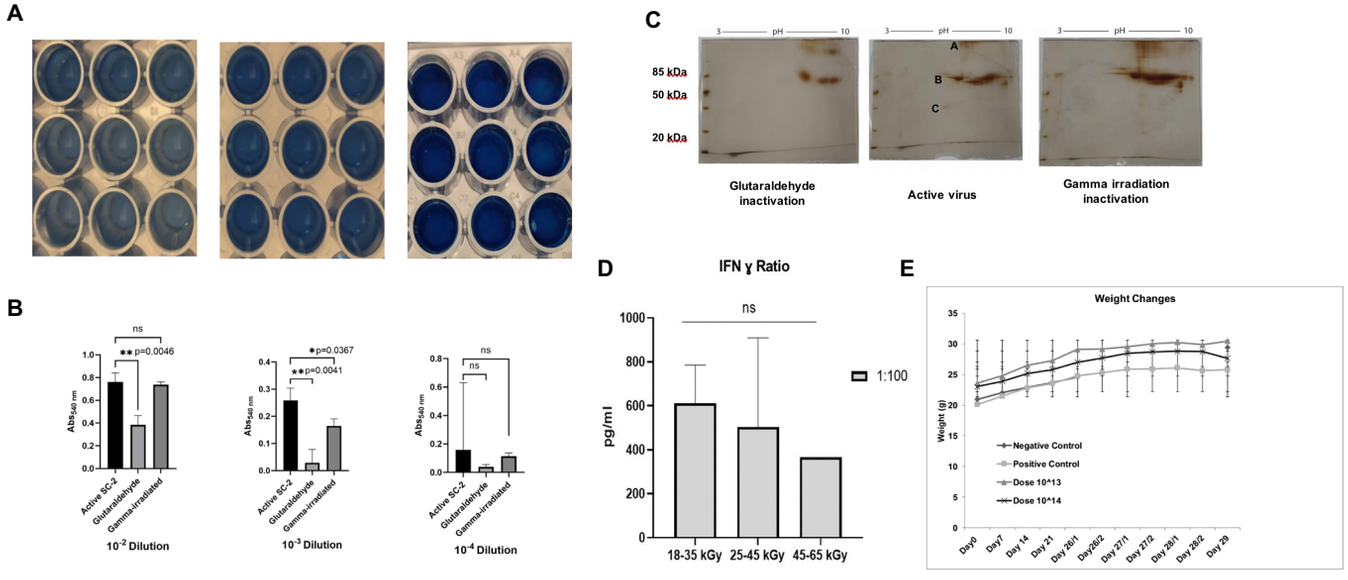

Figure 1. *Coomassie Brillant Blue staining (A), bar graph showing viral spike protein expressions results (B), Two-dimensional gel images of samples with active virus, glutaraldehyde and gamma radiation inactivated virus (A: S Protein Domain; B: N Protein Domain; C: M/RBD Protein Domain) (C), bar graph showing IFNγ ratio of T cells incubated with OZG-38.61.3 for 24 h (D), Body weight changes during challenge experiments (E). * p ≤ .05, ** p ≤ .01.*

3.1.3. The Effect of Gamma Irradiation on the Loss of Viral Proteins

3.1.3.1. Modified Single Immundiffusion Assay Results

After viral spike protein expression, the uptake of SARS-CoV-2 specific spike antibodies by virus particles was followed by the single immundiffusion assay (modified SRID) method. While it was observed that viral spike protein expressions were preserved in an amount similar to the active virus, it was observed that the antigenic expression was significantly decreased in glutaraldehyde-inactivated viral samples. Compared to inactivation with glutaraldehyde, gamma irradiation has been shown to preserve antigenic structure 6 times higher. Gamma-irradiated inactivate vaccine preparation is also a cost-effective approach (Figure 1A, B).

3.1.3.2. 2D Gel Electrophoresis Results

When inactivation by gamma irradiation is compared to inactivation with glutaraldehyde, it is observed that there is a significant loss of protein in inactivation with glutaraldehyde, while proteins are preserved in gamma irradiation. In the analyzes performed after lyophilization, viral proteins could not be clearly evaluated due to the inability to separate from albumin, but a structural change related to protein loss was observed (Figure 1C).

3.1.4. The Effect of Gamma Irradiation on the T cell Stimulation

When different dose of gamma irradiation was evaluated, it was observed that there were no significant changes observed in IFNγ ratios of T cell due to gamma irradiation (Figure 1D).

3.2. Challenge Assay Results

3.2.1. Weight changes

Weight monitoring was carried out at regular intervals. No significant difference was found in the parameter (Figure 1E).

3.2.2. Histopathological Examinations Results

3.2.2.1 H&E Staining

Histopathology analysis showed no significant pathological findings in the histological sections of the heart in all groups. However, few hemorrhagic regions were observed in the H&E-stained lung sections of the PC and vaccine groups. Also, it was observed that less hemorrhagic regions were seen in both vaccine doses compared to PC group. These findings suggested that OZG-38.61.3 vaccine protect the lung against the SARS-CoV-2 virus (Figure 2A-D). Histopathological lesions were not observed in the heart sections of control group. Cardiomyocytes with dense eosinophilic cytoplasm were observed in some areas in the Ch1, Ch2. and PC groups. In addition, hypertrophy was observed in some myocardial cells in the PC group. However, no significant difference was observed in the general myocardial architecture in all groups (Figure 2E-H). Kidney tissue morphology was found normal and intact in NC group consisting of the renal tubules and glomeruli. The dilated blood vessels and neutrophil infiltrations were detected in some regions of the PC group kidneys. However, no tubular or glomerular defects were found. Although dilated blood vessels were also seen in vaccinated Ch1 and Ch2 groups, they were much smaller and sparse compared to the NC group and no neutrophils were observed in these groups (Figure 2I-L).

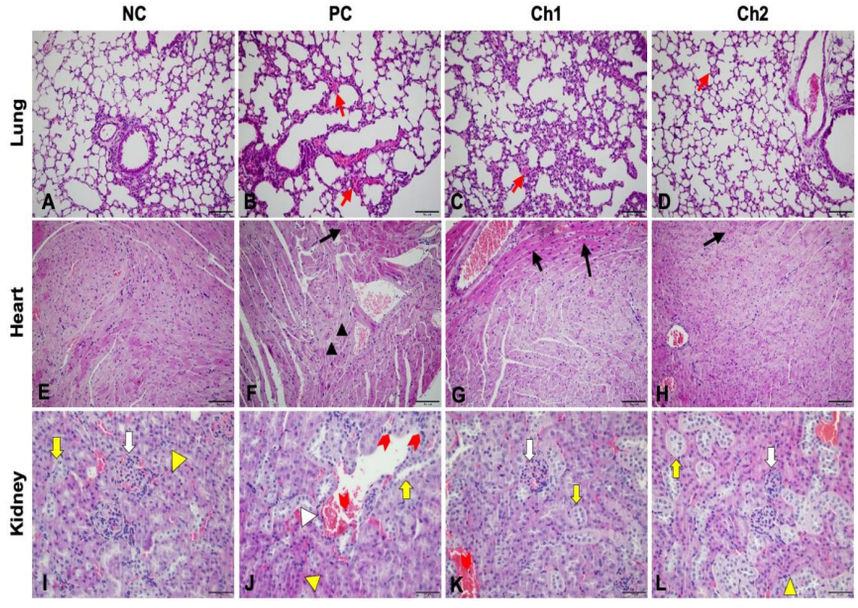

Figure 2. *H&E staining of lung, heart and kidney tissues. Red arrows: hemorrhage, Black arrows: cardiomyocytes with dense eosinophilic cytoplasm, Black arrowheads: hypertrophic cardiomyocytes, yellow arrow: distal tubules, Yellow arrowheads: proximal tubules, White arrow: glomeruli, White arrowheads: dilated blood vessel. Scale bar: A-H: 100 µm, I-L: 50 µm.*

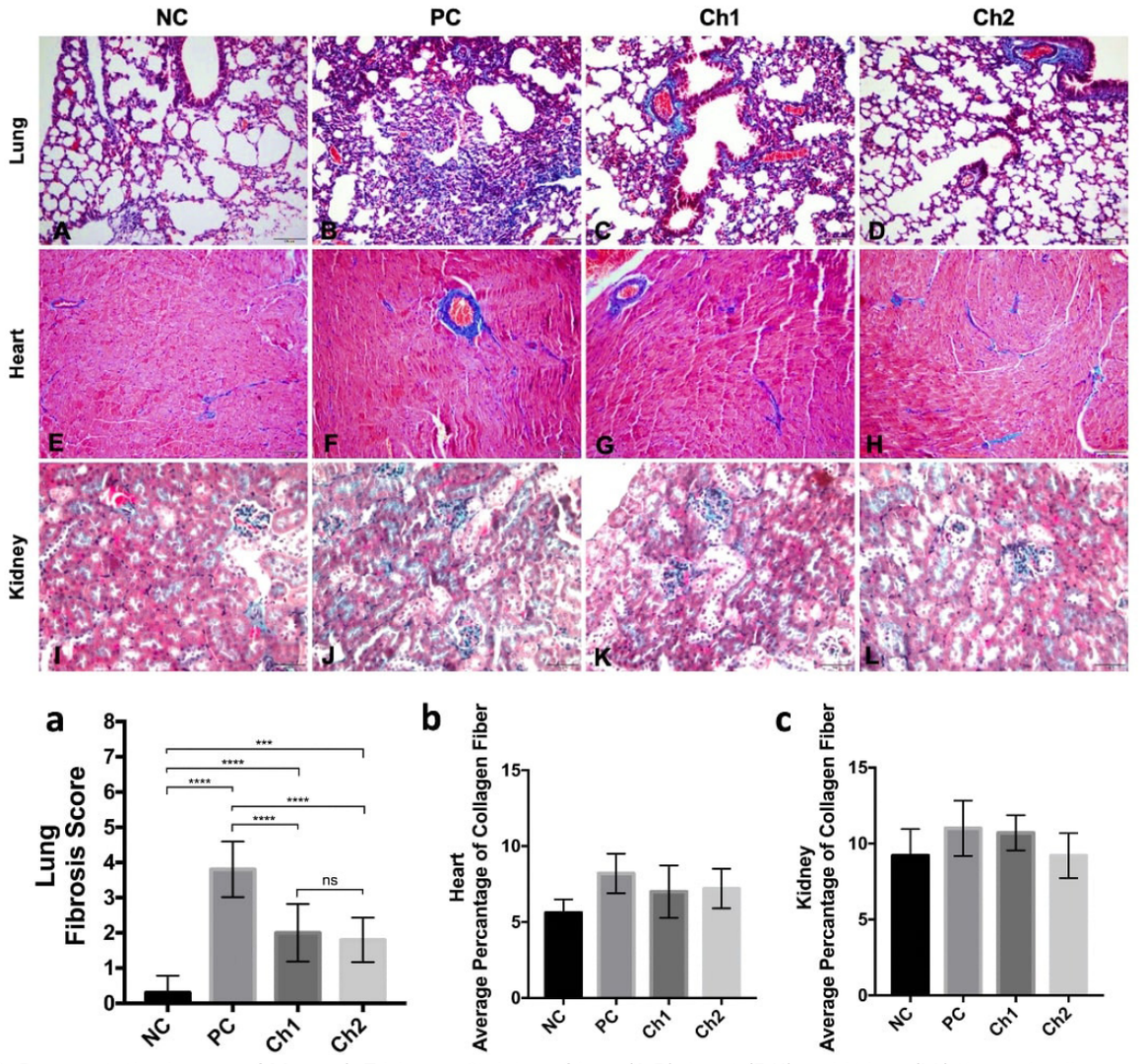

Figure 3. *Representative images of Masson's Trichrome Staining of lung (A-D), heart (E-H) and kidney (I-L) tissues in all groups. Statistical analysis graphs of lung fibrosis (a), average percentage of collagen fiber of heart (b) and kidney (c) tissues. ns: p>.05, *** and **** p<.05. Scale bar: A-H: 100 µm, I-L: 50 µm*

3.2.2.2 Masson's Trichrome Staining

The degree of pulmonary fibrosis was evaluated with ashcroft scoring method as described (13). The highest level of collagen fibers proliferation were observed in the PC group, although vaccine groups had fewer pulmonary fibrosis areas. On the other hand, no abnormal alveolar architecture was noted and a normal distribution of collagen fibers were seen in the NC group. Masson's trichrome staining was demonstrated a moderate thickening of the alveolar septa and increased deposition of collagen in lung tissues in the PC group. Minimal fibrous thickening of the alveolar walls without obvious damage to the lung architecture were observed in the Ch1 and Ch2 groups and the scores of fibrotic lesions were significantly lower in contrast to PC group. Nevertheless, fibrosis scores were similar between vaccine groups. $1x10^{13}$ and $1x10^{14}$ doses of OZG-38.61.3 were hindered the histopathological changes associated with the SARS-CoV-2 virus (Figure 3A-D, a).

Statistical comparison of collagen fibers area percentages in cardiac and renal tissues of mice revealed that no significant difference were found between the whole groups (Figure 3E-H, b for heart and Figure 3I-L, c for kidney, p>.05).

3.2.2.3. Immunohistochemistry and TUNEL Results

SARS-CoV-2 administration significantly increased the Caspase 3 and iNOS immunoreactivities compared to NC groups in the sections of lung, heart and kidney tissues. However, Caspase 3 immunoreactivity dramatically decreased in the $1x10^{13}$ and $1x10^{14}$ doses of OZG-38.61.3 vaccinated groups compared to PC groups in a dose dependent manner in all organs. Although, OZG-38.61.3 vaccination led to a statistically significant reduction in iNOS immunoreactivity in lung tissues, iNOS immunostaining intensities were found similar in vaccinated groups in the

heart and kidney tissues compared to PC groups. eNOS immunoreactivity was highest and statistically significant in the PC group of renal tissue however there was no significant difference observed in alveolar and cardiac tissues among all groups (data not shown). In addition to this, vaccine groups had lower eNOS immunoreactivity in the renal tissue in comparison with the PC group. IL-6 immunoreactivity was found highest in the PC groups, and it was statistically significant compared to NC groups in all organs. In the vaccinated groups, IL-6 immunostaining decreased compared to PC groups. Furthermore, OZG-38.61.3 vaccination reduced IL-6 to a similar level to the NC groups. TNF-α immunoreactivity was found similar in all groups of lung and heart tissues (data not shown). Moreover, TNF-α intensity of renal tissue in PC group was significantly higher than NC group. TNF-α intensity reduced with the vaccine and it was significant according to the NC and PC groups. There was no significance between vaccine doses. While IL-10 immunostaining intensity was significantly elevated by SARS-CoV-2 administration in all organs, OZG-38.61.3 vaccinated groups shown lower IL-10 intensity compared to PC groups. TGF-β1 immunoreactivity was found similar between all groups in cardiac and renal tissues. On the other hand, statistically significant increase of TGF-β1 immunoreactivity was observed in the PC group compared the NC group in the lung tissue. In addition, decreased TGF-β1 immunoreactivity was seen in the vaccinated groups compared to PC group, and vaccinated groups showed similar TGF-β1 intensity but still higher than the NC group. The occludin and claudin-1 expressions were similar in lung and heart tissues for all groups (data not shown). In kidney sections, the occludin and claudin-1 immunoreactivity decreased in PC and vaccinated groups, and it was statistically significant according to NC group. High dose OZG-38.61.3 vaccine administration rose the occludin expressions in kidney tissues and it was statistically significant according to PC group although there was no difference between low dose vaccine and PC groups. Claudin-1 intensity increased significantly in both vaccinated groups according to PC group (Figure 4-7, Supplementary Tables 1-3).

The TUNEL assay revealed that extensive amounts of TUNEL positive nuclei were observed in alveolar and vascular areas in lungs, tubular cells in kidneys and endothelial and surrounding cells in cardiac tissues in the SARS-CoV-2 infected groups compared to uninfected controls. In contrast to PC group, apoptotic indexes were found significantly decreased in the OZG-38.61.3 vaccinated groups in a dose dependent manner in all organs. However, there was no significant difference was observed between Ch1 and Ch2 groups in the heart tissue (Figure 9).

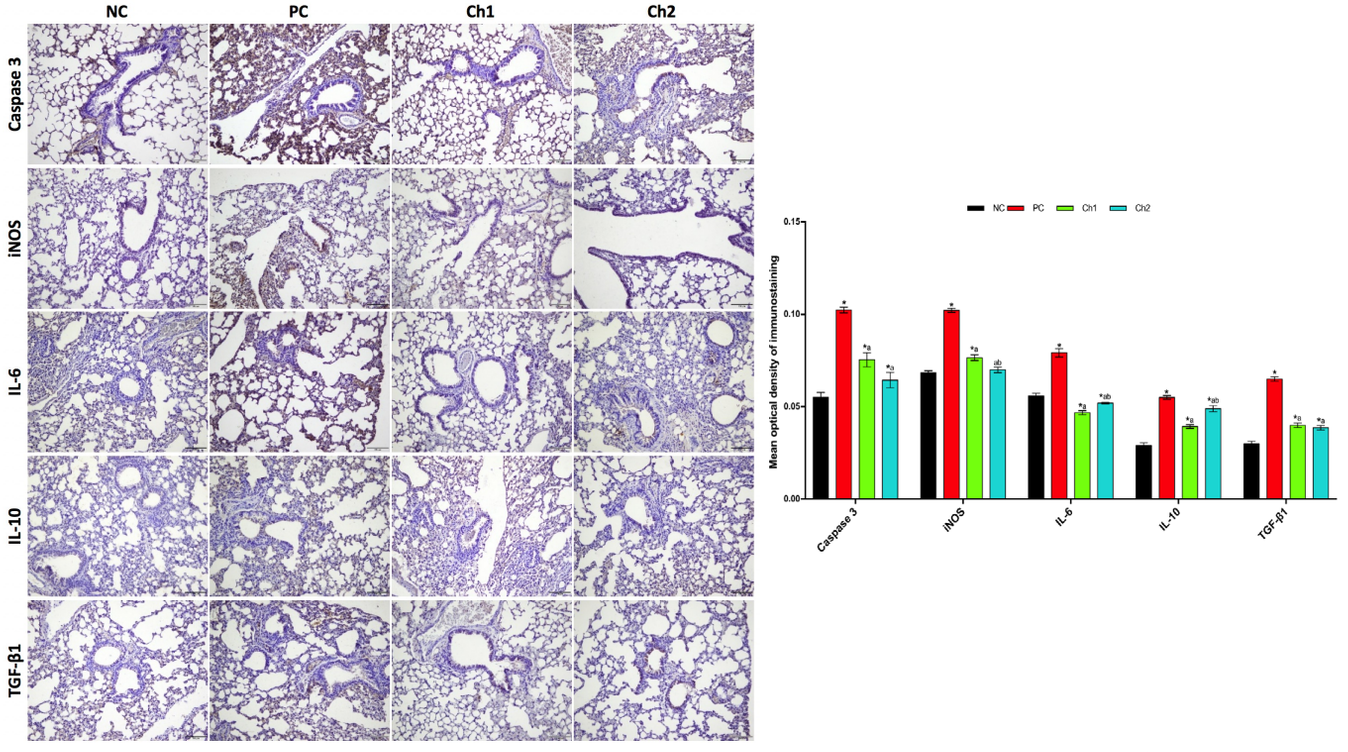

Figure 4. *Immunohistochemical analysis of Caspase-3, iNOS, IL-6, IL-10 and TGF-β in the lung tissues. Scale bar: 100μm (100X magnification). Graphs illustrating the optical density of protein expressions in the lung tissues of all groups. *: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05.*

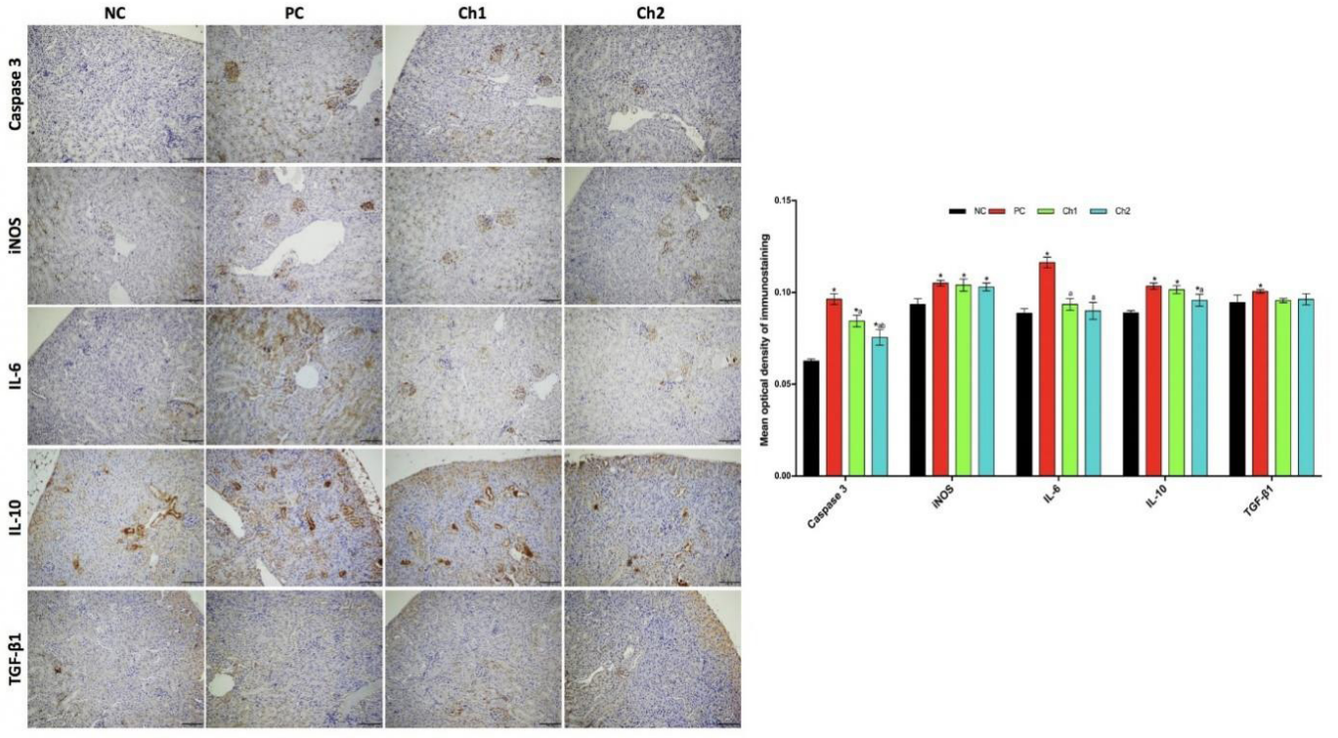

Figure 5. *Immunohistochemical analysis of Caspase-3, iNOS, IL-6, IL-10 and TGF-β in the heart tissues. Scale bar: 100µm (100X magnification). Graphs illustrating the optical density of protein expressions in the heart tissues of all groups. *: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05.*

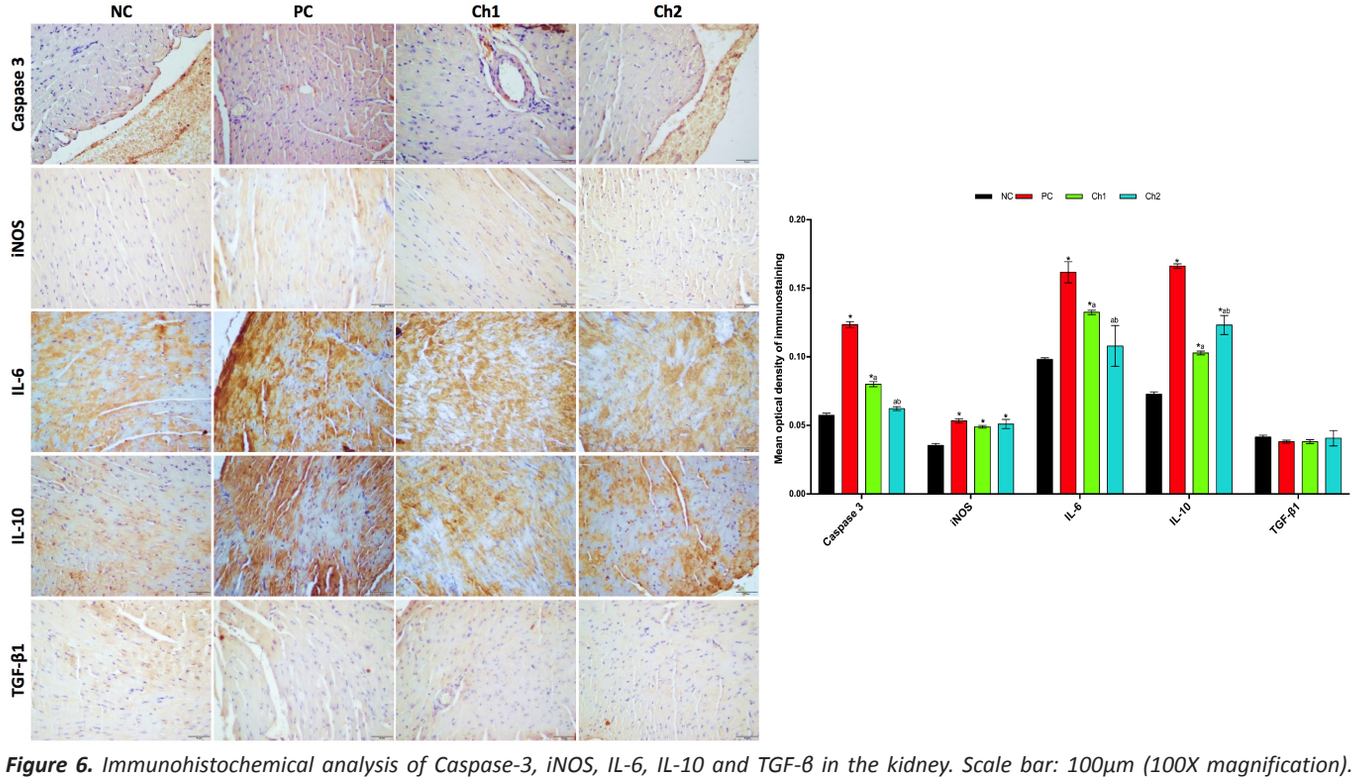

Figure 6. *Immunohistochemical analysis of Caspase-3, iNOS, IL-6, IL-10 and TGF-β in the kidney. Scale bar: 100µm (100X magnification). Graphs illustrating the optical density of protein expressions in the kidney tissues of all groups. *: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05.*

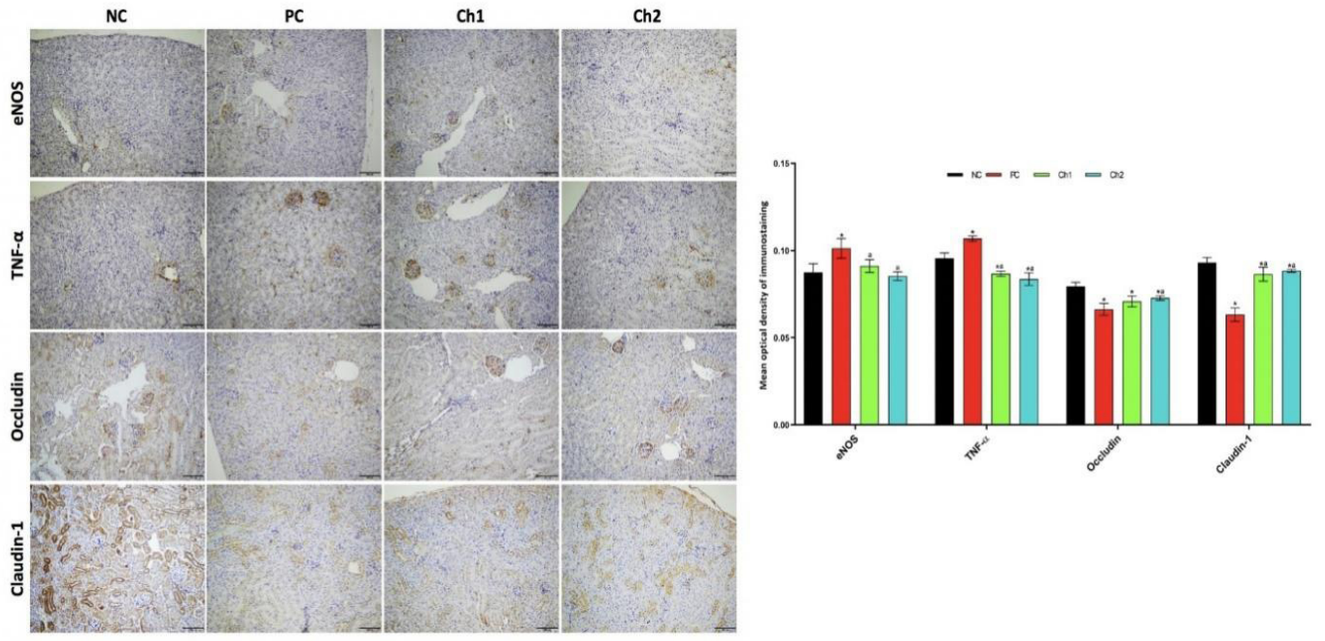

Figure 7. *Immunohistochemical analysis of eNOS, TNF-α, Occludin and Claudin-1 in the kidneys. Scale bar: 100µm (100X magnification). Graphs illustrating the optical density of protein expressions in the kidneys tissues of all groups. *: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05.*

4. DISCUSSION

Several vaccines against SARS-CoV-2 are currently in different stages of pre-clinical or clinical evaluation. In this study, in vivo safety and efficacy analyzes of OZG-38.61.3 lyophilized vaccine candidate, inactivated by gamma-irradiation (25 kGy, determined by kinetics study) were performed. According to comparative analysis carried out by our group, gamma irradiation inactivation was shown to preserve RNA copy number more effectively than chemical inactivation, where 7 times higher viral RNA copy number could be preserved with gamma irradiation. It was also observed that the antigenic expression was significantly decreased in chemically inactivated viral samples, such that gamma irradiation has been shown to preserve antigenic structure 6 times higher. It was seen that there was a significant loss of protein after chemical inactivation, while proteins are preserved in gamma irradiation inactivation. As our results, it has been reported that gamma irradiation method does not cause damage to viral proteins while causing genetic material damage, and therefore it is equivalent to 9 times more antigen gain. These studies also have shown that while chemical inactivation produces only B-cell response, both B-cell and T-cell immunity are induced equally by gamma irradiation (14).

In this viral challenge study, non-lethal doses of SAR-CoV-2 were used for infection. Because, in the past, antibody dependent enhancement (ADE) caused a clinical problem, especially in dengue fever vaccine studies. This ADE effects related to vaccine could not be distinguished in the challenge experiments performed with lethal dose of these studies. Therefore, this study was designed especially considering the ADE effect in addition to observe the ability of infection control.

To determine the antiviral activity and protective effects of the OZG-38.61.3 against SARS-COV-2 in K18-hACE2 mice, microscopic examination was performed for histopathological changes and also immunohistochemistry was conducted to evaluate the expression of inflammatory cytokines, apoptosis, oxidative stress and cell junction markers in the lung, heart and kidney tissues. Our main findings from the SARS-CoV-2-infected lung tissues of hACE2 mice showed hemorrhagic regions, pulmonary fibrosis and moderate thickening of alveolar septa. The OZG-38.61.3 vaccine prevented these defective effects of the virus but still few hemorrhagic regions and rare septal thickening were detected in the vaccinated lung tissues. The virus-infected heart parenchyma showed cardiomyocytes with dense eosinophilic cytoplasm and hypertrophic cell morphology, which reduced with the vaccine application. Dilated blood vessels and the inflammatory cell infiltration were found in kidney tissues of the infected group. The vaccine ameliorated the renal parenchyma, there were no infiltrating cells. Although a previous study found that COVID-19 increased interstitial fibrosis in the postmortem kidney (15), the collagen fiber deposit was affected by neither the virus infection nor vaccine administration in hearts and kidneys of our animal model. Milross et al (16) reported a lot of lung damages consisting of exudative diffuse alveolar disease

featuring prominent hyaline membranes with mild interstitial infiltration by mononuclear cells, alveolar wall congestion, and cellular debris, platelet-rich thrombus causing expansion of an intra-acinar vessel in post-mortem lung biopsies of COVID-19 patients. Shang et al (17) showed necrosis and abscission in bronchiolar endothelial cells, granulocyte infiltration and thickening alveolar septa in SARS-CoV-2-infected lung tissues of hACE2 transgenic mice. Diamond et al (18) detected focal alveolar hemorrhage and immune cell infiltration in SARS-CoV-2-infected Syrian hamsters. Hartman et al (19) reported the severe pericellular interstitial edema around cardiomyocytes and no inflammatory cells in the postmortem heart biopsies of the COVID-19 patients. A similar study was carried out by Pessolani et al (20) and virus-related damage in different organs was examined. They found the necrotic myocytes in myocardium, glomerular capillary microthrombus, tubular dilatation and vacuolisation in kidney tissues of autopsy cases.

Claudin-1 and occludin are tight junction proteins found between epithelial cells. In many studies in the literature, it has been reported that tight junction proteins are disrupted, and their expression decreased after COVID-19 infection (21-23). In particular, it has been reported that the envelope proteins of SARS-CoV-2 disrupt tight junction complexes in cells (21). SARS-CoV-2, by causing tight junction proteins to be damaged, disrupts the barrier function of the respiratory epithelium, thus increasing the spread of the virus rapidly and increasing the severity of the disease (22). In this study, decreased claudin-1 and occludin expressions in kidney tissue were observed in the PC group. There was a significant increase in tight junction protein's expression in the vaccinated groups compared to the PC group. To our knowledge, this is the first study to show the damage of claudin-1 and occludin proteins in kidney tissue after SARS-CoV-2 infection. Herrero et al (24) showed that disruption of the alveleor epithelial barrier as a result of the deterioration of tight junctions causes histopathological findings in the alveoli. Claudin-1 and occludin expressions in kidney tissue were decreased in PC group however, their expressions were increased in the OZG-38.61.3 vaccination groups. These results suggest that the vaccine reduces virus damage.

The specific mechanisms of SARS-CoV-2 infection leading to cell death in different tissues and organs are not fully known. In this study, increased caspase-3 expression and tunnel positive cells were observed in the SARS-CoV-2 positive groups in kidney, lung and heart tissues compared to the control groups. After vaccination with OZG-38.61.3, there was a significant reduction in apoptosis in all tissue types. Increased apoptosis has been reported in postmortem myocardial biopsies of COVID-19 patients. In the study, it is reported that there are very high TUNEL positive cells, especially in endothelial cells (19). Tavazzi et al (25) reported that oxidative stress, hypoxia, intrarenal inflammatory response and renal cell apoptosis are the main mechanisms of sepsis-associated kidney injury. Li et al (26) reported that by providing caspase-8 activation in lung epithelial cells of transgenic HFH4-hACE2 mice infected with SARS-CoV-2, it triggered the inflammatory cytokine storm with intrinsic and extrinsic apoptosis pathways. Initially, virus-induced apoptosis is thought to be beneficial for the host cell in restricting viral replication. However, hyperactivation of these pathways causes serious tissue and organ damage as seen in our study and in the literature.

After SARS-CoV-2 enters the body and replicates rapidly, a rapid cytokine release triggers inflammation. Thus, it has been reported that the main cause of tissue damage is increased inflammation (27,28). Lindner et al (29) reported that severe damage to the myocardium was associated with high TNF-α, IFN, IL-1B, IL-6 levels in the autopsy performed on patients who died as a result of SARS-CoV-2 infection. It is reported that one of the most important triggers of these inflammatory cytokines is NF-κB (27). NF-κB inhibition has been reported to decrease proinflammatory cytokine expressions and increase survival rate in SARS-CoV infected mice (30). In our study, increased TNF-α expression was observed in the PC group. However, a significant decrease in TNF-α levels was observed after the administered OZG-38.61.3 vaccine. This shows that the vaccine candidate is highly effective in eliminating SARS-CoV-2 virus-induced inflammation.

Several studies have suggested that SARS-CoV-2 has an important function in the pathogenesis of the disease by increasing the excessive production of ROS in the cells (31). In our study, iNOS and eNOS, which are oxidative stress markers, showed a significant increase in the PC group compared to the control group. However, although there was a certain decrease in the vaccine group, it was not statistically significant. The role of oxidative stress triggered by COVID-19 infection in the pathogenesis of the disease is still not fully known. On the other hand, oxidative stress induces overactivation of the immune response of infected cells affected by SARS-CoV-2 replication (32). In particular, proinflammatory cytokines IL-1β, IL-6, TNF-α, IL-8 secreted from monocytes and macrophages rapidly trigger an increase in oxidative stress in other cells (33). As shown in our study, it is thought that the increased oxidative stress in the lung, kidney and heart tissues may be caused by the increase in IL-6, TNF-α, IL-10. It has been reported that in addition to immune cells, mast cell activation is also increased after viral infections, and TNF-α released from mast cells plays a role in increasing local inflammation (18).

The Arg-Gly-Asp (RGD)-binding sites required for SARS-CoV-2 to bind to the host cell surface trigger TGF-β activation in the cells and this provides a severe course of the disease (34). TGF-β provides the organization of the extracellular matrix by controlling the fibroblast-myofibroblast transformation. There is evidence that TGF-β is closely related to the fibrogenic process (35). In our study, increased TGF-β expression and increased fibrosis in the lung tissues in the PC group support this situation. At the same time, the increase in TGF-β provides a rapid increase in proinflammatory cytokines, as in our study. The decrease in TGF-β expressions of the vaccine candidate in our study caused a decrease in morphologically fibrotic areas in the lung tissue. Several animal models have

shown that the TGF-β/Smad signaling pathway plays an important role in renal fibrosis (36). In our study, increased TGF-β expression was observed in the PC group kidney tissue compared to the NC group. Although a slight decrease was observed in the vaccinated groups, it was not found to be statistically significant.

5. CONCLUSION

In this study, it was shown that SARS-CoV-2 infection can cause multi-organ involvement other than the lung, and it was determined that the OZG-38.61 vaccine candidate could clearly control the organ dysfunction caused by SARS-CoV-2 infection. And it was observed that there was an infection control with no ADE effect.

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Ethics Committee Approval: *All animal experiments were approved by the Experimental Animal Committee of Acıbadem Mehmet Ali Aydınlar University (ACUHADYEK 2020/36) and Manisa Celal Bayar University (2020/18). The animal research in our study adheres to the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines).*

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Author Contributions:

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Design of the study: MO, HO, DDK, CT, DT, EO

Acquisition of data for the study: DDK, CT, BY, DC, SA, MK, GA, MO, HO, BSH

Analysis of data for the study: DDK, CT, BY, DC, SA, GT, SD, DT, MK, GA, MO, HO, BSH

Interpretation of data for the study: MO, HO, DDK, CT, SA, DT, EO

Drafting the manuscript: MO, HO, DDK

Revising it critically for important intellectual content: EO

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Supplementary Table 1. *Lung tissue optical density of immunostaining (mean ± standard deviation)*

Lung	Negative Control	Positive Control	Challenge 1	Challenge 2
Caspase 3	0.055±0.003	0.102±0.002*	0.080±0.002*[a]	0.062±0.001*[a]
iNOS	0.068±0.001	0.102±0.001*	0.077±0.002*[a]	0.070±0.001[ab]
IL-6	0.056±0.002	0.079±0.002*	0.047±0.001*[a]	0.052±0.000*[ab]
IL-10	0.029±0.001	0.057±0.001*	0.039±0.001*[a]	0.046±0.002*[ab]
TGF-β1	0.030±0.001	0.065±0.001*	0.040±0.001*[a]	0.039±0.001*[a]

**: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05*

Supplementary Table 2. *Heart tissue optical density of immunostaining (mean ± standard deviation)*

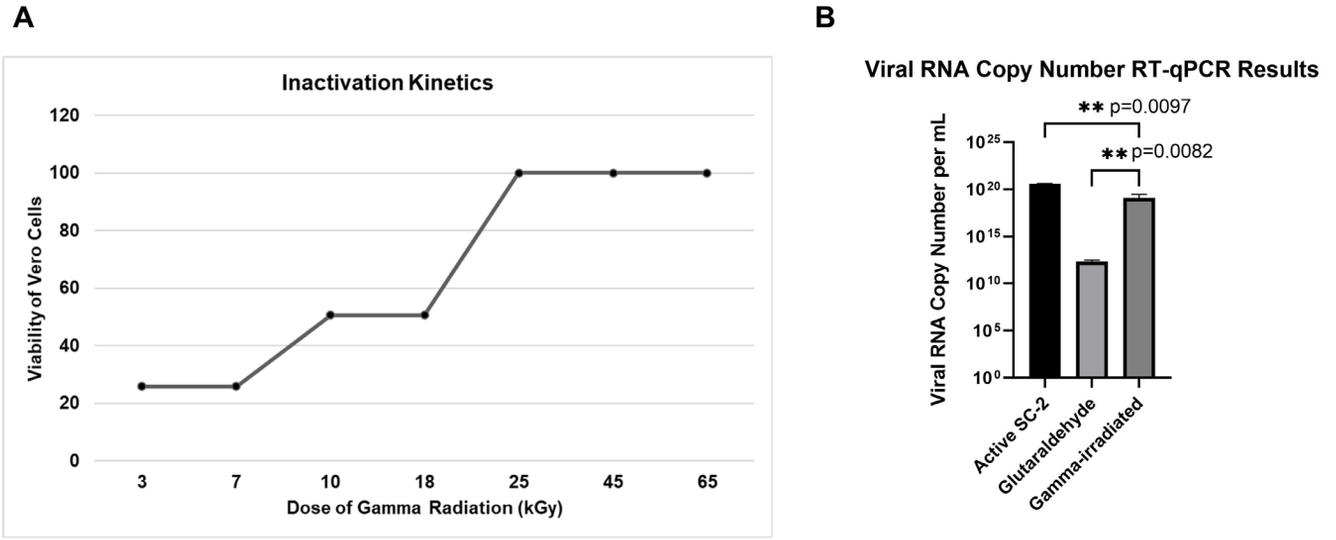
Heart	Negative Control	Positive Control	Challenge 1	Challenge 2
Caspase 3	0.057±0.002	0.124±0.002*	0.078±0.002*[a]	0.062±0.001[ab]
iNOS	0.036±0.001	0.053±0.002*	0.049±0.001*	0.051±0.003*
IL-6	0.098±0.001	0.162±0.008*	0.132±0.002*[a]	0.108±0.015 [ab]
IL-10	0.073±0.002	0.166±0.002*	0.108±0.001*[a]	0.123±0.007*[ab]
TGF-β1	0.043±0.001	0.038±0.001	0.038±0.001	0.041±0.006

**: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05*

Supplementary Table 3. *Kidney tissue optical density of immunostaining (mean ± standard deviation)*

Kidney	Negative Control	Positive Control	Challenge 1	Challenge 2
Caspase 3	0.063 ± 0.001	0.096 ± 0.002*	0.084 ± 0.003*[a]	0.076 ± 0.004*[ab]
iNOS	0.091 ± 0.003	0.105 ± 0.002*	0.104 ± 0.003*	0.103 ± 0.002*
IL-6	0.089 ± 0.002	0.116 ± 0.011*	0.094 ± 0.003[a]	0.090 ± 0.004[a]
IL-10	0.089 ± 0.001	0.103 ± 0.001*	0.102 ± 0.002*	0.096 ± 0.003*[a]
TGF-β1	0.094 ± 0.003	0.101 ± 0.002*	0.096 ± 0.001	0.096 ± 0.002
eNOS	0.087 ± 0.004	0.101 ± 0.002*	0.091 ± 0.003[a]	0.085 ± 0.002[a]
TNF-α	0.095 ± 0.003	0.107 ± 0.010*	0.087 ± 0.001*[a]	0.084 ± 0.003*[a]
Occludin	0.079 ± 0.002	0.066 ± 0.004*	0.071 ± 0.003*	0.073 ± 0.001*[a]
Claudin-1	0.093 ± 0.002	0.063 ± 0.011*	0.086 ± 0.003*[a]	0.088 ± 0.001*[a]

**: Comparison with negative control group * p <.05. a: Comparison with positive control group, a = p <.05; b: Comparison with Challenge 1 group, b = p <.05*



Supplementary Figure *1. Inactivation kinetics of gamma irradiation (A), bar graph showing quantitative RT-PCR results of SARS-CoV-2 viral RNA copy number in log scale (B). ** p <.01.*

Clinical and Experimental Health Sciences

Rational Drug Use and E-Health Literacy Levels of Inpatients of Internal Medicine Services

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ABSTRACT

Objective: Health professionals can provide opportunities to increase the level of e-health literacy by accessing accurate health information on the internet to improve the attitudes and behaviors of rational drug use in the disease management of individuals with chronic diseases. This study aimed to determine the rational drug use and e-health literacy levels of individuals with chronic diseases.

Methods: This descriptive and cross-sectional study was conducted at a training and research hospital of a university in Turkey between March and July 2023. Hospitalized in internal medicine services and with at least one chronic disease were included 381 patients in the study. The study data were collected using the Introductory Information Form, and Rational Drug Use Scale, and E-Health Literacy Scale.

Results: The average age of the patients was 53.44±16.48 years. Of these patients, 60.1% were women and 19.9% had a diagnosis of endocrine system disease. While the average score of the patients on rational drug use was moderate. their e-health literacy levels were found to be low. A weak levels positive correlation was detected between rational drug use and e-health literacy (r=.106. p=.03).

Conclusion: It was concluded that rational drug use among individuals with chronic diseases is moderate and e-health literacy is low. Improving rational drug use and e-health literacy in chronic disease management are important strategies for reducing health expenditures and patient care burden.

Keywords: Rational drug use, health literacy, chronic diseases.

1. INTRODUCTION

Chronic diseases represent a significant social health issue, contributing to mortality and morbidity at global and national levels, reducing life expectancy, altering quality of life, and escalating the financial burden on healthcare services. The growth in the elderly population across the globe, coupled with the rise in stressors associated with urbanisation, sedentary lifestyles and unhealthy habits such as malnourishment– which are a consequence of the technological age – has led to an increase in the prevalence and incidence of chronic diseases (1,2). It is responsible for 41 million deaths worldwide each year, representing 74% of all global deaths. The majority (86%) of those who died before the age of 70 resided in low – and middle-income countries The World Health Organization (WHO) has reported in its non-communicable diseases monitoring report that the mortality rate in Turkey is 86% and that the probability of dying from a non-communicable disease before the age of 70 is 18% (2).

The prevalence of chronic diseases has been linked to an increased frequency of hospitalisation in healthcare institutions and an elevated risk of organ failure. It is estimated that by 2030, 21 trillion dollars of global health expenditure will be spent on the treatment of chronic diseases (3). The unplanned expenditure of budgetary allocations to health services presents a significant challenge to accessibility, particularly in the context of essential medications. This can lead to the emergence of drug resistance, which in turn increases the economic and social burden associated with healthcare services. In this context, the rational use of drugs in chronic diseases increases patient compliance with treatment, reduces drug side effects, prevents drug resistance and reduces healthcare expenditure (4,5). WHO estimates suggest that approximately half of patients worldwide do not use medications correctly, and approximately one-third of the global population lacks access to essential medications (6). These data are corroborated by evidence indicating that, in the context of self-management of chronic diseases, medical treatment preferences without consulting a healthcare professional have been a significant

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factor in shaping health behaviours Consequently, it is crucial that individuals who obtain their health data from digital sources possess the skills to ensure safe internet usage and to interpret health information accurately (7-9).

Rational drug use (RDU) is of vital importance to the success of both diagnosis and treatment of diseases (10). An individual's drug use habits are significantly influenced by a range of sociodemographic characteristics, as well as their level of health literacy, which ensures the appropriate use of the drug in question. E-health literacy (E-HL) has emerged as a consequence of the necessity to conduct research into health-related information whilst utilising technology (10). In this context, the simultaneous measurement and evaluation of RDU and E-HL levels is of great importance for public health. In their study, Tosun and Hoşgör reported a positive correlation between internet usage levels and e-health literacy (E-HL) levels, with the former increasing as the latter does (10). Those with sufficient E-HL are able to access health services with greater ease and security, manage their health conditions more effectively, and develop healthier lifestyle behaviours. In the existing literature, it has been asserted that E-HL fosters the development of health-promoting behaviours in individuals with diabetes and has a positive impact on health outcomes, including health-related quality of life. (7,11) Nurses play a pivotal role in fostering the development of RDU and E-HL levels, thereby facilitating the transformation of individuals' health behaviours through their educational roles (11). A study conducted in India reported that nurses increased drug knowledge and Health Literacy (HL) level, and reduced drug waste and side effects with a mobile application used to educate the public about RDU (12). The dissemination of information to patients about their diseases constitutes a vital component of E-HL and is also effective in improving health management (11). In this regard, it is important to determine the RDU and E-HL levels of individuals with chronic diseases and to develop strategies accordingly. The objective of this study was to determine the RDU and E-HL levels of individuals with chronic diseases.

Questions of this research:

- What is the RDU level of patients hospitalized in internal medicine clinics?
- What is the E-HL level of patients hospitalized in internal medicine clinics?
- Do the RDU and E-HL levels of patients hospitalized in internal medicine clinics differ according to socio-demographic variables?
- Is there a relationship between the RDU and E-HL levels of patients hospitalized in internal medicine clinics?

2. METHODS

2.1. Study Design

The research was conducted in a descriptive manner with the objective of determining the RDU and E-HL levels of individuals with chronic diseases. The study was carried out between March 2023 and July 2023 with patients receiving inpatient treatment in the internal medicine services (internal medicine, neurology, cardiology, chest diseases, infectious diseases, physical therapy, and rehabilitation) of a state university training and research hospital.

2.2. Participants

The study population comprised 6,600 patients admitted to internal medicine clinics between January 2022 and January 2023. The study sample size is as follows: A sample size of 364 patients was calculated using the power analysis method, with a 5% deviation and a 95% confidence interval (https://www.openepi.com). In consideration of the potential for data loss during the course of the study, the sample size was augmented by 5%. A total of 400 patients were reached in the study. However, a total of 19 patients who developed acute problems (such as nausea-vomiting, pain, hypotension) (n=8), whose general condition worsened (n=6), and who did not complete the data collection forms (5) were not included in the study. Consequently, the final sample consisted of 381 patients who met the inclusion criteria for the study. In order to be included in the study, participants had to meet the following criteria: (i) at least 18 years of age, (İİ) literate, (iii) receiving inpatient treatment in internal medicine services. (iv) have at least one chronic disease, and (v) willing to participate in the study. In addition, the following exclusion criteria were applied: (i) communication difficulties, (ii) hearing and vision problems.

2.3. Assessment Tools and Data Collection

The data for the study were collected through the implementation of face-to-face interviews. Each patient who met the inclusion criteria and was hospitalized in the internal medicine services was interviewed separately by the researcher. Prior to commencing the interviews, the researcher provided a detailed explanation of the purpose and rationale for the study, and obtained informed consent from each participant. The research data were collected using the Introductory Information Form, the Rational Drug Use Scale and the E-Health Literacy Scale.

The researchers created the Introductory Information Form by scanning the literature (4,7-11). The form inquired about the patients' sociodemographic characteristics, including age, gender, marital status, presence of children, educational status, place of residence, and employment status. It also explored their disease characteristics, such as the diagnosis of the disease and the presence of another chronic disease. Additionally, the form addressed patients' attitudes regarding drug use, including regular drug use, non-prescription drug use, and the importance of accessing health resources online.

The Rational Drug Use Scale (RDUS) is a tool designed to assess an individual's rationality in their drug use. The scale was developed by Demirtaş et al. (13). The scale comprises a single dimension and a total of 21 items, which are used to ascertain the knowledge levels of individuals in relation to

RDUS. As the scores obtained from the scale increase, so too does the level of knowledge on rational drug use. The cut-off point for the scale was determined to be 34 points. A score of 35 points or above is indicative of knowledge of rational drug use. The Cronbach's alpha value of the RDUS scale is .79, which was calculated as .70 in this study.

The E-Health Literacy Scale (E-HLS): The scale was developed by Norman and Skinner with the objective of determining individuals' skills in utilising health-related information technologies and the interaction between e-health programmes and individuals (14). A Turkish validity and reliability study of the scale in adults over the age of 45 was conducted in 2021 by Uskun et al. (15). The scale comprises a total of eight items, which are evaluated on a five-point Likert scale. The lowest possible score is 8, while the highest is 40. A high score indicates a high level of e-health literacy. The Cronbach's alpha coefficient for the scale is .97. In this study, the Cronbach's alpha coefficient for the scale was found to be .95.

2.4. Ethical Considerations

To conduct the research, approval was obtained from the Non-Interventional Clinical Research Ethics Committee of the İzmir Bakırcay University (Decision number/date: 927/2023). This study was conducted following the ethical standards outlined in the Declaration of Helsinki, and all study participants provided their informed consent. Permission for the use of the scales was obtained from the authors via e-mail.

2.5. Data Analysis

Descriptive and comparative statistical analyses, together with correlation analyses, were employed for the purpose of evaluating the data obtained from the study. The data were analysed using the IBM SPSS Statistics 26 software package. The statistical significance level was set at $p<.05$. The suitability of the measurement tools for normal distribution was evaluated using the Kolmogorov-Smirnov test. As the data did not show normal distribution, Mann Whitney U and Kruskal-Wallis tests and posthoc tests (Dunn test (Bonferroni test) were employed. Spearman correlation analysis was used to examine the correlation between the data.

3.RESULTS

3.1. Descriptive characteristics of participants on sociodemographic, and disease, and drug use

The mean age of the participants in the study was 53.44±16.48 years. 52.2% were 53 years and older. 60.1% of participants were female. 37.5% had completed primary school. 52.8% reported living in the city centre and 69.8% reported not working. When chronic disease diagnoses were examined, in order of frequency, 19.9% had diseases of the endocrine system and 16.8% had diseases of the cardiovascular system, and 70.3% had no other chronic disease. In terms of medication use characteristics, 85% were taking their medication regularly. 55.4% said they did not use over-the-counter medicines. 46.7% thought the internet was 'not very' useful for making health decisions. 40.7% said the Internet was 'not very' important in accessing health resources (Table 1).

Table 1. *Distribution of descriptive and clinical characteristics of participants (n=381)*

Variables	n	%
Age group (year)		
53 years and under	182	47.8
53 years and above	199	52.2
Gender		
Woman	229	60.1
Male	152	39.9
Educational status		
Literate	35	9.2
Primary education	143	37.5
High school	109	28.6
Associate degree	33	8.7
Bachelor's degree and above	61	16.0
Living place		
Village and district	180	47.2
Province	201	52.8
Working status		
Working	115	30.2
Not working	266	69.8
Chronic disease diagnosis		
Endocrine system diseases	76	19.9
CVS system diseases*	64	16.8
Nervous system diseases	54	14.2
GI system diseases**	50	13.1
Urinary system diseases	41	10.8
Respiratory system diseases	31	8.1
Joint connective tissue diseases	18	4.7
Hematological diseases	22	5.8
Cancer	22	5.8
Other	3	0.8
Presence of another chronic disease		
Yes	113	29.6
No	268	70.3
Using medication regularly		
Yes	324	85.0
No	57	15.0
Using non-prescription medication		
Yes	170	44.6
No	211	55.4
Benefits of the internet in health decisions		
None	122	32.0
Little	178	46.7
A lot	81	21.3
The importance of the internet in accessing health resources		
None	107	28.1
Little	155	40.7
A lot	119	31.2

**CVS: Cardiovascular; **GI: Gastrointestinal*

3.2. The findings regarding the participants' average scores on the RDUS and E-HLS, and the relationship between them, are as follows:

The average RDUS scale scores of the participants included in the study were 35.75 ± 4.97. The mean score for the E-HLS was found to be low at 22.96±8.42. In contrast, the mean score for the RDUS was 35 points or above for 64.6% of the participants (Table 2).

The results of the analysis indicated a very weak levels weak positive correlation between the participants' average scores on the RDUS and the E-HLS (r= .10; p=.039).

Table 2. *Distribution of participants' RDUS and E-HLS mean scores (n=381)*

Scales	X±SD	Min-Max
Rational Drug Use Scale	35.75±4.97	14.00-42.00
E-Health Literacy Scale	22.96±8.42	8.00-40.00
	n	%
Rational drug use situation		
Sufficient (<35 points)	135	35.4
Insufficient (>35 points)	246	64.6

3.3. The following findings are presented regarding the participants' average scores on the RDUS and E-HLS

No statistically significant difference was found between the mean scores of the RDUS according to the variables related to the participants' age groups, gender, marital status, place of residence, employment status, regular medication use, and the importance of the internet in health decisions (p>.05). A comparison was made between those with a bachelor's degree or above and those who do not use over-the-counter drugs, with respect to their educational status. Those who indicated that accessing health resources online was of "little" or "very" importance exhibited higher RDUS mean scores than those who rated it as "not at all" important (p<.05).

It was determined that the participants included in the study who were 53 years old or younger, had undergraduate or graduate degrees, and reported that the internet was important in making health decisions and that the internet was very important in accessing health resources had higher E-HLS mean scores (p<.05). There was no statistically significant difference between the mean E-HLS scores of the participants according to their gender, marital status, place of residence, employment status, regular medication use, and non-prescription medication use (p>.05) (Table 3).

Table 3. *Distribution of RDUS and E-HLS mean scores according to some descriptive characteristics and drug use characteristics of the participants (n=381)*

Variables	RDUS X±SD	E-HLS X±SD
Age group (year)		
53 years and under	35.89±4.83	24.28±8.11
53 years and above	35.63±5.11	21.76±8.52
MWU; p	-0.463; .64	-2.902; **.000**
Gender		
Woman	35.89±5.06	22.52±8.46
Male	35.55±4.86	23.63±8.34
MWU; p	-0.837; .40	-1.269; .20
Marital status		
Single	35.80±5.03	23.15±8.63
Married	35.73±4.96	22.88±8.33
MWU ; p	-0.16; .87	-0.338; .73
Educational status		
Literate[1]	34.42±5.61	21.11±9.23
Primary education[2]	35.00±5.05	20.40±7.92
High school[3]	36.55±4.56	22.83±8.15
Associate degree[4]	34.78±5.00	26.69±6.65
Bachelor's degree and above[5]	37.37±4.59	28.26±7.42
KW; p	19.178; **.001** 5>1=2	45.29**; .000** 5=4>1=2; 5>3
Living place		
Village and district	35.61±4.93	22.70±8.06
Province	35.88±5.02	23.20±8.74
MWU ; p	-0.800; .42	-0.724; .46
Regular medication use		
Yes	35.71±4.90	22.75±8.47
No	36.00±5.40	24.19±8.05
MWU ; p	-0.883; .37	-1.447; .14
Using medicine without a prescription		
Yes	35.30±4.89	23.59±7.94
No	36.12±5.02	22.46±8.76
MWU ; p	-2.097; **.03**	-1.156; .24
The benefits of the internet in health decisions		
None[1]	35.02±5.09	18.11±8.76
Little[2]	36.23±4.97	23.65±6.77
A lot[3]	35.81±4.73	28.76±6.94
KW ; p	5.439; .06	82.332**; .000** 3>2>1
The importance of the internet in accessing health resources		
None[1]	34.49±4.90	18.14±8.76
Little[2]	36.38±4.98	22.58±6.82
A lot[3]	36.08±4.87	27.80±7.32
KW ; p	13.319**; .001** 2=3>1	76.060**; .000** 3>2>1

Mann Whitney U test, (MWU) , Kruskal Wallis test (KW), and Post Hoc Dunn test (Bonferroni) were used

4. DISCUSSION

The improvement of rational drug use and e-health literacy in chronic disease management represent important strategies for reducing health expenditures and the burden of patient care. Today, individuals' access to accurate and reliable information about health is of critical importance in terms of making informed decisions and ensuring effective participation in health protection processes. In this direction, the RDUS and E-HLS levels of individuals with chronic diseases were examined in the study, and the relationship between these two variables and the effect of demographic factors were evaluated.

The findings of this study indicate that the participants demonstrated a moderate level of knowledge regarding RDUS (Table 2). A study with findings comparable to those presented here revealed that participants' level of RDUS was shown to be above average (16). In some studies conducted with individuals suffering from chronic disease, it was reported that the participants demonstrated a low level of RDUS knowledge (16-18). In other studies, more than half of the population in Nigeria (19). In Nepal, it was reported that nearly half of the population lacked adequate RDUS knowledge (20). The disparities in the level of knowledge about RDUS may be attributable to a number of factors, including the selection criteria, health status, and sociodemographic characteristics of the sample groups in the studies. While studies conducted in a clinical setting generally include individuals who interact more with health professionals, community-based studies may include individuals with limited access to health services or less exposure to health information.

In some studies, it was reported that the level of E-HLS exhibited by the participants was above average and high (7,10,21). The results of this study indicate that the level of e-health literacy (E-HLS) among the participants was below the average (Table 2). A qualitative study with comparable findings reported that the E-HLS level of the participants was low (21). In the existing literature, there is a clear emphasis on the fact that a low level of E-HLS may lead to medical errors and the adoption of incorrect health behaviours (7-9). It can be posited that the participants' below-average E-HLS level presents an obstacle to the demonstration of positive health behaviours. It was hypothesised that this situation may be related to the individual's knowledge of internet usage, reading comprehension and interpretation, and competence in acquiring health-related knowledge.

It is acknowledged that e-health literacy impacts RDUS and enhances the quality of life of individuals (9,22,23). The results of our study indicated a positive, albeit weak, correlation between the RDUS status and the E-HLS levels of the participants. As the RDUS levels increased, the E-HLS levels also exhibited a corresponding increase. As there is a paucity of studies in the literature that have analysed the relationship between RDUS status and E-HLS level simultaneously, it is not possible to make a meaningful comparison of this study findings (10). As in this study findings, another study reported a weak relationship between E-HLS and RDUS levels (10).

The present study revealed no significant differences in RDUS knowledge levels according to age groups, gender, or marital status (Table 3). Prior research has indicated that there are no significant differences in RDUS knowledge levels according to age groups, gender, or marital status (16-18). Another study has demonstrated that the level of RDUS decreases with increasing age (13). It is hypothesised that this situation may result in complications pertaining to disease and medication management, due to the increased prevalence of comorbidities in advanced age groups and the subsequent polypharmacy that this entails. It should be kept in mind that cognitive, sensory and motor function losses in the elderly may also contribute to this result.

It has been emphasised that an increase in the level of education is an effective method of acquiring information regarding the use, side effects, storage conditions and expiry dates of drugs in the management of chronic diseases. Furthermore, there is a directly proportional correlation between the level of education and the level of RDUS knowledge (8,24). The results of this study indicated that participants with higher educational levels demonstrated a superior level of RDUS knowledge compared to those with less education (Table 3). In other studies that have yielded comparable results, it has been documented that individuals with higher educational attainment tend to demonstrate superior RDUS knowledge (13,16-18). The findings of this study are in accordance with those reported in the existing literature.

The analysis of the data revealed no statistically significant differences in RDUS knowledge levels between participants residing in different locations (Table 3). A study with comparable findings revealed that the place of residence did not influence the level of RDUS knowledge (13). In another study, a significant difference was reported between the RDUS knowledge levels of the participants according to their place of residence. The RDUS knowledge level of those living in the province and district was found to be higher than that of those living in the village (18). In this context, the provision of accurate and appropriate health-related information may contribute to the elimination of the distance barrier to health institutions.

It is of great importance that patients adhere to their regular medication regimen in order to facilitate their adaptation to chronic diseases (18). Nevertheless, this study revealed no statistically significant discrepancy in RDUS knowledge levels between participants who utilized regular medication and those who did not (Table 3). A study with a similar design to our own reported no significant difference between regular drug use and RDUS knowledge level (22). In contrast to this study findings, a previous study demonstrated that individuals who did not use regular medication exhibited a higher level of RDUS knowledge than those who did (18). While regular medication use is a standard aspect of managing chronic diseases, it is important to recognise that this may present

challenges for patients in fulfilling their own responsibilities in medication management. Findings show that knowledge is not enough to put into practice. In this case, individuals need to be supported to put the knowledge into practice.

It has been documented that the utilisation of non-prescription pharmaceuticals, which represents an irrational practice in the context of pharmacotherapy, may culminate in fatality (23). A study conducted in Türkiye revealed that nearly half of the participants exhibited a proclivity for the utilisation of over-the-counter pharmaceuticals (10). The findings revealed that the RDUS knowledge level of those who did not use over-the-counter drugs was higher than those who used over-the-counter drugs. In light of these findings, it is imperative that educational and counselling initiatives be implemented to disseminate awareness about the necessity of physician-prescribed medications and the importance of seeking medical guidance before their use.

It has been reported that barriers to access to information may have a negative impact on the level of RDUS (10). In this study, the RDUS knowledge level of the participants who identified the internet as an important source of health resources was found to be higher than those who did not (Table 3). It is thought that this may be related to the level of technology utilisation of the participants.

The findings of our study indicate that the E-HLS level was higher in participants aged 53 years and younger than in older participants (Table 3). In a separate study, it was demonstrated that the HL level was elevated in participants younger than 51 years of age (23). The relatively low level of E-HLS observed in the older age group may be attributed to physiological changes associated with the ageing process, such as age-related decline in vision and hearing, impaired memory and coordination, which may collectively act as barriers to the utilisation of the internet and technology for information access.

It has been previously documented in the literature that the level of education has an impact on HL, with the level of HL increasing in line with the level of education (8,22,23). The results of this study indicated that individuals with a bachelor's degree or above exhibited higher levels of e-health literacy (Table 3). This study findings align with those of previous studies, which have also demonstrated that an elevated educational level is associated with an enhanced level of HL (23,24-27). It is hypothesised that the level of perception, awareness and knowledge increases in direct correlation with the level of education attained by individuals.

This study findings indicate that individuals who perceive the internet as a crucial resource for health-related decision-making and resource acquisition tend to exhibit higher levels of E-HLS (Table 3). In a study, it was observed that the level of E-HLS was higher in individuals who utilized the Internet more frequently on a daily basis and in those who had recently accessed health-related information on the Internet (10). In light of the paramount importance of access to accurate and reliable health-related information on the Internet, it is imperative to enhance the level of E-HLS. It is obvious that the internet has an active role in protecting, improving and developing health in parallel with E-HLS.

4.1. Limitations and Strengths

The present study is subject to certain limitations. Firstly, the data obtained are limited to the data of adults who volunteered to participate in the study and were hospitalized in the internal medicine clinics of a university hospital in Izmir. Secondly, since the data were collected from a single center, the results may not be generalizable to a larger population. Thirdly, multicentre studies conducted in different health institutions may provide more comprehensive results. Finally, the data were collected through self-reporting by the patients. Despite these limitations, this study also has strengths. The data were collected in a training and research hospital, thus ensuring that the study is directly related to the clinical context. The study evaluates two critical variables (i.e. RDU and E-HL) that are important for individuals with chronic diseases. This provides important information in terms of patient education and health management. The findings emphasize the importance of increasing e-health literacy to reduce health expenditures and ease the burden of patient care.

5.CONCLUSION

The study revealed that individuals with chronic diseases demonstrated a moderate level of RDUS knowledge and a low level of E-HLS. A very weak levels positive correlation was observed between the participants' RDUS and E-HLS levels. Enhancing the E-HLS level of individuals with chronic diseases through access to reliable health information on the internet for RDUS may present cost-effective solutions for disease management and treatment adherence. It is recommended that studies be conducted to examine the contribution of reliable information obtained through health literacy in chronic diseases to disease management, rational drug use, and cost-effectiveness. It is recommended that inaccurate publications be removed from circulation and that the Ministry of Health assume responsibility for monitoring the dissemination of accurate information via the internet. Additionally, the creation of public awareness campaigns on the subject of E-HLS would be beneficial.

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Design of the study: DÇ.
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Analysis of data for the study: DÇ, DE.
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Clinical and Experimental Health Sciences

Evaluation of the Effects of Different Irrigation Solutions on MTA and Dentin Microhardness

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ABSTRACT

Objective: The objective of this study was to compare the dissolution effects of different chemical solutions, which are commonly used as root canal irrigants, on partially or fully set mineral trioxide aggregate (MTA). Furthermore, the impact of these solutions on dentin microhardness was also assessed.

Methods: In this study, a total of 80 extracted single-rooted human teeth were utilized. The roots of the teeth were bisected transversely into slices of 6 mm in length. MTA (NeoPUTTY, Avalon Biomed, USA) was applied in layers of 4 mm in thickness within the cavities, sealed with moist cotton, and stored at 37°C under 100% humidity for 24 hours and 21 days. Forty samples were tested at the 24-hour mark, and the remaining 40 samples were tested at the 21-day mark. The samples were randomly allocated to one of five experimental groups: 17% EDTA, 5.25% NaOCl, 2% CHX, 40% citric acid, and saline (control). The Vickers microhardness test was employed to ascertain the hardness values of the MTA and dentin surfaces that had been exposed to the chemical solutions for a period of 10 minutes. Statistical analyses were performed using MedCalc® v19.7.2 (MedCalc Software Ltd, Belgium), with a significance level set at 0.05.

Results: It was observed that the application of EDTA, NaOCl, CHX, and citric acid solutions resulted in statistically significant reductions in the microhardness of both NeoPUTTY MTA (respectively p= .012; p= .012, p= .010; p= .012) and dentin (respectively p= .011; p= .012; p= .012; p= .012). The citric acid group exhibited the most pronounced reduction of MTA (respectively 52.2±1.6; – 39±0.9). In contrast, no statistically significant change in microhardness was observed in the control group treated with saline (for dentin p= .311; for MTA p= .415). The impact of the solutions on MTA at 21 days was found to be less pronounced than that observed at 1 day (p= .012).

Conclusion: The results of this study indicate that the application of citric acid solutions to MTA results in a statistically significant reduction in microhardness. The highest concentration of citric acid was observed to be more effective than the other solutions in the dissolution of MTA. However, these solutions were also found to significantly reduce the microhardness of dentin. It would be advisable to select solutions that facilitate the removal of MTA without damaging the dentin tissue.

Keywords: Citric acid, dentin microhardness, MTA, retreatment.

1. INTRODUCTION

Mineral trioxide aggregate (MTA) is a biocompatible and biologically active material that has gained recognition for its superior sealing ability and structural integrity in endodontic treatments (1). The high compressive strength of MTA, once its setting process is complete, plays a significant role in preserving structural integrity post-treatment. However, this same property presents a significant challenge during retreatment procedures, as it renders the removal of the material more difficult (2,3). Studies have reported that the compressive strength of MTA reaches 40.0 MPa after 24 hours, and the compressive strength of MTA increases to 67.3 MPa after 21 days in the presence of moisture (4). This high level of durability, particularly in root canals, renders the mechanical removal of MTA nearly impossible, necessitating the use of solvents during retreatment (3).

The solubility of MTA has been reported to be very low or non-existent in numerous studies (5-7). However, some research indicates that low pH can affect MTA's hardness, tensile strength, and push-out bond strength by creating pores and voids (8-10). Furthermore, researchers have noted that low pH reduces the microhardness of MTA, making the

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material, which has hardened and is ready for treatment easier to remove. In order to address this issue, a number of solutions with acidic, alkaline, and neutral pH have been tested (11). These include carbonic acid, hydrochloric acid, chlorhexidine (CHX), sodium hypochlorite (NaoCl), ethylenediaminetetraacetic acid (EDTA), and citric acid (CA). It has been demonstrated in the literature that chelating agents have the potential to compromise the integrity of MTA during retreatment (12,13).

A range of irrigating solutions have been examined for their capacity to dissolve MTA, with each solution exhibiting distinct chemical characteristics that influence both MTA and dentin. EDTA, a well-known chelating agent, is predominantly employed for the removal of the smear layer; however, it has also been documented to soften MTA by chelating calcium ions (14). NaOCl is an oxidizing agent with significant tissue dissolution capacity, although its effect on MTA solubility remains contentious (15). CHX is an antimicrobial agent with limited demineralizing properties (16), whereas CA is a potent acid that has demonstrated efficacy in dissolving calcium-based materials (17). The selection of irrigant is of paramount importance, as these solutions have the potential to influence not only the solubility of MTA but also the microhardness of root canal dentin, thereby potentially compromising its structural integrity. Consequently, a thorough investigation into the impact of these solutions on both MTA and dentin is imperative. Despite the fact that a number of studies have been conducted on the impact of different irrigants on MTA solubility, there remains a paucity of systematic investigations that evaluate and compare their effectiveness under uniform experimental conditions.

It is anticipated that an optimal solution for the removal of MTA will result in a reduction in its microhardness. It is important to note, however, that the use of acidic and chemical agents as root canal irrigants may result in alterations to the microhardness of both radicular and coronal dentin. In this context, it is of great importance to conduct a comprehensive investigation into the effects of various irrigation solutions on both MTA and root canal dentin (18). The objective of this study is to compare and contrast the effects of different chemicals, when used as root canal irrigants, on the dissolution of partially or fully set MTA. Furthermore, the influence of these solutions on dentin microhardness was assessed. The null hypothesis (H_0) of the study posits that the utilization of high-concentration citric acid does not yield superior efficacy in the dissolution of MTA in comparison to alternative solutions.

2. METHODS

The study was conducted in accordance with the ethical standards of the 2008 Declaration of Helsinki and approved by the Ethics Committee of the Institute of Health Sciences at Marmara University (approval number: 24.02.2022-2022/46). The study comprised a total of eighty single-rooted human teeth with straight canals that had been extracted for reasons related to the treatment of the patient. The sample size was determined based on the methodology of previous similar studies. In the existing literature, comparable in vitro studies have commonly utilized 10 samples per group. Accordingly, the present study adopted a similar approach to ensure methodological consistency with prior research (19-22). Teeth exhibiting root fractures, cracks, resorption, calcifications, or anatomical irregularities were excluded from the study. The teeth were disinfected by immersion in a solution of 0.5% NaOCl (Microvem, Altun Medical, Turkey) for a period of 48 hours, after which they were preserved in saline solution (23).

The crowns of the teeth were sectioned using a diamond disc (Tizkavan, Tehran, Iran). Subsequently, transverse sections of 6 mm in height were obtained from the roots using a diamond disc (Buehler Diamond Cut-Off Wheels 114243; Buehler, Lake Bluff, IL) attached to an electric saw (IsoMet Low Speed Saw; Buehler). The 80 samples were then embedded in groups of four in Bakelite moulds (Figure 1). The mechanical preparation was conducted under water cooling via the sequential utilization of Gates Glidden drills (Maillefer, Switzerland) numbered 2, 3, 4 and 5, respectively. Following the use of each instrument, irrigation with 5.25% NaOCl (Microvem, Altun Medical, Turkey) was conducted. The final cavity diameter was standardized at 1.3 mm, which corresponds to the diameter of the last instrument used (Gates Glidden #5). The measurements were verified using a digital caliper with an accuracy of 0.01 mm to ensure consistency across all samples. Specifically, NeoPUTTY (Avalon Biomed, USA) is a premixed, ready-to-use bioceramic material that does not require additional liquid for activation. The material was dispensed directly from its container and placed into the prepared cavities using a sterile spatula. Following placement, it was incrementally compacted to a final thickness of 4 mm using a suitable hand plugger (Dentsply, Switzerland) to ensure uniform adaptation to the cavity walls and minimize void formation. A layer of moist cotton was then placed on top of the MTA. All canals were sealed with a temporary restoration material (Coltosol, Ariadent, Tehran, Iran) and stored at 37°C with 100% humidity. 40 samples were stored for 24 hours, and the remaining 40 for 21 days.

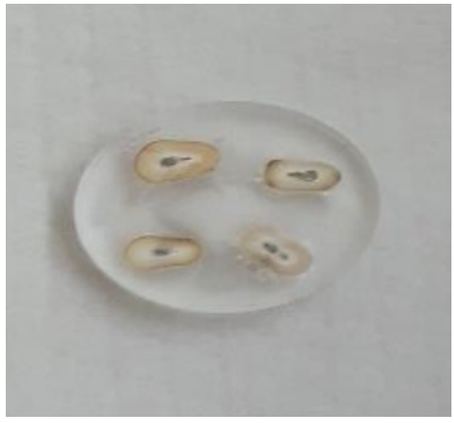

Figure 1. *Samples embedded in bakelite.*

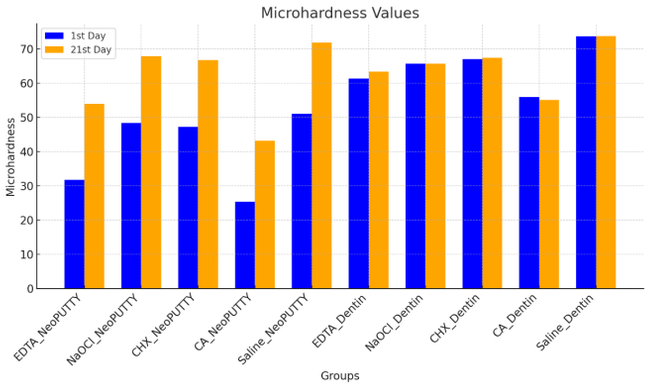

Figure 2. *Comparison of the effects of all solutions on MTA and dentin microhardness on day 1 and day 21.*

A total of 40 samples were subjected to a Vickers microhardness (Buehler Micromet 3 Micro Hardness Tester, Buhler, USA) testing procedure, utilizing a square-based pyramidal diamond indenter to assess the hardness of the MTA and dentin surfaces 24 hours following the initial setup. The indentations were made at a minimum of three similarly positioned points using a 100 g load and a dwell time of five seconds. The Vickers microhardness values were displayed and recorded from the digital readout of the microhardness tester.

Subsequently, the samples were randomly divided into five experimental groups (n = 8) and exposed to the designated solution for 10 minutes, with continuous application (3 drops/5 minutes) (Table 1). Subsequently, the samples were rinsed with distilled water for a period of one minute. Once the samples had been allowed to dry, the Vickers microhardness test was repeated, and the resulting measurements were recorded.

Table 1. *Experimental groups and details of the materials used.*

Experimental Groups	Solution	Manufacturer / Country
Group 1	17% Ethylenediaminetetraacetic Acid Solution (EDTA)	SAVER, Produits Dentaires, Switzerland
Group 2	5.25% Sodium Hypochlorite Solution (NaOCl)	Microvem, Altun Medical, Turkey
Group 3	2% Chlorhexidine Solution (CHX)	Microvem, Altun Medical, Turkey
Group 4	40% Citric Acid Solution (CA)	Prepared in laboratory: 109.58 g citric acid dissolved in water to a final volume of 250 mL
Group 5 (Control)	Saline Solution	House Brand, USA

The aforementioned procedures were then repeated for the remaining 40 samples after a 21-day period of NeoPUTTY MTA (Avalon Biomed, USA) setting. The execution of all experimental procedures was conducted by a single operator. The data were subjected to evaluation, and the mean microhardness values for all groups were determined both before and after chemical exposure.

2.1. Statistical Analysis

The analyses were conducted using MedCalc® Statistical Software, version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Descriptive statistics, including mean, standard deviation, minimum, median, and maximum, were employed to characterize continuous variables. The normality of distribution for continuous variables was evaluated through the implementation of the Shapiro-Wilk test. In instances where two dependent variables did not follow a normal distribution, the Wilcoxon signed-rank test was employed for comparison. In instances where comparisons were to be made between more than two independent groups whose distributions were not normal, the Kruskal-Wallis test was employed. In the event of a statistically significant result, post hoc pairwise comparisons were conducted using the Bonferroni-corrected Mann-Whitney U test. A significance level of 0.05 was employed.

Table 2. *Comparison of the effectiveness of different solutions on MTA microhardness on day1.*

	Day 1/ NeoPUTTY				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	55±1.7	54.7 (53 – 58.4)	31.7±0.8	31.6 (30.6 – 33.1)	**.012**
NaOCl	53.8±0.6	53.7 (53 – 54.8)	48.4±0.4	48.3 (47.8-49)	**.012**
CHX	53.4±0.8	53.7 (52.2 – 54.5)	47.2±0.3	47.2 (46.8 – 47.8)	**.012**
CA	53±1.1	53.1 (51.5 – 54.5)	25.4±0.9	25.4 (24.3 – 26.8)	**.012**
Saline	54±0.9	53.8 (52.8 – 55.4)	51.1±0.5	51.1 (50.4 – 51.6)	.224

Table 3. *Comparison of the effectiveness of different solutions on MTA microhardness on day21.*

	Day 21/ NeoPUTTY				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	71.3±1.4	71.9 (69.3 – 72.7)	54±0.6	53.9 (53.2-55)	**.012**
NaOCl	72±1.2	72.3 (69.6 – 73.7)	67.9±1	67.9 (66.6 – 69.4)	**.012**
CHX	71.7±0.9	71.9 (70.2 – 72.6)	66.8±0.6	66.9 (66-67.6)	**.010**
CA	70.8±1	70.7 (69.5 – 72.4)	43.2±0.7	43.1 (42.1 – 44.6)	**.012**
Saline	73.4±0.6	73.4 (72.6 – 74.4)	71.9±0.6	71.9 (71-72.8)	.415

3. RESULTS

The effects of various solutions on the microhardness of NeoPUTTY and dentin at days 1 and 21 are presented in Tables 2–5. The findings demonstrated that EDTA, NaOCl, CHX, and CA solutions resulted in statistically significant reductions in the microhardness of both NeoPUTTY and dentin at both time points (day 1 for dentin respectively p= .012; p= .012; p= .012; p= .012; day 21 for dentin respectively p= .011; p= .012; p= .012; p= .012) (Fig. 2). The most pronounced reduction on microhardness of MTA (respectively 52.2±1.6; – 39±0.9)

was observed in the CA group. In the control group treated with saline solution, no statistically significant change in microhardness values was observed (for dentin p= .311; for MTA p= .415). Furthermore, the impact of the solutions on MTA at 21 days was found to be less pronounced than that observed at 1 day (p= .012). As demonstrated in Table 6, post hoc pairwise comparisons revealed significant differences between the following groups on both days: differences between the CA-NaOCl (day 1 p= .001; day 21 p= .011), CA-CHX(day 1 p= .028; day 21 p= .011), CA-Saline (day 1 p< .001; day 21 p< .001), EDTA-NaOCl (day 1 p= .013), EDTA-CHX(day 1 p= .014), and EDTA-Saline (day 1 p< .001; day 21 p< .001) groups. Although CA caused a greater reduction in microhardness than EDTA for both NeoPUTTY MTA and dentin, this difference was not statistically significant (p= 1.00).

Table 4. *Comparison of the effectiveness of different solutions on dentin microhardness on day1.*

	Day 1/ Dentin				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	74.1±0.9	74.1 (72.9 – 75.4)	61.4±0.8	61.4 (60.4 – 62.6)	**.012**
NaOCl	73.6±1.1	73.6 (72.2 – 75.2)	66±0.9	65.7 (65.1 – 67.4)	**.012**
CHX	73.9±0.9	73.8 (72.5 – 75.2)	67.1±0.8	67 (66.1-68.2)	**.012**
CA	73.6±0.9	73.6 (72.4 – 74.8)	56±1.2	55.8 (54.5 – 57.8)	**.012**
Saline	74±1	73.9 (72.7 – 75.4)	73.5±0.8	73.7 (72.4 – 74.9)	.216

Table 5. *Comparison of the effectiveness of different solutions on dentin microhardness on day21.*

	Day 21/ Dentin				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	71.3±1.4	71.9 (69.3 – 72.7)	54±0.6	53.9 (53.2-55)	**.012**
NaOCl	72±1.2	72.3 (69.6 – 73.7)	67.9±1	67.9 (66.6 – 69.4)	**.012**
CHX	71.7±0.9	71.9 (70.2 – 72.6)	66.8±0.6	66.9 (66-67.6)	**.010**
CA	70.8±1	70.7 (69.5 – 72.4)	43.2±0.7	43.1 (42.1 – 44.6)	**.012**
Saline	73.4±0.6	73.4 (72.6 – 74.4)	71.9±0.6	71.9 (71-72.8)	.415

Table 6. *Post Hoc comparison of groups for microhardness changes.*

	Day 1 (p value)	Day 21 (p value)
CA-EDTA	1.000	1.00
CA-NaOCl	**.001**	**.011**
CA-CHX	**.028**	**.011**
CA – Saline	**<.001**	**<.001**
EDTA-NAOCL	**.013**	.187
EDTA-CHX	**.014**	.570
EDTA-Saline	**<.001**	**.001**
NaOCl-CHX	.392	1.00
NaOCl-Saline	.104	1.00
CHX-Saline	.131	.401

4. DISCUSSION

The original tricalcium silicate-based products, such as “mineral trioxide aggregate” (MTA), have a number of disadvantages, including difficulty of use, long setting times (>2 hours), a tendency for wash-out during setting, poor adhesion to dentin, relatively high solubility before setting, and the potential for tooth discoloration over time (24,25). In 2010, pre-mixed tricalcium silicate-based cements were introduced with the aim of eliminating the issue of inconsistent consistency that arises during the powder-liquid mixing of traditional tricalcium silicate-based hydraulic cements and to improve ease of application (26,27). NeoPUTTY (NuSmile, Houston, TX, USA), used in our study, is a newly formulated pre-mixed paste version. The product is a bioactive tricalcium silicate-based paste, designed by the manufacturer to overcome the issue of short shelf life associated with similar materials.

The ability of a solution to penetrate an MTA is contingent upon a number of factors, including the concentration of the solution, the exposure time, the type of MTA, and the thickness of the MTA. It has been demonstrated that the application of MTA in a minimum thickness of 4 mm enhances the material’s resistance (28). Consequently, this study employed a 4 mm thickness of MTA. Furthermore, it has been documented that MTA attains its maximum compressive strength and complete chemical setting within a period of 21 days (3). Consequently, to guarantee the full setting of MTA, the acid exposure of the samples was conducted after a 21-day interval.

It has been proposed that reducing the microhardness of MTA may facilitate easier removal compared to fully set, treatment-ready MTA (29). However, no gold standard exists in the literature for evaluating the removability of MTA. Accordingly, the present study employed the microhardness measurement method, which has been utilized in previous research (3,23).

In the present study, three solutions were selected for evaluation: NaOCl, EDTA, and CHX. These solutions are commonly employed as final irrigation agents in endodontic treatments. Prior research has demonstrated that EDTA and NaOCl result in a reduction in the microhardness of both partially and fully set MTA (30,31). Additionally, CHX has been shown to significantly impact the microhardness of partially set MTA (11,16). Citric acid, with a pH of 2.15, is a highly prevalent chelating agent in endodontics. A review of the literature reveals that studies have evaluated the use of citric acid in concentrations ranging from 5% to 50% (32). In the study by Bayraktar et al. (33), it was emphasized that as the acidic strength of the solution increases, there is a greater reduction in MTA microhardness. Consequently, the decision was taken to use 40% citric acid in the present study.

Previous studies have demonstrated that NaOCl and CHX solutions can reduce the microhardness of partially set MTA. In the aforementioned studies, the researchers observed that these solutions did not result in a notable difference in the microhardness of fully set MTA at 21 days (3,11,16). However, in the present study, it was found that NaOCl and CHX solutions significantly reduced the microhardness of NeoPUTTY at both days 1 and 21. This discrepancy may be attributed to the fact that NeoPUTTY is a material that

completes its setting process at a slower rate compared to the tricalcium silicates that have been used in previous studies.

The study conducted by Butt et al. (11) demonstrated that CA significantly reduced the microhardness of both partially and fully set MTA when compared to the CHX and NaOCl groups. These findings are in accordance with our results. The present study demonstrated that CA significantly reduced the microhardness of NeoPUTTY in comparison to the NaOCl, CHX, and saline groups at both day 1 and day 21. A comparison of the CA and EDTA groups revealed that CA caused a numerically greater reduction in microhardness; however, the difference was not statistically significant. In light of these findings, the H_0 of this study, which stated that "High-concentration citric acid does not yield superior efficacy in the dissolution of MTA in comparison to alternative solutions", was rejected.

In assessing the efficacy of solutions employed to facilitate the removal of MTA, it is imperative to consider their impact on dentin tissue. While numerous high-concentration solutions have been demonstrated to effectively dissolve MTA (12,13), it is of paramount importance to ascertain that the selected acid concentration does not precipitate adverse effects on the root canal walls and periodontium. A reduction in dentin microhardness results in a weakening of the mechanical resistance of dentin, rendering it more susceptible to wear, fracture, and caries development. Furthermore, weakened dentin may have a detrimental impact on the success of restorative procedures, as the bonding capacity of restorative materials may be reduced, increasing the risk of post-treatment complications. Consequently, the utilization of biocompatible and safe solutions that do not damage dentin tissue while facilitating the removal of MTA is of paramount importance.

Previous studies have indicated that 2% CHX has a minimal impact on dentin microhardness (11,18). However, the present study has demonstrated that CHX can significantly reduce dentin microhardness. This discrepancy may be attributed to differences in the duration of application. A number of studies have evaluated the effect of NaOCl and EDTA on root canal dentin microhardness. These have consistently demonstrated that both solutions cause a significant reduction in dentin microhardness (11,18,34). This finding is in accordance with the results of the present study, in which all solutions except saline were found to have a reducing effect on dentin microhardness.

It has been demonstrated that EDTA causes demineralization of dentin by dissolving the inorganic components on the dentin surface, leading to a marked reduction in dentin microhardness (35,36). In a study by Cruz-Filho et al. (37), which evaluated the effects of EDTA and 10% citric acid solutions on dentin microhardness, the researchers found that both solutions significantly reduced microhardness, but there was no statistically significant difference between them. Similarly, Eldeniz et al. (36) evaluated the effectiveness of EDTA and 19% citric acid solutions, and the researchers reported a significantly greater reduction in dentin microhardness with citric acid. In the present study, although not statistically significant, the 40% citric acid solution demonstrated a numerically greater reduction in dentin microhardness than EDTA. When all results are considered collectively, it can be inferred that higher concentrations of citric acid led to a greater reduction in dentin microhardness.

The objective of this study was to address a significant shortcoming in the field of endodontic retreatment procedures by examining the impact of various chemical solutions on the dissolution of MTA and the microhardness of dentin. By focusing on the effects of high-concentration citric acid, this study contributes to the existing literature on the removability of MTA. However, it is important to consider the limitations of the study, despite the significant findings. Firstly, it should be noted that the study was conducted under laboratory conditions, which do not fully simulate the biological and dynamic factors present in a clinical environment. Secondly, only specific concentrations and solutions were tested, and the effects of different concentrations and exposure durations were not explored. Furthermore, the biological variability of the human teeth used could lead to individual differences in the results. Finally, in clinical practice, solutions may be applied with varying exposure times and methods, meaning that the results of this study may not fully reflect all clinical scenarios. It is recommended that future studies concentrate on evaluating the effects of varying concentrations, protracted exposure durations, and a range of application techniques under conditions that are more clinically relevant. This would allow for improved simulation of the in vivo environment and greater generalizability of the findings.

5. CONCLUSION

The objective of this study was to evaluate the impact of various chemical solutions on the dissolution of partially and fully set MTA and dentin microhardness. The findings suggest that the high-concentration citric acid solution was the most effective in reducing the microhardness of MTA. However, it was also observed that citric acid significantly reduced dentin microhardness. Furthermore, commonly used endodontic irrigation solutions, including NaOCl, EDTA, and CHX, were found to cause reductions in the microhardness of both MTA and dentin. In light of these findings, solvents aimed at increasing the removability of MTA should also be carefully selected in clinical practice, considering their potential damage to dentin tissue.

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Analysis of data for the study: IO, GB, HSO
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Clinical and Experimental Health Sciences

The Effects of Immunocytochemistry on Diagnostic Accuracy in Thyroid Fine Needle Aspiration Cytology: A Retrospective Study

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ABSTRACT

Objective: In thyroid cytology, ancillary studies are often utilized for intermediate-category cases to better differentiate between benign and malignant lesion. For this purpose, immunocytochemical markers may be preferred because they are cheaper than molecular studies and can be applied in many laboratories. This study retrospectively compares the diagnostic accuracy of cytology samples with and without immunocytochemistry and calculates the frequency of immunocytochemical marker use, as well as sensitivity and specificity rates.

Methods: Between 2015 and 2023, 1816 samples from 1506 patients with a histological diagnosis after cytological examination (thyroidectomy-lobectomy) were included. Cases without a histological diagnosis were excluded. The Thin Prep® method was used for all cytological sample preparations, and cell blocks were obtained. Demographic information, Bethesda system categories, immunocytochemical markers used, and histological diagnoses were recorded. Cases using at least one immunocytochemical marker were re-evaluated, and staining results were categorized as positive, focally positive, or negative. SPSS 15® software was used to assess data normality and perform statistical analyses.

Results: The most frequently used markers were HBME-1 (n=167), CK 19 (n=106), Galectin-3 (n=75), and CD 56 (n=6). Sensitivity rates for HBME-1, CK 19, Galectin-3, and CD 56 were 91%, 94%, 76%, and 75%, respectively; specificity rates were 63%, 61%, 80%, and 50%, respectively. Comparing groups with and without immunocytochemistry, the risk of malignancy was: 6.95%-6.97% for Bethesda category II; 21.7%-19.0% for Bethesda category III; 76.0%-37.1% for Bethesda category IV; 94.0%-95.0% for category V; and 100% for category VI.

Conclusion: In the follicular neoplasm group (Bethesda category IV) the risk of malignancy was higher in the immunohistochemistry applied group. No significant difference in malignancy risk was observed between groups with and without immunocytochemistry in other categories. Considering that immunocytochemical markers were predominantly applied in diagnostically challenging Bethesda categories, the similar malignancy risks across groups may suggest immunocytochemistry aids in accurate categorization. However, according to the results of this study, routine use of immunohistochemical markers in thyroid cytology is unnecessary except for Bethesda category IV.

Keywords: CK 19, Galectin-3, HBME-1, Immunocytochemistry, Thyroid

1. INTRODUCTION

Fine-needle aspiration cytology (FNAC) is a widely used, cost-effective, and minimally invasive diagnostic method that has been employed for decades in the evaluation of thyroid lesions. While FNAC yields high diagnostic accuracy for many thyroid nodules, differentiating between benign and malignant lesions can be particularly challenging in certain cytological samples. Several classification systems aim to provide reliable risk stratification for these intermediate cases; the most widely used is the Bethesda system, updated in 2023.

The 2023 Bethesda system categorizes thyroid cytology into six categories: 1. Nondiagnostic, 2. Benign, 3. Atypia of Undetermined Significance, 4. Follicular neoplasm, 5. Suspicious for malignancy, and 6. Malignant. While detailed cytological features characterize each category (1), routine cytological evaluation can be challenging, leading to difficulties in accurate categorization (2,3). The Bethesda system provides estimated malignancy risks for each category.

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Table 1. *Sensitivity, Specificity, positive predictive value and negative predictive values for HBME-1, CK 19 and Galectin 3 in various studies 1 – Core needle biopsy results of Xiong et al., 2 – Fine needle aspiration and core needle biopsy results of Song et al. 3 – Cazzaniga et al.'s results on histological samples 4 – Xin et al.'s meta-analysis results.*

	HBME-1				CK 19			Galectin-3		
	XIONG (5)	SONG (9)	CAZZANIGA (7)	XIN (10)	XIONG (5)	SONG (9)	XIN (10)	XIONG (5)	SONG (9)	XIN (10)
Sensitivity	63.53	87.5	77.0	92	77.65	51.5	81.6	89.41	96.16	84.2
Specificity	81.81	68.2	83.0	86	63.63	100.0	87.2	50.0	38.46	83.3
PPV	93.10	80.0	-	-	89.19	100.0	-	87.36	95.0	-
NPV	36.73	78.9	-	-	42.42	57.9	-	55.0	60.0	-

To improve diagnostic accuracy, ancillary studies can be used. These studies may include molecular tests (such as mutation analyses, gene expression studies, and miRNA analysis) or immunocytochemical methods. Molecular studies have become increasingly prominent in the updated Bethesda system (4,5). Many immunocytochemical markers are available, including HBME-1, CK19, galectin-3, Cited-1, CD117, CD56, and E-cadherin (4,5). However, the large number of available markers and their varying sensitivity and specificity can lead to different approaches among pathologists (Table 1). There is a growing consensus on standardizing the use of immunocytochemistry. For instance, Margari et al. proposed a diagnostic algorithm that begins with CK19, followed by HBME-1 and galectin-3 (6).

This study focuses on the commonly used markers: HBME-1, which demonstrates higher sensitivity and specificity in papillary carcinoma than in follicular neoplasms (7) and exhibits increased positivity in non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFT-P) and follicular variant papillary thyroid carcinoma (8,9); CK 19, a keratin family member expressed more frequently in papillary carcinomas than in normal thyrocytes (10); galectin-3, an anti-apoptotic protein with potential utility in papillary carcinoma diagnosis (11); and CD56, a marker with expression that decreases in papillary carcinoma (12-14).

The use of markers expected to be positive in papillary carcinoma, in conjunction with negative markers, may improve diagnostic accuracy (14). BRAF mutations are common in papillary thyroid carcinoma and less frequent in anaplastic and poorly differentiated carcinomas (15); immunohistochemical BRAF mutation analysis often correlates with molecular findings (15). While the cytological features of medullary thyroid carcinoma are well-established, preoperative diagnosis can be challenging. Immunocytochemistry using markers such as synaptophysin, chromogranin, CEA, and calcitonin may be necessary to confirm or exclude the diagnosis (16-18).

This retrospective study compares the diagnostic accuracy of thyroid cytology with and without immunohistochemistry and examines the selection of immunocytochemical markers by pathologists.

2. METHODS

Between 2015 and 2023, 1816 samples from 1506 patients whose histological diagnoses were confirmed with thyroidectomy or lobectomy and who had previous thyroid fine needle aspiration cytology (FNAC) at Haseki Training and Research Hospital were included in this study. (This study was approved by Ethics Committee of Haseki Training and Research Hospital, Noninvasive Clinic Ethics Committee (Approval date: 23.05.2024.; Number:26-2024). Thin Prep® method was used in the preparation of all cytological samples and cell blocks were obtained.

Immunocytochemical (ICC) analysis was performed at the discretion of the reporting pathologist during routine diagnostic evaluation. BenchMark®, Clone HBME-1 for HBME-1, BenchMark® A53-B/A2.2b for CK19, BenchMark® 9C4 for Galectin-3 and Ventana® MRQ-42 for CD56 clones were used in routine study in the hospital.

The cases were divided into two groups: those in which immunocytochemistry was used and those in which immunocytochemistry was not used when making a cytological diagnosis. The age, gender, diagnosis groups of these groups according to the Bethesda classification system, and the diagnoses they received after resection were noted. Histological diagnoses were divided into malignant and non-malignant groups according to the WHO Classification of Tumours of Endocrine Organs. Which markers were used in the group where immunocytochemical markers were applied was noted. Sensitivity was defined as the proportion of ICC-positive cases among malignant diagnoses, and specificity as the proportion of ICC-negative cases among benign diagnoses.

The data were evaluated using Microsoft Exel and SPSS programs. Comparisons between groups with and without the use of immunocytochemistry (ICC) were conducted using the chi-square test. In cases where expected cell frequencies were less than 5, Fisher's exact test was applied instead. A *p*-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Patient Demographic

The study included 1816 samples from 1506 patients. Of the 167 patients in the immunocytochemistry group, 132 (79%) were female and 35 (21%) were male; their mean age was 47.0 years (range, 19–84 years). In the non-immunocytochemistry group (n=1339), 1098 (82%) were female and 241 (18%) were male; their mean age was 48.6 years (range, 14–85 years). The mean nodule diameter was 24.07 mm (range, 5–80 mm) in the immunocytochemistry group and 24.07 mm (range, 5–55 mm) in the non-immunocytochemistry group.

3.2. Bethesda Category Analysis

Table 2 presents the Bethesda categories and final diagnoses for cases with non-benign histological diagnoses (excluding follicular nodular disease and thyroiditis).

3.2.1. Bethesda Category I

Immunocytochemistry was not performed (insufficient thyrocytes). Of the 269 cases, histological diagnoses included 23 papillary thyroid carcinomas, 2 anaplastic carcinomas, 1 lymphoma, and 28 microcarcinomas (9% malignancy risk).

3.2.2. Bethesda Category II

In the immunocytochemistry group (n=43), diagnoses included 3 papillary thyroid carcinomas, 5 microcarcinomas, and 1 neoplasm of uncertain malignant potential (6.97% malignancy risk). The non-immunocytochemistry group (n=819) showed 56 papillary thyroid carcinomas, 3 NIFT-P, 1 medullary thyroid carcinoma, and 93 microcarcinomas (6.95% malignancy risk).

3.2.3. Bethesda Category III

The immunocytochemistry group (n=46) showed 1 lymphoma, 9 papillary thyroid carcinomas, 1 medullary carcinoma, and 7 microcarcinomas (21.7% malignancy risk). The non-immunocytochemistry group (n=383) showed 31 papillary thyroid carcinomas, 2 medullary carcinomas, 7 microcarcinomas, and 1 NIFT-P (19% malignancy risk).

3.2.4. Bethesda Category IV

The immunocytochemistry group (n=13) showed 10 papillary thyroid carcinomas and 3 microcarcinomas (76.9% malignancy risk). The non-immunocytochemistry group (n=70) showed 26 papillary thyroid carcinomas and 12 microcarcinomas (37.1% malignancy risk).

3.2.5. Bethesda Category V

The immunocytochemistry group (n=55) showed 30 papillary thyroid carcinomas, 21 microcarcinomas, 1 medullary carcinoma, and 1 NIFT-P (56.3% malignancy risk). The non-immunocytochemistry group (n=81) showed 49 papillary thyroid carcinomas, 1 lymphoma, 1 neoplasm of uncertain malignant potential, and 27 microcarcinomas (61.7% malignancy risk). Including microcarcinomas ≥5 mm in size (radiologically indicated), the malignancy risk increased to 94% in the immunocytochemistry group and 95% in the non-immunocytochemistry group.

Table 2. *Bethesda Categories and histological diagnoses according to the use of immunocytochemistry in neoplastic cases.*

	Histological diagnosis	BK 2	BK 3	BK 4	BK 5	BK 6
Immunocytochemistry Applied	Thyroid papillary carcinoma	3	9	10	30	15
	Microcarcinoma	5	7	3	21	3
	Medullary carcinoma	0	1	0	1	1
	NIFT-P	0	0	0	1	0
	Other	1	1	0	0	0
Immunocytochemistry Not implemented	Thyroid papillary carcinoma	56	31	26	49	13
	Microcarcinoma	93	7	12	27	4
	Medullary carcinoma	1	2	0	0	1
	NIFT-P	3	1	0	0	0
	Other	0	0	0	2	0

Table 3. *Markers applied and number of cases in which they were used.*

Applied immunocytochemistry	Number of Cases (n)
HBME-1	167
CK 19	106
CD 56	6
Galectin-3	75
Calcitonin	26
Synaptophysin	4
Cea	16
Braf	1

Table 4. *Results according to histological diagnoses and Bethesda categories*

		Histological Final Diagnosis			Bethesda Categories				
		Malignant	Benign	Uncertain	BK 2	BK 3	BK 4	BK 5	BK 6
Hbme-1	Positive	67	9	1	3	8	4	45	17
	Focal positive	10	9	1	4	11	1	3	1
	Negative	7	31	4	26	11	5	0	0
	Could not be evaluated	12	16	0	9	11	2	6	0
CK-19	Positive	45	7	1	0	8	3	33	9
	Focal positive	5	5	1	6	2	1	2	0
	Negative	3	19	2	16	7	1	0	0
	Could not be evaluated	8	10	0	6	7	1	4	0
cd56	Positive	0	0	0	0	0	0	0	0
	Focal positive	1	1	0	0	0	0	2	0
	Negative	3	0	0	0	2	0	1	0
	Could not be evaluated	0	1	0	1	0	0	0	0
Galectin-3	Positive	22	2	0	1	0	1	16	6
	Focal positive	7	2	1	0	5	0	5	0
	Negative	9	17	3	14	8	1	5	1
	Could not be evaluated	8	4	0	4	2	2	4	0

Table 5. *Sensitivity and Specificity of ICC markers for Bethesda Categories*

Sensitivity	Bethesda Category	CK-19	Galectin-3	HBME-1
	BK 2	0.00	0.07	0.10
	BK 3	0.83	0.38	0.52
	BK 4	0.80	0.50	0.50
	BK 5	1.00	0.81	0.89
	BK 6	1.00	0.86	1.00
Specificity	Bethesda Category	CK-19	Galectin-3	HBME-1
	BK 2	0.84	1.00	0.76
	BK 3	0.73	0.62	0.53
	BK 4	0.50	1.00	0.71
	BK 5	0.00	0.50	0.00
	BK 6	N/A	1.00	N/A

3.2.6. Bethesda Category VI

Both the immunocytochemistry group (n=19) and the non-immunocytochemistry group (n=18) showed 100% malignancy risk. The immunocytochemistry group showed 1 medullary carcinoma, 15 papillary carcinomas and 3 microcarcinomas. The non-immunocytochemistry group showed 1 medullary carcinoma, 13 thyroid papillary carcinomas and 4 microcarcinomas.

3.3. Marker Usage

The relevant pathologist decided during the routine procedure whether immunocytochemistry would be used and which markers would be used. While in some cases, a single marker was used, it was often observed that several markers were used together (Table 3). The most frequently used markers were HBME-1 (n=167), CK 19 (n=106), Galectin-3 (n=75) and CD56 (n=6). While the most used marker alone was HBME-1, the most frequently used markers were the combination of HBME-1, CK19 and Galectin-3 (n = 74). In some cases, CD 56 appears to be added as a negative marker. Synaptophysin, chromogranin, monoclonal CEA, and calcitonin were used to exclude or confirm medullary carcinoma. In one case, immunohistochemical studies, such as ER, PR and CDX2 were used together with TTF-1 to exclude the possibility of metastasis in a case where papillary structures were clearly observed but nuclear features of thyroid papillary carcinoma were unclear.

3.4. Marker Performance

Table 4 summarizes the immunocytochemical markers used, Bethesda categories, and final diagnoses. Sensitivity and specificity rates for HBME-1, CK 19, Galectin-3, and CD 56 were 91%, 94%, 76%, and 75%, respectively, and 63%, 61%, 80%, and 50%, respectively. Table 5 shows the sensitivity and specificity rates according to Bethesda categories.

3.5. Overall Malignancy Risk

When malignancy rates were compared between immunocytochemistry and non – immunocytochemistry

group across Bethesda categories, a statistically significant difference was observed only in Bethesda Category IV (p = 0.019). In this category, ICC use was associated with a substantially higher rate of malignancy detection (76.0% vs. 37.1%). In contrast, no significant differences were found in categories II (p = 1.000), III (p = 0.813), V (p = 1.000), and VI (p = 1.000), likely due to either low baseline risk (in categories II and III) or universally high malignancy rates (in categories V and VI). These findings suggest that ICC may provide the most diagnostic utility in the indeterminate Bethesda IV category.

4. DISCUSSION

In thyroid cytology, a distinction can often be made between benign and malignant nodules. However, pathologists may have a challenge deciding between Bethesda categories for various reasons, such as autoimmune thyroiditis, reactive changes and degenerative changes around the cyst, Graves' Disease, granulomatous thyroiditis, and thyroid lesions in the intermediate category in the follicular pattern (2,3).

Cytological assessment of thyroid nodules may occasionally present interpretative challenges, particularly when nuclear enlargement and pleomorphism are observed in otherwise benign-appearing samples. These features may lead to diagnostic uncertainty between the benign category and atypia of undetermined significance (AUS). An illustrative case is shown in Figure 1, where negative immunoreactivity for HBME-1 and CK19 likely contributed to the cytological interpretation of the nodule as benign. The final histopathological diagnosis in this case was follicular nodular disease. A second example, presented in Figure 2, demonstrates similar cytological and immunostaining findings; however, the histological diagnosis was follicular adenoma. In this case, the dominance of microfollicular architecture may have been overlooked in the cytological evaluation, potentially leading to misclassification. In the case shown in Figure 3, a cluster of thyrocytes exhibits nuclear enlargement, membrane irregularities, and nuclear grooves. In this instance, positive immunostaining for HBME-1 and CK19 may support a cytological classification of suspicious for malignancy. The histological diagnosis of this case was thyroid papillary carcinoma.

In cases where there is difficulty between the Bethesda categories, molecular studies can guide the distinction between benign and malignant. Molecular studies are excluded from the scope of this study. The reason for this is that molecular study was performed on only one cytology sample (next-generation sequencing was performed in the patient with BK3 twice and BRAF V600E mutation was detected). As molecular testing has only recently been introduced at the institution where the study was conducted, it is likely that clinicians will increasingly request such analyses in a greater number of cases over time.

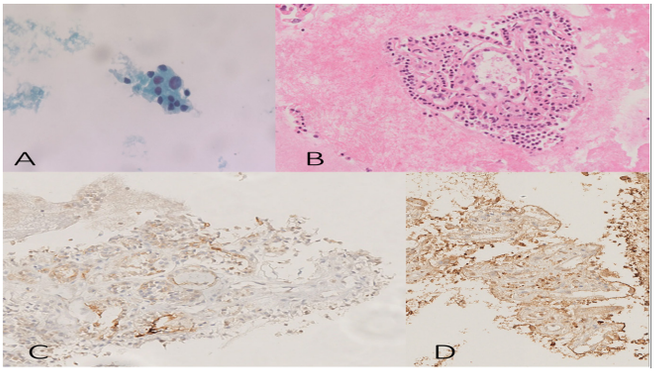

Figure 1. *A sample evaluated as Bethesda Category II. A: Nuclear enlargement and pleomorphism in thyrocytes (ThinPrep®, PAP stain, ×400), B: Benign-appearing thyrocytes with abundant cytoplasm and pleomorphism in the cell block (Hematoxylin & Eosin, ×400), C: Negative immunostaining for CK19, D: Negative immunostaining for HBME-1.*

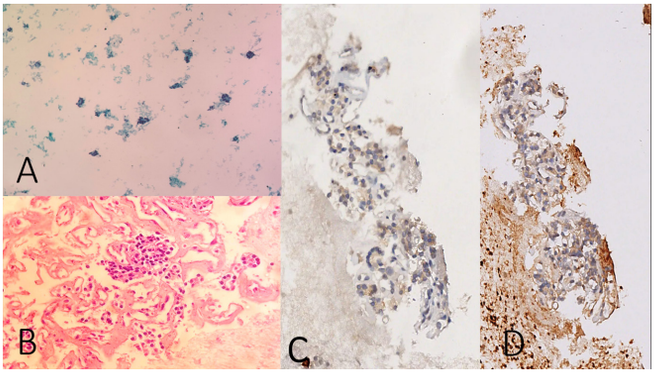

Figure 2. *A case of follicular adenoma evaluated as Bethesda Category II in routine practice. A: Benign-appearing, microfollicular-dominant thyrocytes (ThinPrep®, PAP stain, ×400), B: Benign-appearing thyrocytes in the cell block (Hematoxylin & Eosin, ×400), C: Negative immunostaining for CK19, D: Negative immunostaining for HBME-1.*

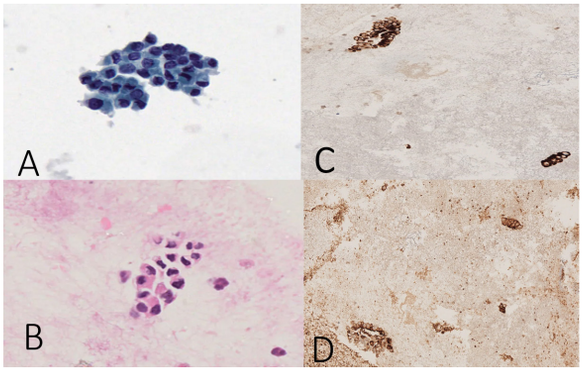

Figure 3. *A case of papillary thyroid carcinoma evaluated as Bethesda Category V in routine practice. A: Thyrocytes exhibiting nuclear enlargement, membrane irregularities, and grooves (ThinPrep®, PAP stain, ×400), B: A few atypical cells observed in the cell block (Hematoxylin & Eosin, ×400), C: Positive immunostaining for CK19, D: Positive immunostaining for HBME-1.*

As mentioned previously, the use of immunocytochemistry in thyroid cytology has been examined in various studies. In a study by He et al., Cyclin D1 was investigated and found its sensitivity to be 88.5% and specificity to be 100% (19). Dixit et al. suggested that the combined use of CD117 and Galectin-3 eliminates the problem of distinguishing between benign and malignant intermediate lesions (14). What does BRAF, CD173, Cadherin-16 (CDH16), somatostatin and CXCR4 receptor, S100 calcium binding protein A1 (S100A1), p53 and MDM-2, Bcl2, PD-1 and PD-L1 and periostin, which are mostly studied in histological samples, play a role in thyroid cytology? Time will tell how much space it can find (20-26). As the number of antibodies that can be used for this purpose and the experience with these antibodies increase, pathologists' preferences may also change. Although these antibodies were not used in our study, the results are not as promising as the result of Dixit et al. (14). In our current study, sensitivity rates for HBME-1, CK 19, Galectin-3 and CD 56 were calculated as 91%, 94%, 76% and 75%, respectively, and specificity rates were calculated as 63%, 61%, 80% and 50%, respectively.

A factor that causes immunocytochemical markers to give false positive and negative results in cytological samples may be sampling errors. Since FNAB samples represent only a part of the thyroid lesion, focal staining may cause difficulty in evaluation. A higher number of evaluated cells will allow for more appropriate evaluation. It can be suggested that more successful results can be obtained in core biopsies, where the number of cells is higher than in fine needle aspiration cytology, but there is not enough evidence to say that the diagnostic performance is better, and since there were no cases in which core biopsy was performed in this study, an evaluation on this subject could not be made (27,28).

One of the limitations of this retrospective study is that pathologists evaluated immunocytochemical results together with cytological findings in the decision-making process, and it was not possible to determine which findings they prioritized when determining Bethesda categories. The subjective nature of Bethesda categorization and differences in ICC assessment can be considered limitations of this study.

Bethesda Category IV (Follicular neoplasm) is one of the intermediate categories and may represent hyperplastic nodule, follicular adenoma, NIFT-P, and follicular carcinoma (29,30). However, in the follicular subtype of thyroid papillary carcinoma, papillary carcinoma nucleus features may not be evident and may be classified in this category. In this study, the diagnoses that increase the risk of malignancy in the Bethesda Category IV group where immunocytochemistry was applied are the follicular subtype of papillary carcinoma. Therefore, it can be suggested that the use of immunocytochemistry may be beneficial in the group considered to be Bethesda Category IV. The absence of a case diagnosed with follicular carcinoma in this study is one of the limitations of this study.

5. CONCLUSION

This study highlights the potential diagnostic value of immunocytochemistry in the evaluation of thyroid nodules, particularly in cytologically indeterminate cases. While the use of ICC did not significantly alter malignancy detection rates in Bethesda categories II, III, V, and VI, a statistically significant increase in malignancy detection was observed in Bethesda Category IV. These findings suggest that ICC may serve as a useful adjunctive tool in improving diagnostic accuracy and guiding clinical decision-making in this diagnostically challenging subgroup.

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Design of the study: AM,CBI,EGU
Acquisition of data for the study: AM,SGB,,EGU
Analysis of data for the study: AM,SGB,CBI
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Drafting the manuscript: AM,CBI,EGU
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Clinical and Experimental Health Sciences

Evaluation of The Gap Volume Following Temporomandibular Joint Ankylosis Surgery: A Preliminary Study

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ABSTRACT

Objective: This retrospective study quantitatively evaluated linear and volumetric dimensions of the joint space created following gap arthroplasty (GA) and interpositional arthroplasty (IA) in temporomandibular joint ankylosis (TMJA) management, analyzing 10 patients (7 female, 3 male).

Methods: Demographic, etiological, and surgical data were extracted from institutional archives. Pre – and postoperative cone beam computed tomography (CBCT) scans enabled joint space assessment, with linear measurements at anterior (AJS), superior (SJS), and posterior (PJS) joint spaces in coronal views. Three-dimensional volumetric analysis was performed using Mimics Innovation Suite Software.

Results: GA demonstrated a mean interosseous distance of 5.92 mm (range: 3.56-9.78 mm) with corresponding space volumes ranging from 560.46 mm^3 to 2194.66 mm^3. IA showed mean interosseous distances at 6.85 mm (range: 2.8-11.83 mm), with volumetric measurements between 910.73 mm^3 and 2738.08 mm^3. All patients achieved clinically significant improvements in maximum interincisal opening, with a mean increase of 28.09 mm. Notably, no cases of reankylosis were observed throughout the mean follow-up period of 16.2 months.

Conclusions: Both GA and IA effectively restored TMJ function, with early physiotherapy initiation being a critical success factor. While cost-effective, procedure selection should be individualized based on age, ankylosis type, and anatomical considerations. Although limited by sample size and follow-up duration, these findings validate both techniques, warranting further large-scale studies to establish definitive guidelines.

Keywords: Temporomandibular joint ankylosis, CBCT, Gap arthroplasty, Interpositional arthroplasty, Joint space volume.

1. INTRODUCTION

Temporomandibular joint ankylosis (TMJA) is a pathologic condition characterized by fibrous and/or bony adhesion of the joint components (1). These components may be affected partially or completely by ankylosis. The etiology of TMJA is multifactorial, with trauma being the most common causative factor (13%-100%), followed by local or systemic infections, or systemic diseases such as rheumatoid arthritis, ankylosing spondylitis, and psoriasis. Temporomandibular joint (TMJ) surgeries may also lead to ankylosis. TMJA can occur at any age but usually develops before the age of 10 years (2). Unilateral ankylosis is more common, with a slight male predominance (3).

TMJA can be classified according to three main criteria: location (intraarticular or extraarticular), type of tissue involved (bony, fibrous, or fibro-osseous), and extent of fusion (complete or incomplete). Tripathy further classified TMJA as true or false (4). Several classification systems have been proposed, including Topazian's classification describing the stage of ankylosis, Sawhney's classification based on radiographic findings, and He et al.'s more recent classification using coronal CT scans. The Topazian classification describes the dimensions of ankylosis, while the Sawhney and He classifications characterize the ankylotic mass, providing guidance for treatment (3).

Patients with TMJA suffer from mandibular hypomobility, limited mouth opening, difficulties in speech and mastication, poor oral hygiene maintenance, airway complications, and facial deformities, which may lead to psychological distress (4, 5).

The diagnosis of TMJA is typically made through clinical examination combined with imaging studies, including plain radiographs, orthopantomograms, computed tomography (CT) scans, MRI, and three-dimensional reconstruction (3).

The primary goals of management are to alleviate patient suffering and prevent reankylosis. TMJA is always treated surgically. Various surgical approaches have been suggested, including gap arthroplasty (GA), interpositional

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arthroplasty (IA), total joint reconstruction (TJR), and distraction osteogenesis (DO) (6). GA, the oldest treatment method, involves releasing the ankylosis by resecting the ankylotic mass to create a space. IA differs from GA by the additional insertion of an interpositional material into the created space (3). Various autogenous grafts have been used as interpositional materials, including masseter muscle, temporalis muscle and fascia, fascia lata, dermis, fat, auricular cartilage, and costochondral grafts. Synthetic materials such as silastic, silicon, and T-plates may also be used (4).

The aim of this retrospective study was to evaluate the linear and volumetric measurements of the space created after GA and IA, and to investigate whether this space volume was sufficient to achieve the required maximum interincisal opening and prevent reankylosis.

2. METHODS

The protocol of this retrospective study was approved by the Institutional Review Board of the Hacettepe University, Ankara, Turkey (IRB No. GO 19/369), and informed consent was obtained from the patients.

Table 1. *Patient Demographics, Etiology, and Duration of Ankylosis*

	Gender	Age	Etiology	Affected site	Duration of ankylosis (yrs)	Previous surgery
1	K	9	Trauma	Left	4	-
2	E	12	Trauma	Right	8	+
3	K	11	Trauma	Right	7	-
4	K	30	Trauma	Left	19	-
5	E	61	Trauma	Right Left	NA	-
6	K	62	Trauma	Right	53	+
7	E	13	Trauma	Left	5	-
8	K	14	Trauma	Right	8	-
9	K	18	Trauma	Left	8	-
10	K	38	Trauma	Left	28	-

Patient 5 had bilateral temporomandibular joint ankylosis.

The study included 10 patients (7 females, 3 males) diagnosed with partial or complete TMJA via cone beam computed tomography (CBCT) and treated with either gap arthroplasty (GA) or interpositional arthroplasty (IA) using abdominal fat grafts. Patient demographics, including age, gender, etiology of ankylosis, affected joint, and duration of ankylosis, were summarized in Table 1. Two patients had undergone previous TMJ ankylosis surgeries at other institutions. At the time of presentation, reankylosis was already established; however, due to incomplete surgical records, the specific cause, whether insufficient gap creation or inadequate postoperative physiotherapy, could not be determined (Table 1). Type of ankylosis, stage and type of ankylosis, surgery technique and follow up periods (Table 2) were obtained from instutional archives.

Table 2. *Ankylosis Type, Surgical Technique, and Follow-Up Duration*

	Type of ankylosis	Stage (by Topazian)	Type (by Sawhney)	Surgery technique	Ipsilateral coronoidectomy	Follow-up (mth)
1	Osseous	1	3	GA	-	37
2	Osseous	1	4	IA	-	61
3	Fibroosseous	1	3	IA	-	6
4	Osseous	2	4	IA	-	3
5	Osseous	2	4	GA	-	24
6	Fibroosseous	2	4	GA	+	12
7	Osseous	2	4	IA	+	6
8	Osseous	2	4	IA	-	5
9	Fibroosseous	1	1	GA	-	5
10	Fibroosseous	1	2	IA	-	3

In patient 5 with bilateral temporomandibular joint ankylosis, the type of ankylosis and the surgical technique were the same on both sides.

CBCT scans were retrieved from the institutional diagnostic image database. CBCT is routinely performed for diagnostic and follow-up purposes in TMJ ankylosis patients at specific intervals: preoperatively, in the early postoperative period (within the first month) using panoramic radiographs, and subsequently with CBCT at the 3rd month, 6th month, 1st year, and annually thereafter. Only preoperative and available postoperative follow-up CBCT images were included in this study.

All surgical procedures were performed under general anesthesia administered via nasal fiberoptic intubation. A preauricular incision was used for all patients. Dissection was carried out through the subcutaneous layer down to the deep temporal fascia, with careful preservation of the superficial temporal artery, vein, and the temporal branch of the facial nerve. The dissection was extended inferiorly to expose the zygomatic arch. The deep temporal fascia was incised, followed by an inverted L-shaped osteotomy on the zygoma, distal to the articular eminence and anterior to the external auditory meatus. The ankylotic mass was exposed and excised to create an adequate gap, and the glenoid fossa was reshaped as needed. In six patients, an abdominal fat graft was placed as interpositional material. For Patients 6 and 7, ipsilateral coronoidectomy was performed to achieve sufficient mouth opening, following Kaban's protocol. Contralateral coronoidectomy was not required in any case. Maximum mouth opening was measured postoperatively and showed significant improvement in all patients. The preauricular incisions were closed primarily, and De Meurechy et al.'s physiotherapy protocol (7) was initiated during the first postoperative week. The physiotherapy protocol consisted of three distinct phases. In the first postoperative week, exercises focused on reducing inflammation and pain while maintaining mandibular mobility. Patients performed 3-5 low-intensity exercises daily, with emphasis on condylar rotation within a pain-free range. Cold application and appropriate pain management were recommended to support recovery during this initial phase. From weeks 1-3, the exercise frequency increased to 5-10 sessions daily, with a gradual shift in focus toward improving range of motion, coordination, and muscle performance. During this

intermediate phase, heat therapy was introduced to enhance tissue flexibility, and stretching exercises were systematically initiated. Beyond four weeks postoperatively, the therapy prioritized achieving smooth, symmetrical jaw movement through muscle strengthening and restoration of normal joint kinematics. In this advanced phase, resistance exercises and joint mobilization were progressively intensified, accompanied by a gradual transition to a normal diet. Throughout all phases, the exercise regimen was carefully tailored to individual patient tolerance to avoid excessive strain while ensuring optimal rehabilitation outcomes.

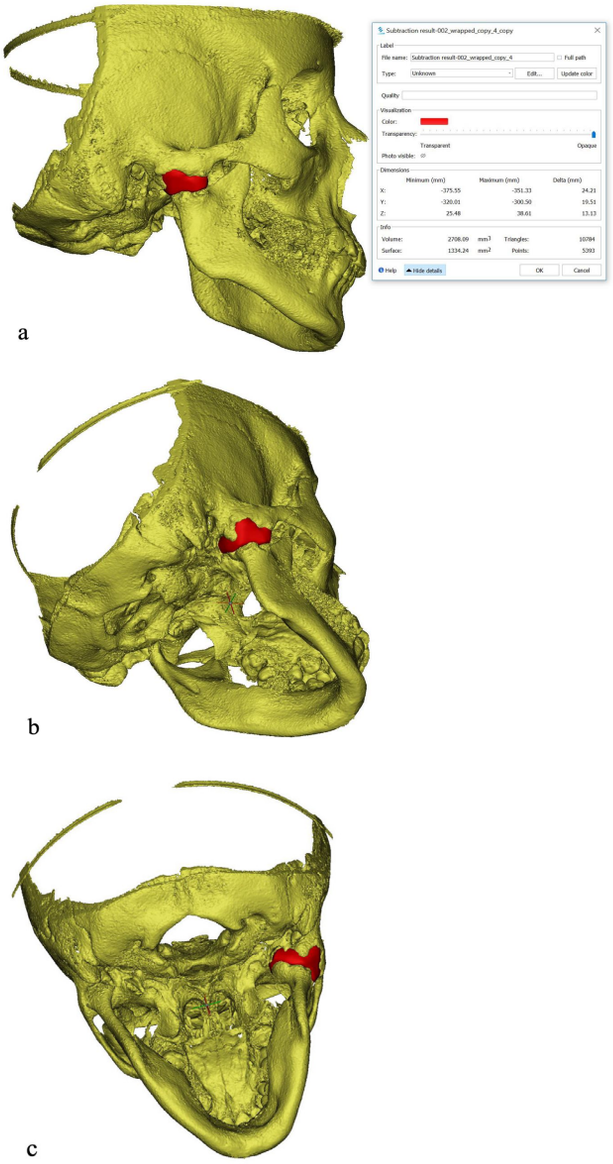

Figure 1.a, 1.b, and 1.c.: *3D Volumetric Measurement of the Gap Following Surgery*

The 3D volumetric measurement of the gap was assessed in postoperative CBCT scans using Mimics Innovation Suite Software. Linear and volumetric measurements were made by a single examiner (Figure 1). For the linear measurement of joint space, anterior joint space (AJS), superior joint space (SPS) and posterior joint space (PJS) was measured in coronal view (Figure 2). For standardization, CBCT images were acquired and analyzed using a consistent 1 mm slice thickness, with the narrowest measurements recorded for analysis.Descriptive statistics were used to summarize patient demographics and clinical characteristics. Continuous variables, such as volumetric and linear joint space measurements and maximum interincisal opening (MIO), were presented as means, minimums, and maximums. Due to the small sample size (n=10), formal inferential statistical comparisons between GA and IA groups were not conducted. All data analyses were performed using Microsoft Excel (Version 16.78.3, Microsoft Corporation, Redmond, WA, USA). The measurements were evaluated by a single calibrated examiner to ensure consistency.

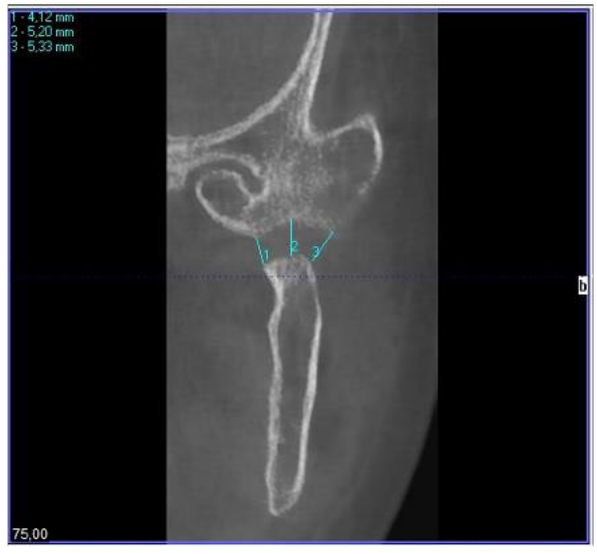

Figure 2: *Coronal View of Anterior, Superior, and Posterior Joint Space (AJS, SPS, PJS) for Linear Measurement*

Figure 3: *Post-operative Minimum and Maximum Interosseous Distance*

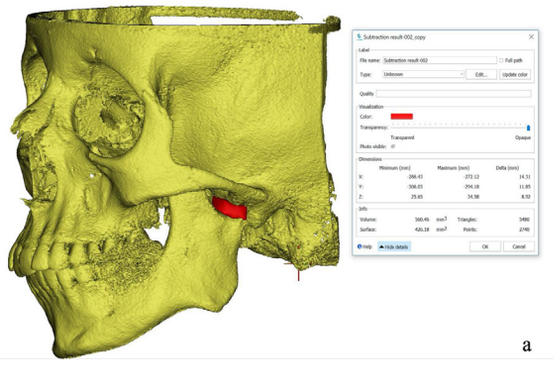

Figure 4.a: *Minimum Space Volume After Gap Arthroplasty (GA)*

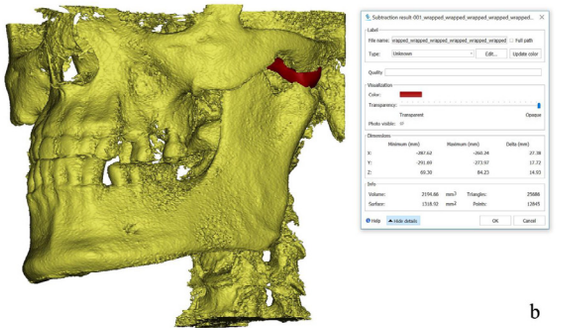

Figure 4.b: *Maximum Space Volume After Gap Arthroplasty (GA)*

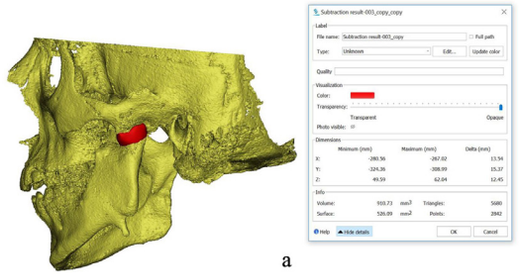

Figure 5.a: *Minimum Space Volume After Interpositional Arthroplasty (IA)*

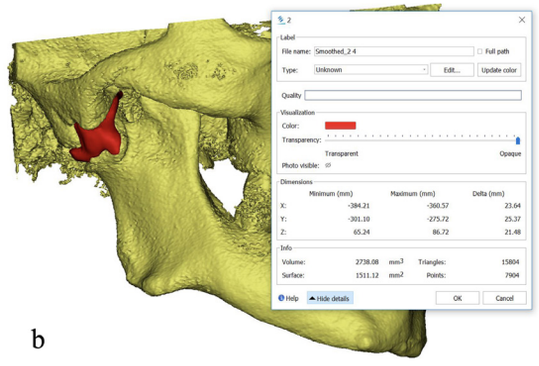

Figure 5.b: *Maximum Space Volume After Interpositional Arthroplasty (IA)*

3. RESULTS

The study evaluated ten patients with follow-up periods from 3 to 61 months (mean: 16.2 months). Postoperative CBCT scans for all patients demonstrated significant increases in interarticular space. No clinical or radiographic evidence of reankylosis was observed during the follow-up period, and none of the patients required secondary surgical intervention.

Preoperative and postoperative MIO measurements are presented in Table 3. The minimum improvement in mouth opening was 23 mm (Patient 1), while the maximum improvement reached 34 mm (Patient 2). Interosseous distance measurements revealed a minimum value of 2.8 mm (superior joint space, SJS) and a maximum value of 11.83 mm (anterior joint space, AJS), with a mean measurement of 6.43 mm across all patients (Figure 3).

The volumetric analysis showed that GA procedures resulted in space volumes ranging from 560.46 mm³ to 2194.66 mm³ (mean: 1377.56 mm³), as illustrated in Figures 1 and 4. IA cases demonstrated space volumes between 910.73 mm³ and 2738.08 mm³ (mean: 1824.41 mm³), shown in Figures 5 and 6. Detailed quantitative results for all patients are provided in Table 3.

Table 3. *Postoperative Interosseous Distances and Space Volumes for GA and IA*

	MIO (mm)		Gap volume (mm^3)	Joint space (mm)			
	Preop	Postop		Anterior	Superior	Posterior	Mean+ss
1	11	34	560.46	5.23	4.2	4.92	4.78+0.53
2	6	40	2738.08	11.83	2.8	3.68	6.1+4.98
3	11	38	2708.09	7.92	6	3.23	5.72+2.36
4	12	39	1706.84	7.09	11.29	10.14	9.51+2.17
5	12	42	2194.66	6.91	3.82	3.56	4.76+1.86
			2106.4	5.06	4.2	6.44	5.23+1.13
6	13	38	2178.7	8.1	6.03	5.69	6.61+1.3
7	8	40	910.73	5.33	5.2	4.12	4.88+0.66
8	13	40	2290.4	9.61	2.41	9.25	7.09+4.06
9	12	41	1367.42	6.53	8.4	9.78	8.24+1.63
10	13	38	2179.72	7.22	9.61	6.53	7.79+1.62

In patient 5 with bilateral temporomandibular joint ankylosis, the top row shows the values for the right temporomandibular joint and the bottom row shows the values for the left temporomandibular joint..

4. DISCUSSION

TMJA represents a pathological fusion between the condylar head and glenoid fossa, mediated by fibrotic or osseous tissue (8). Imaging plays a crucial role in TMJA evaluation, with osseous components best visualized by conventional CT and CBCT, while soft tissue assessment requires MRI. CBCT was selected as the imaging modality due to its optimal balance of short scan time, reduced radiation exposure, and high spatial resolution – characteristics particularly advantageous for maxillofacial applications (9). Therefore, CBCT images were used in this study.

The temporal aspects of TMJA management warrant special consideration. Early disease onset and delayed intervention correlate with progressive dentofacial deformity and functional impairment. While the peak incidence occurs between 20-30 years, most cases originate in childhood, with 90% of the cohort developing trauma-induced ankylosis before age 10 (10). This epidemiological pattern aligns with established literature, though one 11-year-old patient and another with indeterminate onset timing represent notable exceptions in this case series.

The surgery is mandatory in the treatment of ankylosis and early interventions are necessary when the ankylosis is diagnosed. The choice of the surgical methods and interpositional material depends on the surgeon's own experiences and skills. Comparative analysis of the two principal approaches – GA and IA – reveals distinct advantages and limitations. GA offers technical simplicity, reduced operative duration, and cost-effectiveness but carries higher risks of reankylosis (11.3-27%) and malocclusion secondary to ramus shortening (11). IA mitigates these concerns through interpositional materials but introduces donor site morbidity (12–18%), graft resorption (5–15%), and prolonged surgical time (12). The emerging gold standard, total joint replacement (TJR), provides anatomical reconstruction with lower recurrence rates (3–8%) and enables faster physiotherapy initiation and asymmetry correction via concomitant orthognathic surgery. However, its adoption remains constrained by cost and device-related complications (13). Within the cohort, GA was performed in 4 patients (5 joints) compared to IA in 6 patients (6 joints), though the retrospective design limited analysis of selection criteria. Factors such as the patient's age, surgical tolerance, and likely cooperation with postoperative exercises should all be considered in selecting the appropriate surgical approach (12). It was observed that none of the patients in this study accepted TJR as a treatment option. According to the records, this decision was primarily driven by financial constraints.

The relationship between ankylosis type and surgical outcomes remains clinically debated. Current literature suggests Sawhney types I and II may favor gap arthroplasty (GA), while types III and IV could benefit more from interpositional arthroplasty (IA) (3). However, these findings challenge this conventional paradigm. Among two patients with types I/II ankylosis, equivalent functional outcomes were achieved with both GA (n=1) and IA (n=1). Similarly, in types III/IV cases (n=9), comparable success rates were observed between GA (4/9) and IA (5/9) approaches. All patients demonstrated clinically significant improvements in mouth opening (mean increase: 28.09 mm) with no instances of reankylosis during follow-up. These results suggest surgical technique selection may have less prognostic significance than previously hypothesized, though the small cohort size (n=10) and moderate follow-up duration (mean 16.2 months) prevent definitive conclusions. Notably, the absence of reankylosis across all cases underscores the potential importance of postoperative management in determining outcomes.

Inadequate removal of heterotopic bone remains one of the primary causes of failed TMJ surgery (14). Current literature contains limited studies specifically addressing the optimal gap width following GA and IA procedures. Kaban et al. strongly advocate for aggressive resection (15-20 mm) of the ankylotic mass during GA as essential for preventing reankylosis, a recommendation supported by numerous authors (15-17). However, emerging evidence suggests that more conservative gaps of 8-10 mm may be equally effective for GA (18, 19). This moderated approach helps avoid the disadvantages associated with extensive resection, including ramus mandible shortening that can lead to mandibular deviation and malocclusion (manifesting as open bite or premature occlusion), while also reducing the need for subsequent vertical height reconstruction.

The requirements differ significantly for IA procedures, where the interpositional material's presence eliminates the necessity for wide gaps. This technique allows for better preservation of the TMJ's anatomical structure, consequently reducing risks of mandibular deviation and malocclusion. Fewer studies have specifically examined gap dimensions in IA, though Babu et al. demonstrated that minimal gap interpositional arthroplasty (5-8 mm), when combined with complete mediolateral ankylotic mass removal, proves both viable and effective in preventing recurrence (20). A comprehensive meta-analysis further clarified this distinction, establishing that while gaps exceeding 10 mm are crucial for GA success, IA typically achieves comparable results with smaller gaps, often less than 5 mm (21). Chidzonga's work importantly identified inadequate jaw-opening exercises as a key factor in relapse, highlighting the critical role of postoperative physiotherapy in ensuring long-term success (22). In the present study, measurements of interosseous distances at three anatomical points (anterior, superior, and posterior) revealed mean gap distances ranging from 4.76±1.86 mm to 8.24±1.63 mm for GA procedures, while IA procedures demonstrated mean gap distances between 4.88±0.66 mm and 9.51±2.17 mm. Although these values fall below many literature recommendations for GA, the complete absence of reankylosis may be attributed to the early intensive physiotherapy protocol. However, the relatively short mean follow-up period of 16.2 months should be considered as a potential limiting factor when interpreting these results.

TMJ ankylosis continues to present substantial challenges due to the lack of standardized surgical protocols. The frequent occurrence of surgical failure and reankylosis, particularly after repeated procedures, carries significant psychological consequences and increases healthcare utilization. The findings highlight the critical importance of comprehensive perioperative counseling and vigilant postoperative monitoring. Early detection of recurrence signs, particularly morning joint stiffness and progressive reduction in mouth opening, remains essential. Graduated intensification of physiotherapy frequency is strongly emphasized, with the implemented protocol maintaining intensive exercises for 6–12 months postoperatively. Meticulous maintenance of the

surgically achieved maximum interincisal opening through consistent follow-up remains paramount for minimizing recurrence risk (23).

A novel aspect of this study involves volumetric analysis of the created joint space, an approach not previously documented in GA/IA literature. Normal TMJ anatomy features an articular disc dividing the joint into upper (1200 mm^3) and lower (900 mm^3) spaces (24), with Kim et al. reporting total joint space volumes averaging 1500 mm^3 (25). Results demonstrate created volumes ranging from 560.46-2194.66 mm^3 (mean 1681.53 mm^3) for GA procedures and 910.73-2738.08 mm^3 (mean 2088.98 mm^3) for IA procedures. When considering that literature values represent adult norms, these measurements appear anatomically appropriate for maintaining joint function and preventing reankylosis.

Several limitations should be considered when interpreting these findings. The modest cohort size (n=10) and variable follow-up duration (3-61 months) restrict broader generalizations from the data. The retrospective design introduced unavoidable gaps in surgical decision-making documentation. Standardization challenges with postoperative imaging prevented meaningful longitudinal volumetric comparisons. Future prospective studies incorporating larger patient cohorts, controlled follow-up intervals, and standardized imaging protocols would help validate these observations.

5. CONCLUSION

GA and IA persist as clinically effective and economically viable treatment options for TMJA, notwithstanding the potential for superior functional and aesthetic outcomes with advanced reconstructive procedures. The immediate postoperative period proves most critical for successful outcomes, particularly regarding the timely initiation of physiotherapy protocols. Selection of interpositional materials may help prevention of recurrence in the long-term.

These findings collectively suggest that GA, whether employed with or without interpositional materials, constitutes a sufficient treatment approach for the majority of ankylosis cases. More sophisticated surgical interventions may warrant consideration in specific clinical scenarios, including recurrent cases or those requiring latent reconstruction with particular anatomical considerations.

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Acquisition of data for the study: C.A.
Analysis of data for the study: C.A.
Interpretation of data for the study: C.A., E.T., H.H.T
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Clinical and Experimental Health Sciences

Breast Self-Examination Practices and Influencing Factors among Healthcare Professionals Consisting of Nurses and Midwives

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ABSTRACT

Objective: This study was conducted to determine the status of breast self-examination practices and the influencing factors among healthcare professionals consisting of nurses and midwives.

Methods: Descriptive study was conducted with 500 healthcare professionals, who are nurses and midwives working in four state hospitals. The data were collected using a personal information form, Health Belief Model Scale, and Scale to Measure Factors Influencing Women's Breast Cancer Prevention Behaviors. Descriptive statistics, t-test in independent groups, chi-square test, and logistic regression analysis were used to evaluate the data.

Results: The findings showed that 49.8% of the participants practiced breast self-examination. The risk associated with the lack of breast self-examination practice was two fold higher in those whose income was less than their expenses, who did not experience pregnancy, and who had no history of breast cancer among first-degree relatives. A one-unit decrease in self-efficacy perception and information seeking score increased the risk of not performing breast self-examination 1.1 times.

Conclusions: The results showed that the rate of breast self-examination practices among healthcare professionals were low and income status, pregnancy history, family history of breast cancer, perception of self-efficacy and information-seeking behavior influenced breast self-examination.

Keywords: Breast self-exam, healthcare professionals, practices, associated factors.

1. INTRODUCTION

Breast cancer stands as the most prevalent form of cancer among women on a global scale. In the year 2020, approximately two million women received a diagnosis of breast cancer, with one in eight individuals diagnosed with cancer having breast cancer (1). Globally, breast cancer ranks fifth in terms of cancer-related fatalities, claiming the lives of 685,000 women annually (1).

While the risk of mortality remains elevated in breast cancer, the likelihood of survival significantly improves through early diagnosis and prompt treatment (2). Cultivating awareness about cancer and promoting regular screenings are pivotal strategies in the battle against this disease. The American Cancer Society advocates for women to engage in monthly breast self-exams (BSE), undergo an annual clinical breast exam, and receive a mammography every two years, particularly for women aged 40 to 69 (3).

Around 80-90% of breast masses in women are identified through BSE which emerges as an uncomplicated, reliable, and cost-effective method devoid of invasive procedures (4). Despite its significant advantages in breast cancer prevention, the prevalence of BSE among women of reproductive age is between 15-44% (5-8).

Women constitute a significant majority, accounting for 67% of the workforce in the health sector (9). Nurses and midwives form the largest group among the employees in this sector (10). In Türkiye, nurses and midwives collectively make up approximately one-fourth of the health workforce (11). These professionals are considered a high-risk group for breast cancer due to the nature of their work, working conditions, and exposure to carcinogenic factors within the health sector (12,13).

Studies have indicated that nurses and midwives, despite their heightened awareness of breast cancer, exhibit a variable and

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generally low prevalence of BSE, ranging between 47-75%. This underscores the importance of targeted interventions and awareness campaigns within this specific occupational group to enhance early detection practices and overall breast health (14-17).

The factors influencing breast self-exam and the early detection of breast cancer among female healthcare workers are highly diverse. Research indicates that socio-demographic characteristics, the perception of barriers and benefits associated with the exam (8), insufficient knowledge (18), and a family history of breast cancer (19) significantly impact the practice of BSE and the timely detection of breast cancer. Understanding and addressing these varied factors are crucial for developing effective interventions and educational initiatives aimed at improving breast health practices among female healthcare professionals.

Research on breast self-examination has predominantly cantered on examining prevalence, knowledge, and attitudes toward BSE, often encompassing women of reproductive age and students (18-21). However, studies specifically exploring the factors influencing BSE in healthcare workers and the predictive value of these factors are limited (22,23). This study examines the rate of BSE and its influencing factors specifically for healthcare professionals who are nurses and midwives. This study contributes to the literature by revealing the breast cancer prevention behaviors of this group, which play a critical role in the implementation of preventive health services and screening programs. The study is considered to have a high awareness of breast cancer, and the factors that influence this behavior. Furthermore, unlike the existing literature, the combined use of the Health Belief Model Scale and the Scale to Measure Factors Influencing Women's Breast Cancer Prevention Behaviors allowed for a multifaceted assessment of the factors influencing BSE.

This research aimed to identify the factors influencing breast self-exam by assessing the BSE practices among healthcare professionals employed in public hospitals within a specific province. A comprehensive range of factors, including demographic information, obstetric characteristics, health beliefs, and attitudes towards behaviors related to breast cancer prevention was explored to better understand the influencing factors of BSE in this professional group.

1.1. Research questions

1. What is the rate of BSE in healthcare professionals who are nurses and midwives?
2. What are the factors influencing BSE among healthcare professionals?

2. METHOD

2.1. Setting and Participants

The research was conducted across all public hospitals in a province situated in the northwest of Türkiye. The study population comprised of 775 female nurse and midwife healthcare professionals, employed in four hospitals. In the study, the known universe sampling method was used ($N= nt^2pq/d^2(n-1)+t^2pq$), p:0.5 was taken and the minimum sample size was calculated as 257 people. Considering that the representation of the universe increases even more when the sample size is increased, it was aimed to reach more participants. A total of 275 participants were excluded from the study, including 73 on leave, 171 who declined participation, and 31 who incompletely filled out the questionnaires. The study was completed with a total of 500 female healthcare professionals, 399 nurses and 101 midwives. Inclusion criteria involved being women, holding the position of nurse or midwife, and providing consent to participate. Exclusion criteria encompassed a history of breast cancer and male nurses, who were not considered in the study.

2.2. Data Collection Tools

The research data were collected by the personal information form, Health Belief Model Scale and Scale to Measure Factors Influencing Women's Breast Cancer Prevention Behaviors:

2.2.1. Personal Information Form: The Personal Information Form consisted of 15 questions about demographic (age, occupation, etc.) and obstetric characteristics (pregnancy, breastfeeding, etc.), status of practicing breast exams, and factors relevant to BSE (presence of breast cancer in first degree relatives, benign breast disease, etc.).

2.2.2. Health Belief Model Scale (HBMS): The scale utilized in this study was originally developed by Champion in 1984 to assess women's attitudes and beliefs regarding breast cancer and breast self-exam. Its Turkish validity and reliability were established by Karayurt and Dramalı in 2007 (24). The scale is a 5-point Likert-type scale (1 = strongly disagree, 2 = disagree, 3 = undecided, 4 = agree, 5 = strongly agree). Possible scores from the scale are calculated differently for each sub-dimension and cannot be combined. Higher scores indicate greater sensitivity and importance, greater perceived benefits for perceived benefits, and greater perceived barriers for perceived barriers. The scale comprises 42 items distributed across six sub-dimensions, namely risk susceptibility, risk severity, benefits to action, barriers to action, self-efficacy, and cues to action. The Cronbach's Alpha values for the scale range from 0.58 to 0.89, as reported by the original developers. In the current study, the Cronbach's Alpha was determined to be between 0.75 and 0.91, indicating a high level of internal consistency and reliability.

2.2.2. Scale to Measure Factors Influencing Women's Breast Cancer Prevention Behaviors (ASSISTS): The scale employed in this study was originally developed by Khazaee-Pool et al. in 2016 to assess factors influencing breast cancer prevention behaviors (18). The validity and reliability study for the Turkish version of the scale was conducted by Turan and Yiğit in 2021 (25). The scale is a 5-point Likert type (1= never, 2= rarely, 3= sometimes, 4= often, 5= always). Comprising

33 items distributed across seven sub-dimensions attitude, supportive systems, self-efficacy, information seeking, stress management, stimulant and self-care. The scale ranges from a minimum score of 33 to a maximum score of 165. An increase in the scores for each sub-dimension indicates a positive behavior related to that particular aspect. The Cronbach's Alpha for the scale is reported as 0.75, while in the present study, the Cronbach's Alpha was determined to be 0.79, indicating a higher level of internal consistency and reliability.

2.3. Data Collection

The study data were gathered from December 15, 2021 to January 30, 2022. The researcher visited the units where participants were stationed, provided information about the research, and distributed questionnaire forms to voluntary participants. The process of completing the questionnaires by female health workers took approximately 15 minutes.

2.4. Data Analysis

The data were analyzed using IBM SPSS Statistics 23 software. The normal distribution of the data was assessed through Skewness and Kurtosis values, ensuring they fell within the range of <-1.5 to 1.5>. Various statistical methods, including number, percentage, mean, standard deviation, independent groups *t*-test, and chi-square test, were employed in the data analysis. Logistic regression analysis was applied to identify the factors influencing BSE practices. The results were assessed at a 95% confidence interval, and statistical significance was considered at P < .05.

2.5. Ethics

This study adhered to the principles outlined in the Helsinki Declaration. Written approval was granted by the Clinical Research Ethics Committee of the Bolu Abant İzzet Baysal University (Approval Date: 29.11.2021/Number: 2021/363) and the Provincial Health Directorate (Approval Date: 01.12.2021/Number: 49769843). The study strictly adhered to the principle of voluntary participation, and written consent was obtained from all participants. Additionally, approval was obtained from the authors for the use of the selected scales.

3. RESULTS

Table 1 presents a comparison of the descriptive characteristics healthcare professionals who performed breast self-examination and those who did not. The average age of the participants was 32.44±7.60 years, with 61.2% being married. It was observed that 49.8% of the participants engaged in BSE, 14.2% had a history of breast cancer among first-degree relatives and 82.2% possessed knowledge about BSE and breast cancer. A comprehensive overview of all descriptive characteristics is provided in Table 1.

Table 1. *Comparison of the introductory characteristics of healthcare professionals who do and do not do breast self-examination (N=500)*

Characteristics	Breast Self-Examination						Test	p
	Yes		No		Total			
	n	%	n	%	n	%		
Breast Self – Examination	249	49.8	251	50.2	500	100.0		
Education								
High school	43	17.3	25	10.0	68	13.6	5.683	.058**
Graduate	177	71.1	194	77.3	371	74.2		
Postgraduate	29	11.6	32	12.7	61	12.2		
Marital status								
Married	171	68.7	135	53.8	306	61.2	**11.671**	**.001****
Single	78	31.3	116	46.2	194	38.8		
Income								
Income > expenses	41	16.5	31	12.4	72	14.4	**8.359**	**.015****
Income = expense	158	63.5	142	56.6	300	60.0		
Income < expenses	50	20.1	78	31.1	128	2.6		
Smoking								
Yes	95	38.2	63	25.1	158	31.6	**9.853**	**.002****
No	154	61.8	188	74.9	342	68.4		
Alcohol use								
Yes	43	17.3	25	10.0	68	13.6	**5.683**	**.019****
No	206	82.7	226	90.0	432	86.4		
Pregnancy								
Yes	167.0	67.1	118.0	47.0	285.0	57.0	**20.515**	**.001****
No	82.0	32.9	133.0	53.0	215.0	43.0		
Breastfeeding								
Yes	145.0	86.8	100.0	84.7	245.0	86.0		
No	22.0	13.2	18.0	15.3	40.0	14.0	**0.248**	**.618****
Contraceptive use								
Yes	119.0	47.8	93.0	37.1	212.0	42.4	**5.903**	**.015****
No	130.0	52.2	158.0	62.9	288.0	57.6		
Menstruation								
Continuing	244.0	98.0	237.0	94.4	481.0	96.2	**4.357**	**.037****
Menopause	5.0	2.0	14.0	5.6	19.0	3.8		
Presence of breast cancer in first degree relatives								
Yes	49.0	19.7	22.0	8.8	71.0	14.2	**12.220**	**.001****
No	200.0	80.3	229.0	91.2	429.0	85.8		
Benign breast disease								
Yes	16.0	6.4	13.0	5.2	29.0	5.8	0.355	.551**
No	233.0	93.6	238.0	94.8	471.0	94.2		
Getting education on BSE and breast cancer								
Yes	220.0	88.4	191.0	76.1	411.0	82.2	**12.836**	**.001****
No	29.0	11.6	60.0	23.9	89.0	17.8		
	Mean± SD		**Mean± SD**		**Mean± SD**			
Age/*year*	32.45±7.56		32.43±7.65		32.44±7.60		0.040	.968*
Breastfeeding duration/ *month*	12.47±6.28		14.59±8.18		13.33±7.18		**-2.185**	**.030***

**: Independent sample t-test; **: Chi square test.*

A statistically significant difference was also identified in relation to participants' pregnancy experiences, duration of breastfeeding, use of contraceptive pills, menstruation status, history of breast cancer in first-degree relatives,

training on BSE and breast cancer, and BSE practices (P < .05). Conversely, no statistically significant difference was noted in terms of participants' age, education, presence of diagnosed breast disease, and BSE practices (P > .05) (Table 1).

Table 2 illustrates the comparison of HBMS and ASSISTS mean scores among healthcare professionals who practiced BSE and those who did not. Participants received the highest mean score from the HBMS in the confidence/self-efficacy dimension (36.58±6.35) and the lowest mean score from the sensitivity sub-dimension (8.04±2.25). The participants' total mean ASSISTS score was 106.26±14.25. The study concluded that health professionals who practiced BSE demonstrated higher scores in self-efficacy and cues to action, while exhibiting lower scores in barriers to action (P < .05). Regarding the ASSISTS scale, individuals who performed BSE recorded higher mean scores in stimulant, self-efficacy, supportive systems, information seeking, self-care, stress management, and the overall ASSISTS total scale scores compared to those who did not perform BSE, with the differences being statistically significant (P < .05).

Table 2. *Comparison of the mean scores of HBM and ASSISTS Scales of healthcare professionals who did and did not do breast self – examination (N= 500)*

Scales		Breast Self – Examination				t*	p
		Yes		No			
	Mean+SD	Mean	SD	Mean	SD		
HBM							
Sensitivity	8.04±2.25	8.11	2.28	7.97	2.23	0.696	.487
Seriousness/ importance	21.40±4.86	21.25	4.81	21.55	4.91	-0.683	.495
Benefit	15.62±3.01	15.68	2.94	15.55	3.08	0.494	.622
Barrier	25.83±5.96	24.94	5.66	26.72	6.12	**-3.384**	**.001**
Confidence/ self-efficacy	36.58±6.35	38.98	5.27	34.19	6.43	**9.119**	**.001**
Health motivation	26.57±3.85	27.51	3.75	25.64	3.73	**5.596**	**.001**
ASSISTS							
Attitude	20.25±4.58	20.63	4.56	19.87	4.57	1.856	.064
Motivation	15.71±2.59	16.20	2.52	15.21	2.57	**4.366**	**.001**
Self-efficacy	14.66±2.46	15.09	2.42	14.23	2.43	**3.973**	**.001**
Support systems	12.86±3.57	13.38	3.68	12.35	3.39	**3.270**	**.001**
Searching for information	13.82±2.76	14.48	2.76	13.17	2.61	**5.450**	**.001**
Self care	18.59±4.04	19.41	4.10	17.77	3.81	**4.633**	**.001**
Stress management	10.37±2.22	10.62	2.14	10.12	2.27	**2.525**	**.012**
ASSISTS total	106.26±14.25	109.82	14.34	102.73	13.27	**5.757**	**.001**

**: Independet sample t-test was used*
HBM: Health Belief Model; ASSISTS: Scale to Measure Factors Influencing Women's Breast Cancer Prevention Behaviors

Table 3 presents the factors influencing BSE practices as revealed by logistic regression analysis. The results indicate that individuals with an income less than their expenses had a 2.1 times higher risk of not engaging in BSE compared to those with income exceeding expenses. Similarly, individuals who had not experienced pregnancy faced a 2.1 times higher risk of not performing BSE compared to those with a history of pregnancy. Participants without a family history of breast cancer among their first-degree relatives had a two times higher risk of not performing BSE than those with a family history of breast cancer. Additionally, a one-unit decrease in self-efficacy and information-seeking score was associated with a 1.1 times increased risk of not performing BSE.

Table 3. *Factors influencing breast self – examination among healthcare professionals (N= 500)*

Characteristic	β	SH	p	Exp(β)	95%C.I.	
					Lower limit	Upper limit
Income status			.064			
Income and expense equal	0.355	0.302	.239	1.427	0.789	2.579
Income less than expenses	0.762	0.339	**.024**	2.143	1.103	4.162
Pregnancy status	0.716	0.206	**.001**	2.047	1.367	3.064
First degree recent history of breast cancer	0.679	0.307	**.027**	1.972	1.080	3.602
Confidence self-efficacy	-0.122	0.019	**.001**	1.129	1.089	1.171
Searching for information	-0.116	0.039	**.003**	1.123	1.041	1.211

β:regression coefficient

4. DISCUSSION

It was observed in this study that almost half of participants practiced BSE (Table1). Research from various countries has indicated BSE rates ranging from 47-75% among female healthcare professionals (14,16,17) and 15-44% among women of reproductive age (5-7). The higher prevalence of BSE among female healthcare professionals, as compared to women of reproductive age, can be attributed to their specialized education and increased exposure to cases of breast cancer. Nevertheless, despite being a group with heightened awareness on the subject, the fact that only half of the participants practiced BSE underscores the importance of further attention to this issue, considering the simplicity and cost-effectiveness of BSE in the early detection of breast cancer.

Health beliefs, which express individuals' thoughts and behaviors about health or illness, can lead to positive or negative health-related behaviors (26). Studies evaluating health beliefs regarding BSE have shown that a low perceived barrier and a high perceived benefit and self-efficacy support behaviors aimed at early diagnosis (8, 27-29). The lower the perceived barrier, which represents the individual barriers to BSE, the higher the likelihood of BSE (27-29). In this study, the mean scale score for barrier perception among participants who did not perform BSE was higher than those who did, and the difference was statistically significant (Table 2). This finding is consistent with the literature, and a high perceived

barrier stands out as a strong barrier that hinders behavior. Another factor influencing BSE is health motivation, which indicates a woman's desire and intention to perform BSE for the early diagnosis of breast cancer (30). In our study, health motivation was found to be higher among healthcare professionals who performed BSE than among those who did not (Table 2). Similar to the current study, meta-analysis studies that included both healthcare professionals and female students found that high health motivation increased the intention and behavior to perform BSE (27,28).

In the study, participants who performed BSE had significantly higher mean self-efficacy scores than those who did not (Table 2). Self-efficacy is a critical psychological force in initiating and sustaining behavior. The positive relationship between self-efficacy and BSE behavior is supported by the literature (27,29).

In our study, the mean ASSISTS scale scores were also higher in healthcare professionals who performed BSE than in those who did not (Table 2). This finding is similar to studies showing that individuals with higher ASSISTS total scores are more likely to perform BSE (25,31,32).

The demographic and obstetric characteristics can influence women's practice of BSE (8). This study found that participants with lower income than expenses had a 2.1 times higher risk of not performing BSE compared to those with higher income than expenses (Table 3). Other researchers have also observed that individuals with lower income engage in less frequent BSE compared to those with higher income (33,34). Getu et al. (2022) determined that individuals with better economic status place greater importance on health preservation and improvement. In this context, the findings of the present study findings align with the results in the existing literature (35-37).

Another factor affecting the practice of BSE in this study is related to pregnancy. The risk of not practicing BSE was found to be two times higher in individuals who have not experienced pregnancy higher compared to those who have been pregnant (Table 3). The absence of pregnancy experiences is considered to be a risk factor for breast cancer, emphasizing the importance of nulliparous individuals practicing BSE (3). Koç et al. (2014) found in their study with nurses that had not given birth at all is perceived as a risk factor for breast cancer (38). However, there are also different research findings. Similar to our study, Moey et al. (2020) observed that individuals who have experienced pregnancy perform BSE more frequently than those who have not; on the other hand, Özmen et al. (2019) did not find a difference in BSE practices between nulliparous and multiparous individuals (39, 40).

Another significant factor influencing the practice of BSE is the presence of breast cancer in a first-degree relative. In our study, the likelihood of participants with a first-degree relative having breast cancer performing BSE was found to be two times higher compared to those without such a family history (Table 3). In their study conducted in Ethiopia, Dagne et al. (2018) found that the probability of nurses and midwives with a family history of breast cancer performing BSE was 6.5 times higher than those without such a history, while Negeri et al. (2017) reported a 5 times higher probability (41, 42). In the present study, the higher frequency of BSE among those with a family history of breast cancer suggests that participants perceive genetic predisposition to breast cancer as a risk and exhibit positive behavior regarding BSE. The American Cancer Society (2022) also reported that the risk of breast cancer is approximately 1.5 times higher in women with a first-degree relative with breast cancer, and it increases to 2-4 times higher in women with more than one first-degree relative affected by breast cancer, compared to women without a family history of breast cancer (3).

Higher self-efficacy perception indicates individuals' confidence in performing the desired behavior (43). In the present study, those who practiced BSE demonstrated a higher level of self-efficacy perception compared to those who did not practice BSE. A one-unit decrease in the self-efficacy perception score increased the risk of not performing BSE by 1.1 times (Table 3). In a study conducted in Ethiopia, Tewelde et al. (2022) found that a one-unit increase in self-efficacy score increased the likelihood of performing BSE by 6.3 times, while Dagne et al. (2018) observed that a one-unit increase in self-efficacy score increased the likelihood of performing BSE by 4.7 times (41,44). In contrast to the study by Dagne et al., the fact that the effect of knowledge on performing BSE was lower in the study conducted despite the similarity of the sample group suggests that individual or environmental barriers may be effective in preventing the behavior despite knowledge (45).

Inclination towards protective behaviors is influenced by individuals' information-seeking behaviors (46). In the present study, a one-unit decrease in the knowledge-seeking score of participants increased the risk of not performing BSE by 1.1 times (Table 3). Researchers have found that participants who actively seek and acquire information about breast cancer are more likely to participate in screening programs and have higher awareness of these programs (47, 48). Similar to the present study, Dagne et al. (2018) observed that healthcare professionals who were knowledgeable about BSE were 4.7 times more likely to perform BSE compared to those who were not informed about BSE (41).

This study has several limitations. Firstly, the collected data relied on subjective responses, which may have influenced the participants' answers. Secondly, the study's participants were restricted to midwives and nurses present in the hospital during the study period, limiting the generalizability of the findings to other institutions and employee groups. Thirdly, the research is limited to the responses given to the scales used as data collection tools.

5. CONCLUSION

This study revealed a low rate of BSE practices among participants, emphasizing the significance of considering

the factors such as self-efficacy and information-seeking attitudes alongside demographic and obstetric characteristics in promoting BSE. Despite healthcare professionals who are nurses and midwives being generally aware of breast cancer, it is crucial for institutional managers to establish supportive mechanisms that enhance the self-efficacy and information-seeking attitudes of female healthcare professionals.

Nurses and midwives are among the healthcare professionals most frequently contacted and consulted on women's health issues (30). There is a strong relationship between the behavior of healthcare personnel and public attitudes (49). Training nurses and midwives on BSE can empower them to transition into educator and counsellor roles with the knowledge and skills they acquire, thus impacting public awareness of breast cancer and their approach to screening programs.

Recommendations for future initiatives include planning in-service training programs to raise awareness and promote BSE among nurse and midwife health professionals. Moreover, qualitative studies should be conducted to identify the factors influencing breast cancer prevention behaviors. It is advisable to organize activities aimed at enhancing knowledge about breast cancer and BSE. Additionally, conducting multicentered studies involving a broader spectrum of healthcare professionals would contribute to a more comprehensive understanding of the subject.

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Clinical and Experimental Health Sciences

Investigation of the Effects of Mouthwashes on the Microhardness and Color Stability of Bulk-fill Composite Resins

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ABSTRACT

Objective: The current study aimed to investigate the effects of mouthwashes on the microhardness and discoloration of bulk-fill resin composites by comparison with a conventional resin composite.

Methods: In the study, 22 disc-shaped samples (8*2 mm) were prepared from four different composite resins, Clearfil Majesty Esthetic (CME), Estelite Bulk-fill Flow (EBF), Filtek One Bulk-fill Restorative (FBF) and SonicFill-2 (SF2). Half of the samples for each material were stored in Listerine Cool Mint and the other half in Colgate Optic White mouthwashes for 24 hours (n=11). The color and microhardness of the samples were measured baseline and after 24 h incubation in mouthwashes. After calculating color change (ΔE00) and microhardness changes (ΔVHN), the obtained data were analyzed in SPSS software at α=.05 by Two-Way ANOVA, One-Way ANOVA, Post-hoc Tukey, Independent and Paired-Samples t test.

Results: The effect of composite material type (p=.016) and mouthwash type (p=.008) on color change was statistically significant. All materials were more colored in Listerine Cool Mint than in Colgate Optic White but statistically significant only for FBF (p=.017). Discoloration of the materials in both mouthwashes didn't exceed the 50:50% acceptability threshold (ΔEAT=1.8). For all materials, microhardness decreased significantly after storage in mouthwashes compared to baseline state (p<.05). There was no significant difference in ΔVHN between the mouthwashes (p>.05).

Conclusion: Although the discolorations of materials were at an acceptable threshold for the tested mouthwashes, a significant decrease in microhardness is very important for long-term clinical success of the materials. The uncontrolled use of mouthwash should be avoided.

Keywords: Bulk-fill, Color stability, Microhardness, Mouthwashes, Spectrophotometer

1. INTRODUCTION

Resin composites are frequently preferred in direct restoration of teeth (anterior/posterior) due to their aesthetic and mechanical properties (1,2). In recent years, bulk-fill composite resins have become popular, offering bulk application and curing of up to 4-5 mm thickness in a single step (3). Bulk-fill composites have the advantage of less polymerization shrinkage, preventing the formation of interlayer gaps and contamination risks in conventional methods (4). It also provides advantages for both the physician and the patient, as it reduces the time it takes to complete the restoration (5). Additionally, for the bulk-fill technique, concerns have been reported regarding aesthetics, mechanical properties, degree of conversion, and polymerization stress, as well as disadvantages such as less satisfactory handling compared to traditional resin composites (6).

There are different type of bulk-fill materials available on the market with low and high viscosity that may or may not require coating with a conventional resin composite (7). However, some companies have also produced materials with high viscosity that offer the advantage of use by reducing viscosity with sonic activation (8). In addition to the advantages they provide, materials are affected by oral environmental conditions at different levels. Controlled clinical studies examining bulk-fill composites over the long term are limited literatures (9,10), but existing studies have reported that they exhibit similar clinical performance to conventional composite resins (11-13). In general, fracture toughness, marginal adaptation, adequate wear resistance, microhardness, and color stability are important factors affecting the long-term success of composite resin restorations (14). Microhardness is influenced by the composition and degree of polymerization of the resin composite. A low degree of polymerization of the composite can cause secondary caries and discoloration due to reduced microhardness and increased plaque accumulation (15).

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Increased microhardness affects the clinical success by the increasing resistance to wear and surface scratching (16). Another prerequisite for clinical success is color stability (17). After restorations are made properly in accordance with the shade selection, it is expected that the restoration will maintain its color and optical properties for a long time (18). However, composite resins can easily deteriorate when exposed to various conditions due to their polymer base (19).

In oral conditions, oral hygiene practices such as tooth brushing and use of various mouthwashes can have an impact on the mechanical and optical properties of composite resins (20,21). The preventive use of mouthwashes, which is extremely important in maintaining public oral health, has increased in recent years, especially due to the restrictions on face-to-face treatment of patients after the Covid-19 pandemic (22). Mouthwashes can be used for various purposes, including the treatment of periodontal disease, as a caries preventive in cases of high caries risk such as orthodontic treatment, in special cases such as bad breath, and for the expectation of whitening (23). These products are easy to obtain, and their use is often outside professional supervision (24). A previous study suggested that more than half of adults aged 25 to 34, and nearly a quarter of all age groups, use mouthwash daily (25).

Mouthwash solutions contain various components such as organic acids, emulsifiers, detergents, dyes and alcohol (26). While some mouthwashes used for whitening purposes contain hydrogen peroxide as the active ingredient (27), some contain alternative substances such as tetrasodium pyrophosphate, sodium hexametaphosphate, phthalimido-peroxy-caproic acid, and sodium citrate for this purpose (28). Variations in mouthwash composition, including ingredient concentration and pH, can influence their effects on restorative materials (29). Previous studies have reported that mouthwashes with lower pH and higher alcohol content can reduce the microhardness of composite resins, affect the bond between the matrix and inorganic fillers, leading to reduced erosion resistance and increased staining of the material (26,29-31). In addition, the literature provides varying results regarding the effects of hydrogen peroxide (HP)-containing and HP-free mouthwashes on restorations (15,23). The differences in results make the evaluation of the effects of mouthwashes on resin-based materials clinically important and inevitable.

The effects of mouthwashes on resin-based materials have often been evaluated on conventional resin composites (15,21,23). Especially, studies on the effect of mouthwashes on bulk-fill composite resins are limited (32,33). In this context, the aim of this study was to evaluate the effects of two different mouthwashes on the microhardness and color stability of three bulk-fill composite resins compared to conventional composite resin.

The null hypotheses of the study were, 1. Mouthwash type and material type does not affect the color stability of the tested materials, 2. Mouthwashes does not affect the microhardness of the materials, and 3. There is no difference between the two mouthwashes in terms of microhardness change of the tested materials.

2. METHODS

In this in vitro study, the following restoration materials were used: nanohybrid universal composite (Control: Clearfil Majesty Esthetic (CME), Kuraray, Japan), supranano-filled flowable bulk-fill (Estelite Bulk-fill Flow (EBF), Tokuyama, Japan), nano filled high viscosity bulk-fill (Filtek One Bulk-fill Restorative (FBF), 3M ESPE, USA), and sonic-activated bulk-fill composite (SonicFill-2 (SF2), Kerr, USA). For mouthwashes, Listerine Cool Mint (Johnson & Johnson, Neuss, DE, Germany) and Colgate Optic White (Colgate–Palmolive Swidnica, Poland) were used. The chemical compositions of the composite resins and mouthwashes used in the study are presented in Table 1 and Table 2, respectively. The study design is summarized in Figure 1. Minimum sample size was calculated for 2 sub-experimental groups of each material with 0.85 power and 0.40 effect size in G Power 3.1.9.2 software. A total of 88 samples, 22 from each material and 11 from each sub-group, were found sufficient (n=11). In the current study, a total of 176 samples were prepared, 88 samples for color stability and 88 samples for microhardness.

Table 1. *Restorative materials used in the study*

Material	Type	Contents	Filler Load	Manufacturer
Clearfil Majesty Esthetic	Nanohybrid universal composite	Bis-GMA, TEGDMA, hydrophobic aromaticdimethacrylate Silanated barium glass filler, prepolymerized organic filler, Filler size: 0,7 µm Photoinitiator:CQ	78 wt% 66 vol%	Kuraray, Okayama, Japan LOT: 7E0228
Estelite Bulk-Fill Flow	Supranano fil flowable bulk – fill composite	Bis-GMA, Bis-MPEPP, TEGDMA, SiO2, and ZrO2 fillers Filler size:200nm Photoinitiator: RAP technology™	70 wt% 56 vol%	Tokuyama, Tokyo, Japan LOT: 011E97
Filtek One Bulk-Fill Restorative	Nanofill high viscosity bulk-fill composite	AUDMA, AFM, DDDMA, UDMA Silica (20 nm), zirconia (4–11 nm), ytterbiumtrifluoride (100 nm), zirconia/silica Photoinitiator: CQ	76,5wt% 58,4 vol%	3M, St.Paul, USA LOT: NA29615
SonicFill-2	Sonic activated hybrid bulk-fill composite	Bis-GMA, TEGDMA, Bis-EMA Zirconium oxide, glass Filler size: 25-400nm Photoinitiator: not disclosed	81,3 wt% 66 vol%	Kerr, Orange, USA LOT: 6689419

Bis-GMA: Bisphenol A-glycidyl methacrylate, Bis-MPEPP: Bisphenol A polyethoxymethacrylate, TEGDMA: Triethylene glycol dimethacrylate AUDMA: Aromatic urethane dimethacrylate monomer, AFM: Additional fragmentation monomer, DDDMA: 1,10-decanediol dimethacrylate, UDMA: Urethane dimethacrylate, Bis-EMA: Ethoxylated Bisphenol A dimethacrylate, RAP: Rapid amplified photopolymerization

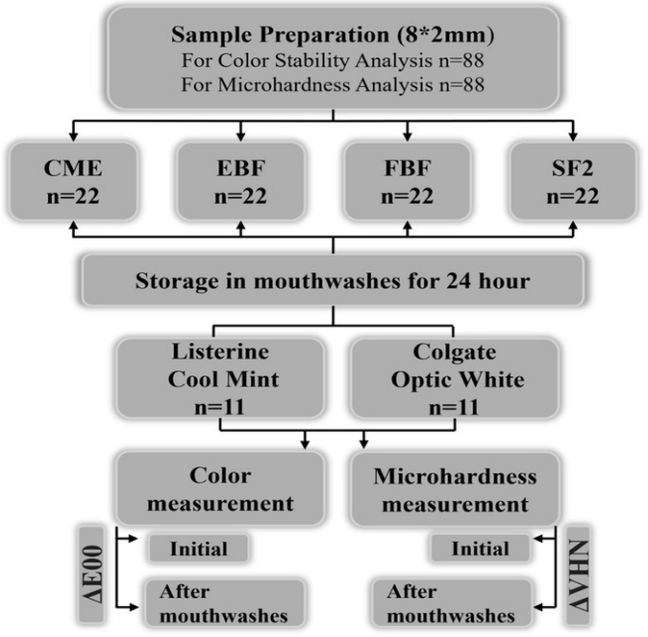

Figure 1. *Summary of the study design*

Table 2. *Characteristics of the mouthwashes used in the study*

Material	Type	Contents	pH	Manufacturer
Listerine Cool Mint	Whitening –	Aqua, Alcohol, Sorbitol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Eucalyptol, Methyl Salicylate, Thymol, Sodium Benzoate, Menthol, Aroma	3.89	Johnson & Johnson, Neuss, DE, Germany
Colgate Optic White	Whitening+	Aqua, Glycerin, Propylene Glycol, Sorbitol, Tetrapotassium Pyrophosphate, Polysorbate 20, Tetrasodium Pyrophosphate, Zinc Citrate, PVM/MA Copolymer, Aroma, Benzyl Alcohol, Sodium Fluoride, Sodium Saccharin	4.92	Colgate – Palmolive Swidnica, Poland

The samples were prepared in Teflon molds of 2 mm depth and 8 mm diameter. The resin composite was placed in the Teflon mold on a glass slide, gently pressed over the transparent strip and the excess resin was removed with a spatula. Then, a glass slide was placed on top, and the sample was polymerized. Polymerization was performed with a third-generation LED curing unit (Valo Grand, Ultradent Products, South Jordan, USA) in standard mode (1000 mW/cm^2 for 20 s). The tip of the curing device touched the glass to standardize the exposure distance during polymerization of all samples. For surface standardization, samples were polished by the same researcher using aluminum oxide discs (Sof-Lex Discs, 3M, St.Paul, MN, USA) of coarse, medium, fine and super fine sizes, respectively, for 5 seconds each at 10.000 rpm. The prepared samples were kept in lightproof bottles in distilled water at 37°C for 24 hours and the post-polymerization period was completed.

Prepared composite resin samples were divided into 2 groups according to mouthwashes. For each type of resin composite, half of the samples were stored in Listerine Cool Mint and the other half in Colgate Optic White (n=11). The samples were kept in lightproof bottles containing 20 ml of mouthwash solution for 24 hours. The bottles were numbered in order of sample. The pH of the mouthwashes was checked with a pH meter (Mettler-Toledo GmbH, Schwerzebbach, Switzerland) during the experiment (Listerine pH=3.89 and Colgate pH=4.92).

Color coordinates of the samples in the CIEL*a*b* system were measured from the upper surfaces with a spectrophotometer (VITA Easyshade V, VITA Zahnfabrik, Bad Säckingen, Germany) on a standard white background at baseline and after 24 h storage in the mouthwashes. L* indicates lightness and ranges from 0 for black to 100 for white, a* indicates green (a*<0) or red (a*>0) color components and b* indicates blue (b*<0) or yellow (b*>0) color components (21). To minimize the effect of environmental conditions, the measurements were performed by the same clinician under D65 standard light, with the tip of the device placed perpendicular (90°) to the sample surface, and the device was calibrated at each measurement. The color of the samples was measured 3 times and the average L, a, b values were recorded. Second color measurements followed the same protocol as the beginning. The following CIEDE2000 formula was used to calculate the color change (21):

$$\Delta E_{00}=\sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2+\left(\frac{\Delta C}{K_C S_C}\right)^2+\left(\frac{\Delta H}{K_H S_H}\right)^2+R_T\left(\frac{\Delta C}{K_C S_C}\right)\left(\frac{\Delta H}{K_H S_H}\right)}$$

In this formula, the letters "Δ_L", "Δ_C" and "Δ_H" indicate the differences in brightness, chroma and hue between the initial and subsequent measurements, respectively. S_L, S_C and S_H are functions used to calibrate the lack of visual inhomogeneity for the aspects of lightness (L), chroma (C) and hue (H). The environmental correction parameters used, K_L, K_C and K_H, were set to 1. RT is a rotation function included to consider the interaction between chroma and hue differences in the blue region.

Microhardness measurements of the samples were carried out baseline and after storage in mouthwashes using a Vickers device (Duramin, Struers, USA) under a load of 2.94N for 15 seconds at ×40 magnification. Three measurements were obtained for each sample, from the right, left and center of the upper surface and their averages were recorded. The change in microhardness was calculated with the formula $\Delta VHN=VHN_{after} - VHN_{before}$. Negative values in the results indicate a decrease in microhardness.

Data were analyzed in SPSS (v22.0, IBM-SPSS Inc. Chicago, Illinois, USA) software. In the evaluated parameters, the conformity of the data belonging to each group to normal distribution was determined with Shapiro Wilk Test, and the homogeneity was determined with Levene Test. The effects of composite material type, mouthwash type and composite*mouthwash interaction on color stability and

microhardness were examined using Two-Way ANOVA. Differences of materials for each mouthwash in terms of ΔE00 and ΔVHN were examined with One-Way ANOVA. Pairwise comparisons were evaluated by Post-hoc Tukey and Post-hoc Tamhane's T2 test. Differences in color change between two mouthwashes were determined with Independent-Samples t test. The microhardness of each material at the baseline and after storage in the mouthwashes was examined with different Paired-Samples t test. (α=.05, statistically significant).

3. RESULTS

The color change (ΔE00) findings of the tested materials in the mouthwashes are listed in Table 3. The effects of material type (p=.016) and mouthwash type (p=.008) on color change were statistically significant, while the material*mouthwash interaction (p=.251) was not significant (Table 4).

Table 3. *Color changes (ΔE00) and microhardness change (ΔVHN) of tested materials in mouthwashes*

Parameter	Materials	Mouthwashes		p value*
		Listerine Cool Mint	Colgate Optic White	
ΔE00	CME	0.99±0.38 [a]	0.89±0.29 [ab]	.533
	EBF	1.62±0.75 [a]	1.39±0.79 [a]	.483
	FBF	1.48±0.90 [a]	0.71±0.37 [b]	.017
	SF2	1.34±0.45 [a]	1.08 ±0.35 [ab]	.148
	p value**	.151	.02	
ΔVHN	CME	-5.10±2.37 [a]	-3.60±1.16 [a]	.075
	EBF	-4.77±1.05 [a]	-4.10±3.23 [a]	.530
	FBF	-4.10±2.03 [a]	-4.05±1.60 [a]	.945
	SF2	-5.73±2.45 [a]	-4.64±2.03 [a]	.270
	p value**	.324	.730	

Different lowercase letters in the columns represent statistical significance, while at least one of the same letters indicates no significant difference (Separately for the ΔE00 and ΔVHN parameters). Lettering applies to columns only. Independent t test, **One-Way ANOVA. Statistical significance p<.05*

Table 4. *Two-Way ANOVA test results for color change and microhardness*

ΔE00	Sum of Squares	Mean Square	F value	p value
Materials type	3.725	1.242	3.637	.016*
Mouthwashes type	2.537	2.537	7.432	.008*
Materials*Mouthwashes	1.427	0.476	1.394	.251
VHN	**Sum of Squares**	**Mean Square**	**F value**	**p value**
Materials type	9730.496	3243.499	171.958	.000*
Mouthwashes type	3.641	3.641	0.193	.662
Materials* Mouthwashes	225.439	75.146	3.984	.011*

**Statistically significant. p<.05*

When the color change of the materials in the mouthwashes was examined, the difference between of the materials in Listerine Cool Mint mouthwash was not significant (p=.151), but the difference between of the materials in Colgate Optic White mouthwash was significant (p=.02). The least discoloration was obtained in FBF (0.71±0.37) kept in Colgate Optic White, which was not significantly different when compared to CME (0.89±0.29) and SF2 (1.08±0.35) (p>.05). This value was significantly lower compared to EBF (1.39±0.79) (p<.05) (Table 3).

Among the tested materials, discoloration of FBF was significantly lower in Colgate Optic White mouthwash (0.71±0.37) compared to Listerine Cool Mint (1.48±0.90) (p=.017). For the other materials, discoloration levels were slightly lower in Colgate Optic White than in Listerine Cool Mint, however, the differences were not statistically significant (p>.05) (Table 3)

The effects of material type (p<.001) and material*mouthwash interaction (p=.011) on microhardness were statistically significant, while the mouthwash type (p=.662) wasn't significant (Table 4). The microhardness of the tested materials at the baseline and after storage in mouthwashes are shown in Table 5. The microhardness of the materials decreased significantly after 24 hours of storage in both mouthwashes compared to the baseline state (p<.05). For both mouthwashes, the difference in microhardness between the materials was found significant (p<.05). The highest microhardness value was obtained in the Listerine Cool Mint group of SF2 (baseline: 74.34±3.34, after: 68.60±3.69, respectively), the lowest microhardness value was obtained in the Colgate Optic White group of CME (baseline: 45.14±2.22, after: 41.54±1.92). The microhardness order between the materials in Colgate Optic White mouthwash was SF2>FBF>EBF>CME. The order in Listerine Cool Mint was SF2>FBF>CME>EBF, however, the difference between the microhardness of CME and EBF in Listerine Cool Mint was not statistically significant (p>.05).

Table 5. *Microhardness values of the tested materials baseline and after mouthwashes*

Materials	Listerine Cool Mint		p value*	Colgate Optic White		p value*
	Baseline	After		Baseline	After	
CME	51.09±4.79 [a]	45.99±5.03 [a]	.000	45.14±2.22 [w]	41.54±1.92 [w]	.001
EBF	48.50±4.70 [a]	43.74±4.17 [a]	.000	51.88±3.10 [x]	47.77±3.18 [x]	.006
FBF	64.98±6.05 [b]	60.87±7.05 [b]	.000	67.27±3.31 [y]	63.21±4.00 [y]	.018
SF2	74.34±3.34 [c]	68.60±3.69 [c]	.000	72.94±4.91 [z]	68.30±3.85 [z]	.023

Different lowercase letters (a, b, c, d) in the same column indicate significant differences between the materials in Listerine Cool Mint mouthwash. Different lowercase letters (w, x, y, z) in the same column indicate significant differences between the materials in Colgate Optic White mouthwash. Lettering applies to columns only. Paired-samples t test. Statistical significance p<.05*

The changes in microhardness (ΔVHN) of the tested materials in the mouthwashes are listed in Table 3. The difference between the materials in terms of ΔVHN was not significant (p>.05). Besides, while the differences were not significantly different, the decrease in microhardness was greater in Listerine Cool Mint when compared to Colgate Optic White but it wasn't statistically significant (p>.05).

4. DISCUSSION

Mouthwashes are recommended to patients under the supervision of a dentist for the control of periodontal

diseases and oral health (34); however, considering that these products are sold without a prescription in the dental market worldwide, they can often be used uncontrolled according to individuals' own preferences and application methods (24). Therefore, examining the effects of mouthwashes on resin composites, which are widely used restorative materials in the mouth, is an important issue in terms of the reliability of these products. According to the results of the current study, the first null hypothesis was rejected because the color stability of the tested materials was statistically significantly affected by the type of materials and the mouthwashes (Table 4).

All the tested composites tested in this study exhibited numerically greater discoloration in Listerine Cool Mint compared to Colgate Optic White; however, the increase in staining in Listerine Cool Mint mouthwash was statistically significant only in FBF (Table 3). Resin composites can be intrinsically or extrinsically colored due to aging from oral conditions or exposure to external agents such as mouthwashes (24). Previous studies have reported that acidic pH and alcohol content in mouthwashes can cause deterioration of the surface smoothness of composite resins and increased stainability, depending on the concentration (35-37). Staining after exposure to mouthwashes may occur internally due to rapid degradation of the polymer matrix resulting from the formation of alcohol and carboxylic acids resulting from hydrolysis of ester bonds in the materials due to the low pH (30), or externally due to adsorption of colored pigments from mouthwashes onto the surface of the composites (24,38). Both tested mouthwashes in this study were blue in color, however the pH of Listerine Cool Mint (3.89) was lower than that of Colgate Optic White (4.92). Additionally, it has been reported that tetra potassium pyrophosphate and tetrasodium pyrophosphate, which are also present in the composition of Colgate Optic White, are included in HP-free whitening mouthwashes to prevent stain adhesion (28). The greater staining observed in the materials tested in this study with Listerine Cool Mint mouthwash may be due to the lower pH and higher alcohol concentration (21.6%) of this mouthwash, as well as and the pyrophosphate content in Colgate Optic White, which prevents stain adhesion.

Regarding the findings on a per-material basis for color stability, no difference was observed between the materials in Listerine Cool Mint, while a significant difference was found between the materials in Colgate Optic White. The least discolored material was FBF, and its level of discoloring was significantly lower than EBF. The color change in the samples can be attributed to different resin formulations (26,39). It has been reported that materials using urethane dimethacrylate in the resin matrix exhibited more color stability than others due to their lower water absorption properties (40). Additionally, for the performance of resin composites in aqueous environments such as mouthwash solutions, uniform particle distribution throughout the polymer network in essential (41,42), as voids or unbonded areas at the filler/matrix interface can increase the water absorption of the composites (26). The smaller the filler particle, the less water absorbed by the polymer network, resulting in less disruption of the interface matrix/particle and hence lower color change (43). Among the tested materials, FBF, which showed the lowest coloration, was the only material containing UDMA without Bis-GMA in the resin matrix. Furthermore, the filler particle size (4-11nm) was lower than the other materials and the filler volume percentage (58.4%) was lower . This indicates that the particles are better compacted and can perform more stably in liquid environments. All of these may have contributed to the less staining of FBF than other materials in the current study.

The clinical perceptibility and acceptability of the coloration findings presented by the materials in the mouthwashes are important in terms of the contribution of the results to oral conditions. In this study, the 50:50% perceptibility threshold (ΔE_{PT}=0.8) and 50:50% acceptability threshold (ΔE_{AT}=1.8) reported by Paravina et al. were used to evaluate color changes (44). Morais Sampaio GA et al. reported in their systematic review that most studies under different in vitro conditions concluded that mouthwashes do not cause clinically unacceptable color changes in composite resins (42). Consistent with the results of previous studies, none of the materials in the current study exhibited unacceptable discoloration. In their study, where Toz Akalin et al. tested a Listerine-derived mouthwash with similar content to our study with a 12-hour immersion period, Sonicfill showed a color change above the clinically acceptable threshold, unlike the current study (45). The differences in results may be attributed to variations in the polishing procedures of the samples. Surface roughness can influence outcomes by facilitating the precipitation of coloring agents from the mouthwash onto the sample surface. The results of studies compare the discoloration levels of bulk-fill composites with conventional composites vary from lower (46) to higher (24). In this study, there was no significant difference in the discoloration levels of bulk-fill composites compared to the control group (CME), which was the conventional composite. Differences in results may be due to the composition of the tested materials and the behavior of the material in various mouthwashes. In addition, the fact that EBF with low viscosity is significantly more discolored than FBF with high viscosity bulk-fill is consistent with the results of previous studies in the literature (46). This may be related to the TEGDMA composition, which provides lower viscosity and at the same time causes more water absorption and solubility (43,46).

In the second step of the experiment, the effect of mouthwashes on the microhardness of the tested materials was investigated. The findings were that both mouthwashes significantly reduced the microhardness of the materials compared to the baseline (for all groups $p<.05$, Table 5), so the second null hypothesis of the study was rejected. It has been previously reported that mouthwashes contain water, essential oils, emulsifiers, alcohol and sometimes hydrogen peroxide (in the case of whitening mouthwashes), and this can result in hydrolysis of ester bonds in the polymer matrix of composite resins, softening of the polymer matrix and a

decrease in microhardness (15,26,27,36). In this context, the decrease in microhardness of the tested materials after 24 hours of storage on both mouthwashes was an expected result. Considering the experimental procedure and material variety, it is difficult to compare the findings with previous studies; however, in line with our study, Alessa NA reported a significant decrease in microhardness in low pH mouthwashes post-immersion compared to pre-immersion (47). Similarly, Jyothi K. et al. (48) and Miranda Dde et al. (36) reported a significant decrease in microhardness, especially for alcohol-containing mouthwashes. Hamdy et al. (15) reported that Colgate Optic White mouthwash significantly reduced microhardness compared to artificial saliva, while Lepri et al. (39) reported that mouthwashes did not significantly differ from artificial saliva in terms of microhardness in their studies using Listerine. It has been reported that 24-hour storage under in vitro conditions simulates two years of clinical, assuming 2 minutes of daily mouthwash use is considered (49). When comparing the results with previous studies, it is important to note that the immersion protocol and duration are important factors for microhardness due to the aging of composite resins. In this study, the highest microhardness was obtained in SF2 both baseline and after storage in mouthwashes. The obtained result can be attributed to the increased filler content of SF2. The microhardness of the conventional composite CME was significantly lower than that of the other bulk-fill composites in the baseline and post-treatment measurements in two mouthwashes. This result may be related to the fact that bulk-fill composites contain translucent fillers to increase the depth of polymerization, which likely results in higher monomer conversion (7).

When examining the microhardness changes (ΔVHN) in the mouthwashes of the materials, the decrease in microhardness of all materials was slightly higher in Listerine Cool Mint compared to Colgate Optic White, but the difference between the two mouthwashes was not significant. Since the result for Listerine Cool Mint covers all materials, we can say that the third null hypothesis of the study was partially rejected. The lower pH and higher alcohol content of Listerine Cool Mint may have been effective in the obtained result. In addition, the HP-free form of Colgate Optic White was used in the study. Hamdy et al. (15) tested the HP-containing form of the same mouthwash and found a greater decrease in microhardness. Different forms of the same product on the market may cause different results due to chemical composition.

When the changes in microhardness (ΔVHN) were evaluated for each material, SF2 exhibited the greatest reduction in microhardness in both mouthwashes, although the differences were not statistically significant. It was an interesting finding that SF2 exhibited the highest hardness values both at baseline and post-immersion measurements and also demonstrated the greatest decrease in microhardness. Despite the increased filler content of Sonicfill2, it has been reported that sonic activation during placement significantly increased the internal void volume of the material (50). This may affect the matrix stability in liquid environments, leading to increased monomer release and a further decrease in microhardness, and may explain the results obtained in the present study.

Mouthwash exposure is an important factor to consider in the long-term performance of restorative materials. Although the mouthwashes tested in this study did not cause unacceptable changes in the color stability of the materials, they significantly reduced microhardness. Clinically, a decrease in microhardness may compromise the material's wear resistance, leading to increased surface roughness (42). This, in turn, can promote plaque accumulation, staining of the restoration, and ultimately reduce its longevity. Patients should be advised to avoid tooth brushing immediately after using mouthwashes, particularly those with low pH, to minimize potential adverse effects on restorative materials (15). When evaluating the clinical significance of the results obtained in this in vitro study, it is necessary to consider the limitations of the study such as the use of artificial saliva, the absence of brushing simulation, thermal cycles, and the lack of representation of patient' nutritional habits. Various intraoral factors and patient behaviors may either increase or mitigate the effects of mouthwashes on restorative materials.

5. CONCLUSION

Within the limitations of the study, the conclusions can be summarized as follows;

1. Although the color stability of the tested materials varied depending on the examined mouthwashes, the color changes were at an acceptable level for both mouthwashes. In this context, it can be suggested that the examined mouthwashes are safe in terms of color stability of the materials; however, the results should be validated by studies representing longer periods of clinical use.
2. The decrease in microhardness is a significant finding in terms of the effect of mouthwashes on the long-term clinical success of restorative materials. Therefore, the uncontrolled and prolonged use of mouthwashes should be avoided.
3. The discoloration and microhardness of the same material differed depending on the mouthwashes. Therefore, the restorative material selection should be based on the specific clinical situation, including the location of the restoration (anterior/posterior) and the expected performance properties.

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Clinical and Experimental Health Sciences

Attitudes Towards Breastfeeding and Related Factors Among Mothers with Infants Aged 0-6 Months

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ABSTRACT

Objective: The study aims to evaluate the attitudes towards breastfeeding and the related factors among mothers with infants aged 0-6 months.

Methods: This descriptive study was conducted with 455 voluntary mothers who have infants aged 0-6 months. The research data were collected using the Descriptive Information Form and the Breastfeeding Attitude Evaluation Scale (BAES).

Results: The mean total score on the BAES was 110.2±14.2. It was found that 87.0% of the mothers had breastfeeding knowledge, and 90.3% were positively influenced by the breastfeeding process. Additionally, 34.4% of the mothers breastfed for 2-3 months, 25.6% for 0-1 month, 25.4% for 6 months, and 14.6% for 4-5 months. The top three sources of support were partners (78.9%), midwives/nurses (78.7%), and the mothers' own mothers (76.0%). Mothers who had a university education, delivered vaginally, were not negatively influenced by breastfeeding, had no negative sources around them, and had partner support had higher BAES scores ($p<.05$).

Conclusion: Mothers have breastfed knowledge and a positive attitude towards breastfeeding. However, breastfeeding rates remain low. Midwives and nurses should provide support in clinical practice to enhance positive attitudes towards breastfeeding and breastfeeding success, considering the mothers' individual characteristics, needs, and experiences.

Keywords: Breastfeeding, breastfeeding attitude, mother, infant

1. INTRODUCTION

Exclusive breastfeeding is recommended for the first six months. However, in developing countries, only 39% of infants under six months old are exclusively breastfed, and just 58% continue breastfeeding until 20-23 months (1). The rates of exclusive breastfeeding for the first six months are 1% in the UK, 16.4% in the USA, 12% in Azerbaijan, 16% in Afghanistan, 51% in China, and 53% in Egypt (2). Given the Türkiye Health Statistics Yearbook 2023, the exclusive breastfeeding rate for the first six months in Türkiye is 40.7% (3).

Various factors influence breastfeeding behavior, attitudes, and success, including the mother's sociodemographic and obstetric characteristics, psychological state, beliefs, habits, family and environmental support, perceptions of breastfeeding, and access to healthcare services (4, 5).

Breastfeeding is a cultural phenomenon for mothers from mental, social, and societal aspects. Current evidence suggests providing community-based breastfeeding support to women (6), as well as counseling supporting and encouraging mothers and their family members (7). Mothers receiving family and environmental support, having the backing of their partners, and learning positive breastfeeding experiences from their surroundings are more successful in breastfeeding (8,9). Particularly, the roles of midwives and nurses in care, education, and counseling are crucial for breastfeeding support (10). This study aims to evaluate the attitudes towards breastfeeding and the related factors among mothers with infants aged 0-6 months.

For this purpose, the questions to be answered are as follows:

1. What are the breastfeeding rates among mothers?

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2. What are mothers' attitudes toward breastfeeding?
3. Do mothers' attitudes toward breastfeeding differ according to certain variables?

2. METHOD

2.1. Study Type

This descriptive study was conducted in a public hospital located in a city in northern Türkiye.

2.2. Study Universe and Sample

The study universe consisted of voluntary mothers with infants aged 0-6 months who visited the newborn and pediatric clinics of the relevant hospital between December 2022 and April 2023 and did not have any physical/psychological illnesses. Mothers who did not know how to read or write Turkish, had babies older than 6 months, had communication difficulties, had a physical or psychological illness, and did not want to participate in the study were excluded from the study. The sample size was determined using the G*Power 3.1.9.7 program. Considering the mean total Breastfeeding Attitude Evaluation Scale (BAES) score (107.95±12.74) reported by Güney and Uçar (2018) (11), the effect size was calculated as d=0.15, and the power of the test was set at Power (1-β err prob)=0.85 for a two-tailed hypothesis method. A confidence interval was set at 95% and a margin of error at 5%. Then, the minimum sample size was determined to be 420. Considering potential data losses, the study was completed with 445 mothers.

2.3. Data Collection Method and Instrument

"Descriptive Information Form" and "Breastfeeding Attitude Evaluation Scale (BAES)" were used as data collection tools. The data were collected through face-to-face interviews with mothers who visited the newborn and pediatric clinics and had infants aged 0-6 months.

***Descriptive Information Form**:* The form, created by the researchers based on literature review (3,6,11), includes 31 questions about demographic and obstetric characteristics, certain characteristics related to breastfeeding, environmental and partner support during breastfeeding, and problems experienced with breastfeeding.

Breastfeeding Attitude Evaluation Scale (BAES): Developed by Arslan Özkan in 1999, the scale was revised and republished in 2015 (12). The scale consists of 46 items on a 5-point Likert scale, with responses ranging from "Strongly Agree" to "Strongly Disagree," scored as 4-3-2-1-0 respectively. Positive items (3, 4, 6, 7, 8, 11, 13, 15, 19, 23, 24, 26, 27, 28, 29, 30, 31, 32, 37, 38, 42, 43) are scored as 4-3-2-1-0, while negative items (1, 2, 5, 9, 10, 12, 14, 16, 17,18, 20, 21, 22, 25, 33, 34, 35, 36, 39, 40, 41, 44, 45, 46) are reverse scored. The total score ranges between 0 and 184, with higher scores indicating more positive attitudes. In the validity and reliability study of the scale, the Cronbach's alpha value was reported as .63 (12). In this study, the Cronbach's alpha coefficient of the scale was found to be .91.

2.4. Data Analysis

Research data were analyzed using SPSS 22.0 software and descriptive analyses (number, percentage, mean, standard deviation, minimum, maximum). The skewness and kurtosis values for the total BAES score were checked, and since these values were within ±1, a normal distribution was assumed. The independent samples t-test was used to compare independent two-group variables for the BAES total score mean. One-Way Analysis of Variance (ANOVA) was used to compare three or more independent groups. In cases with differences found in ANOVA, the LSD post-hoc multiple comparison method was used. The relationship between quantitative variables and scale scores was analyzed using Pearson's Correlation test. The significance level was set at $p<.05$.

2.5. Ethical Considerations

Necessary ethical approval for the study was obtained from Tokat Gaziosmanpaşa University Social and Humanities Research Ethics Committee (Approval date:16.11.2022; Number: E.218723) indicating that the study conformed with the provisions of the Helsinki. Administrative permission, written and verbal consent of participants, and permission for the scale were also obtained.

3. RESULTS

Some demographic and obstetric characteristics of the participants are described (Table 1). Of mothers, 78.9% received support from their spouse, 78.7% from midwives/nurses, 76.0% from their mothers, 65.6% from peers, 64.3% from social media, 50.3% from friends, 45.4% from doctors, and 36.2% from their mother-in-law. Most women reported that their spouses showed love and affection during pregnancy and the postpartum period (93.7%), were always by their side when needed (90.8%), supported breastfeeding (85.6%), participated in baby care and breastfeeding decisions (78.2%), and supported them when they were unable to breastfeed (85.6%). The most frequent breast problems in early postpartum were nipple pain (67.2%), nipple cracks (62.7%), and breast engorgement (60.9%). Besides, 86.3% of the mothers reported not being separated from breastfeeding at the hospital, and 78.9% reported that their baby did not experience any health problems at the hospital.

The mean total BAES score of the participants was 110.2±14.2 (Min=53, Max=153). University graduate mothers had higher BAES scores than mothers with high school or lower education levels ($p<.05$). Mothers who had vaginal deliveries had higher BAES scores higher than those of mothers who had cesarean deliveries ($p<.05$) (Table 2).

Table 1. *Demographic and obstetric characteristics (N=445)*

Sociodemographic Characteristics	Min-Max	x̄ ±SD
Mother's age	21-43	28.60±4.20
Length of marriage	0-19	4.10±3.20
Spouse's age	21-50	30.90±4.50
Age of first marriage	17-35	23.90±3.30
Age of first pregnancy	17-38	25.10±3.60
Obstetric Characteristics		**n (%)**
Parity	Primiparous	266 (59.8)
	Multiparous	179 (40.2)
Intentional Pregnancy	Yes	355 (79.8)
	No	90 (20.2)
Delivery	Vaginal	221 (49.7)
	Cesarean	224 (50.3)
Breastfeeding duration	0-1 months	114 (25.6)
	2-3 months	153 (34.4)
	4-5 months	65 (14.6)
	6 months	113 (25.4)
Breastfeeding Knowledge	Yes	387 (87.0)
	No	58 (13.0)
Positively affected	Yes	402 (90.3)
	No	43 (9.7)

Mothers reporting not being negatively affected in terms of breastfeeding and those indicating the absence of negative sources in their environment have higher BAES scores (p<.05). (Table 2). There is no significant difference in BAES scores according to the type of environmental support provided to mothers (p>.05).

Women reporting that their spouse supported breastfeeding, participated in baby care, and supported them when they had breastfeeding difficulties due to breast problems have higher BAES scores (p<.05). Conversely, women who reported that their spouse did not support breastfeeding, did not participate in baby care, and did not take responsibility in baby care have lower BAES scores (p<.05) (Table 3).

Mothers who experienced breast engorgement problems have higher BAES scores than those who did not experience this problem (p<.05). Moreover, mothers who did not separate from breastfeeding in the hospital and whose newborns did not experience health problems have higher BAES scores (p<.05) (Table 4).

Table 2. *Comparison of baes scores by demographic, obstetric and breastfeeding characteristics (N=445)*

Characteristics		n	χ ±SD	Test	p
Sociodemographic Characteristics					
Education	*High school or lower*	124	107.2±15.9	-2.955	**.003**
	University	321	111.4±13.4		
Employment	Unemployed	312	110.1±14.3	-0.122	.903
	Employed	133	110.3±14.1		
Spouse's education	High school or lower	139	108.4±14.5	-1.737	.083
	University	306	111.2±14.1		
Spouse's employment	Unemployed	19	111.3±15.3	0.354	.723
	Employed	426	110.1±14.2		
Income level	Income<Expenses	89	107.7±14.3		
	Income=Expenses	227	111.5±14.5	2.563	.768
	Income>Expenses	129	109.5±13.6		
Obstetric Characteristics					
Breastfeeding duration	0-1 month	114	107.6±13.1	2.049	.106
	2-3 months	153	112.3±13.2		
	4-5 months	65	110.4±17.1		
	6 months	113	110.2±14.7		
Parity	Primiparous	266	109.2±14.2	-1.848	.065
	Multiparous	179	111.7±14.2		
Miscarriage	Yes	72	113.3±14.2	2.013	.065
	No	373	109.6±14.2		
Live birth	Single child	307	109.4±14.2	-1.694	.091
	Multiple	138	111.9±14.1		
Living child	Single child	308	109.4±14.2	-1.772	.077
	Multiple	137	112.4±14.2		
Delivery	Vaginal	221	111.5±13.7	2.003	**.046**
	Cesarean	224	108.8±14.6		
Breastfeeding Characteristics					
Breastfeeding Knowledge	Yes	387	110.4±14.3	0.786	.432
	No	58	108.8±13.8		
Negatively affected	Yes	169	107.6±14.5	-3.032	**.003**
	No	276	111.8±13.9		
Negative source	Yes	160	108.2±14.7	-2.471	**.014**
	No	285	111.4±13.9		
Positively affected	Yes	402	110.5±14.1	1.374	.172
	No	43	107.3±16.5		
Positive source	Yes	430	109.1±13.1	1.271	.275
	No	15	108.5±13.8		
Total BAES		445	110.2±14.2		

Independent Samples t-test and ANOVA test were used.

Table 3. *BAES scores by spousal support approaches (N=445)*

Spousal Support		n	X±SD	Test	p
My spouse showed me love and compassion during my pregnancy and the postpartum period.	Yes No	417 28	110.4±14.3 107.7±13.9	0.961	.337
My spouse and I acquired information about breastfeeding together.	Yes No	234 211	109.5±13.7 110.9±14.9	-1.064	.288
My spouse supported me whenever I had physical, social, emotional, or economic needs.	Yes No	404 41	110.5±13.8 106.7±17.7	1.666	.096
My spouse supported my breastfeeding efforts.	Yes No	429 16	110.5±14.1 102.8±15.6	2.117	**.035**
My spouse participated in the care of the baby after birth.	Yes No	348 97	111.3±13.8 107.3±15.5	2.257	**.024**
My spouse does not consider baby care to be his responsibility.	Yes No	94 351	107.6±17.4 110.9±13.3	-2.022	**.044**
My spouse and I made the decision to breastfeed together.	Yes No	306 139	110.3±14.3 109.9±14.1	0.246	.806
My spouse is not willing to participate in the care of our baby.	Yes No	90 355	106.9±15.8 111.2±13.7	-2.462	**.014**
My spouse does not want me to breastfeed our baby.	Yes No	19 426	100.6±14.4 110.6±14.1	-3.015	**.003**
My spouse supported me when I experienced breastfeeding difficulties due to breast problems.	Yes No	381 64	110.7±14.2 107.5±14.2	1.928	**.045**

Independent Samples t-test was used.

Table 4. *Comparison of BAES scores by early breast problems and some variables (N=445)*

Early Breast Problems and Some Variables	Group	n	X±SD	Test	p
Nipple Pain	Yes No	299 146	109.8±14.6 111.3±13.5	-0.825	.410
Nipple Crack	Yes No	279 166	109.7±14.4 110.9±14.2	-0.866	.387
Breast Engorgement	Yes No	271 174	111.8±14.5 107.7±13.4	3.006	**.003**
Breast Occlusion	Yes No	114 331	108.3±16.1 110.8±13.6	-1.679	.094
Breast Apnea	Yes No	52 393	108.4±14.8 110.4±14.2	-0.958	.339
Leaving Hospital Without Breastfeeding	Yes No	61 384	104.4±14.5 111.1±14.6	-3.470	**.001**
Health Problem of the Newborn	Yes No	94 351	106.4±14.1 111.2±14.3	-2.921	**.004**

Independent Samples t-test was used

4. DISCUSSION

In this study, it was observed that 25.4 % of mothers breastfed their babies for 6 months (Table 1). The rate of exclusive breastfeeding was reported to be 60% by Yılmaz (2017) and 49.4% (12) by Güney and Uçar (2018) (13). According to WHO data, the rate of exclusive breastfeeding for infants under six months was 37% in 2017 and 40% in 2022 (14). Türkiye Health Statistics Yearbook 2023 data showed that only 40.7% of infants were exclusively breastfed for the first six months (3). It can be seen that the breastfeeding rates of mothers are still quite low.

It was determined in this study that the majority of women had breastfeeding knowledge and were positively influenced for breastfeeding. Consistent with the literature, women's breastfeeding knowledge is high (15). Despite the high breastfeeding knowledge, the low breastfeeding rates are also noteworthy. It is thought that merely having knowledge is not sufficient for breastfeeding rates; multidimensional motivational sources are important to improve breastfeeding behaviors.

Women primarily received support from their spouses. Most women reported that their spouses showed love and affection during pregnancy and the postpartum period, were always by their side when needed, supported breastfeeding, participated in baby care and breastfeeding decisions, and supported them when they were unable to breastfeed (Table 2). It has been observed that women receive support primarily from their spouses during the breastfeeding period (14,16), and this support is very important.

In the present study, 78.7% of women received breastfeeding support from midwives and nurses. Dündar and Özsoy (2020) reported this rate at 49.5% (17) and Arbour et al. (2019) (18) at 61%. The literature reports different types of breastfeeding support provided by midwives and nurses. The studies reported different results regarding breastfeeding support from midwives and nurses (19,20). The differences in results may be due to sample diversity.

In this study, 76% of women received breastfeeding support from their mothers, while 36.2% received support from their mothers-in-law. Yılmaz and Taşpınar (2017) reported mother and mother-in-law support at 71.8% (13). The difference between studies can be explained by the individual approach and different birth experiences of the mothers or mothers-in-law.

Among the participants, 65.6% received support regarding breastfeeding from peers, 64.3% from social media, and 50.3% from friends. The studies reported different results regarding the support received from peers, social media, and friends (17,21). Women's social media usage habits and peer or friend relationships may vary.

In this study, the most common breast problems experienced by women during the postpartum period were nipple pain (67.2%), nipple cracks (62.7%), and breast engorgement (60.9%). Although the prevalence of breastfeeding problems during the postpartum period varies by population (22,23), similar issues are observed. Breastfeeding support is critical in resolving breast problems.

The mean total score for mothers on the BAES was 110.2±14.2 (Table 2). Most of the mothers have positive breastfeeding attitudes. Güney and Uçar (2018) reported a mean score of 107.95±12.74 (11), and Kurnaz and Uyar (2021) reported 110.86±18.14 (24). Mothers' breastfeeding attitudes have become more positive in recent years, consistent with the literature.

Breastfeeding success increase with increasing education levels (13,24). However, a study in Iran revealed that women with a high school education or higher were more likely to stop breastfeeding earlier than illiterate women (25). Differences in education level affect individuals' attitudes from awareness and understanding aspects (24). This study revealed that mothers with a university education had a more positive breastfeeding attitude. However, no significant difference was found between breastfeeding attitudes and other demographic variables (Table 2). Similarly, maternal age was reported to not affect breastfeeding attitude and success (24). However, some studies reported that family income, the spouse's education level, and the spouse's employment status affect breastfeeding attitude (13). Breastfeeding attitudes are influenced by multiple factors, so variability in research findings is expected.

In the present study, there was no significant difference in breastfeeding attitudes by duration of breastfeeding, total number of pregnancies, number of live births, and number of living children (Table 2). Similar results in the literature indicate that the total number of pregnancies, number of live births, and number of living children do not affect breastfeeding success (24).

In this study, mothers who had vaginal deliveries showed more positive breastfeeding attitudes (Table 2). This difference is thought to be due to the impact of the mode of delivery on the bonding between mother and baby and the effects on milk secretion. Buran et al. (2022) reported higher breastfeeding success in mothers who had vaginal deliveries (25). A literature review (2021) found lower breastfeeding success in mothers who had cesarean deliveries. In comparison with natural childbirth, C-section can delay the start of breastfeeding and shorten the duration of exclusive breastfeeding. The planned C-section is considered the most critical factor affecting breastfeeding (26).

In this study, the breastfeeding attitudes of mothers who reported not being negatively influenced by their environment were more positive (Table 2). A supportive environment or positive resources encouraging breastfeeding can undoubtedly have a positive effect on a woman's breastfeeding attitude. Various studies reported that peer support positively contributes to breastfeeding process (27). It was reported that grandmothers' past breastfeeding experiences and knowledge significantly influence mothers, supporting them either positively or negatively (28). Literature indicates that the opinions and thoughts of spouses, family elders, peers, friends, midwives, nurses, and doctors regarding breastfeeding are influential (29). This study determined that the breastfeeding attitudes of women were similar regardless of the support they received from their environment. These results suggest that positive breastfeeding support, rather than the type of support, is more significant for mothers.

In this study, women who reported that their husbands supported breastfeeding, participated in baby care, and provided support when they experienced breastfeeding difficulties showed more positive breastfeeding attitudes. However, women whose husbands did not support breastfeeding, did not participate in baby care, and did not take responsibility for baby care exhibited more negative breastfeeding attitudes (Table 3). Similarly, Abageda et al. (2024) reported that mothers whose husbands did not take responsibility for baby care displayed negative breastfeeding attitudes (30). A study in Taiwan revealed that spousal support positively affected the first eight weeks of the breastfeeding period (31), whereas a study carried out in Malaysia reported that spousal support positively influenced the duration of breastfeeding (32). Despite the differences in the types of support provided by spouses, the literature indicates positive effects on initiating and maintaining breastfeeding (33).

In this study, the breastfeeding attitudes of mothers leaving the hospital without breastfeeding (13.7%) and those whose newborns had health problems (21.1%) were more negative (Table 4). A study carried out in China reported that 45% of mothers left the hospital without breastfeeding their babies (34). The rates of newborns not breastfed within the first hour postpartum were reported as 32.6% by Kurnaz and Uyar (2021) (24). Mothers who do not or are unable to breastfeed in the early postpartum period are expected to have insufficient mother-baby interaction and negative breastfeeding attitudes.

Breastfeeding problems commonly encountered during the postpartum period were reported to negatively affect breastfeeding, leading to early cessation (35). A study reported that mothers who experienced nipple pain had lower breastfeeding attitude scores than those who did not experience pain (23). Unlike previous results, this study found no significant difference in breastfeeding attitudes by problems such as nipple pain, nipple cracks, breast engorgement, or breast abscess. However, mothers experiencing breast fullness exhibited more positive breastfeeding attitudes. The positive breastfeeding attitude among mothers with breast fullness may be attributed to sufficient milk production and the belief that they can adequately feed their babies. However, the key is to ensure effective and successful breastfeeding before breast fullness occurs.

This study has some limitations. The limitations of this study include its conduct in a single public hospital located in a city center in the northern region of Türkiye. This research topic could be studied in larger samples.

5. CONCLUSION

Although mothers have knowledge about breastfeeding and a positive attitude towards it, breastfeeding rates remain low. Women receive support primarily from their husbands, midwives, nurses, and their own mothers. Mothers who are university graduates, have vaginal births, are not negatively influenced regarding breastfeeding, have no negative sources around them, and receive spousal support exhibit more positive breastfeeding attitudes. Those who leave the hospital without breastfeeding and whose newborns have health problems tend to have more negative attitudes towards breastfeeding. Ensuring and maintaining successful breastfeeding from birth and fostering positive breastfeeding attitudes among postpartum women are the responsibilities of midwives and nurses. In clinical practice, positive environmental support should be provided, considering the mothers' individual characteristics, desires, experiences, and close circles.

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Author Contributions:

Research idea: SNA,DÖ
Design of the study: SNA,DÖ
Acquisition of data for the study: SNA,DÖ
Analysis of data for the study: SNA,DÖ
Interpretation of data for the study: SNA,DÖ
Drafting the manuscript: SNA,DÖ
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Clinical and Experimental Health Sciences

Determinants of Sexual Dysfunction in Allergic Rhinitis According to the Unpleasant Symptoms Theory: A Comparative Study

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ABSTRACT

Objective: Sexual dysfunction, a key component of adults' quality of life, is under-studied in allergic rhinitis. This study aimed to compare sexual dysfunction in individuals with and without allergic rhinitis and to examine the effects of physiological, psychological, and situational factors, as conceptualized by the Theory of Unpleasant Symptoms.

Methods: This descriptive and between-groups comparative study was conducted between September 2020 and September 2021 in the otorhinolaryngology outpatient clinic. Data were collected from 249 patients with allergic rhinitis and 235 healthy individuals using validated scales that assess depression, anxiety, fatigue, sleep quality, nasal symptoms, and sexual function. Logistic regression analysis was used to identify factors associated with sexual dysfunction.

Results: Compared to the healthy group, individuals with allergic rhinitis had more sexual dysfunction, sleep problems, fatigue, depression, anxiety, and nasal and non-nasal symptoms ($p<.01$). Based on the results of logistic regression analysis, factors contributing to sexual dysfunction in women with allergic rhinitis included anxiety, depression, the presence of asthma, and the severity of physiological symptoms (Nagelkerke R^2: 0.345; Model: x^2= 18.759 p= .000). Factors affecting sexual dysfunction in men with allergic rhinitis were determined as sleep quality, depression, the severity of physiological symptoms, fatigue, and smoking (Nagelkerke R^2: 0.194; Model: x^2= 17.761; p= .023).

Conclusions: Sexual dysfunction in allergic rhinitis patients is influenced by a range of symptoms, as outlined by the Unpleasant Symptoms Theory. These findings highlight the need for targeted intervention programs to manage sexual dysfunction by addressing both illness symptoms and related factors.

Keywords: allergic rhinitis, sexual dysfunction, unpleasant symptoms theory, symptoms

1. INTRODUCTION

Allergic rhinitis (AR) is a chronic inflammatory disease of the nasal mucosa mediated by IgE, characterized by symptoms of nasal congestion, runny nose, sneezing, and nasal itching triggered by inhaled allergens. AR is among the most prevalent chronic illnesses globally, impacting 10-30% of adults and as many as 40% of children. The prevalence of asthma and allergic rhinitis continues to rise, particularly in Asian countries (1). The disturbing nature of AR symptoms can seriously affect individuals' daily activities, work performance, quality of life, and psychosocial well-being (2-5). AR is also associated with significant economic costs. The indirect costs of AR-related work and school absenteeism are estimated to be higher than those caused by diabetes, migraine, anxiety, or asthma (6-8). For these reasons, it is emphasized that AR has become an important public health problem worldwide.

Besides the nasal symptoms, individuals with AR also experience additional non-nasal symptoms like fatigue, disrupted sleep, mood disorders, anxiety, depression, and reduced work efficiency (9-11). Nasal congestion is among the most troublesome symptoms contributing to sleep disturbances, particularly in cases involving irregular

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breathing patterns such as snoring and sleep apnea (3, 12). It has been reported that sleep disorders commonly seen in individuals with AR are associated with poor quality of life, fatigue, irritability, memory deficit, anxiety and depression, and sexual dysfunction (13-15).

While numerous studies have explored the physical and social dimensions of allergic rhinitis and its impact on quality of life, sexual function, a vital aspect of adults' quality of life, has frequently been overlooked. Limited studies identified that individuals with allergic rhinitis may encounter sexual dysfunction (16-18). Even though its pathophysiology remains poorly understood, there is a suggestion that the connection between sexual dysfunction and allergic rhinitis may be bidirectional. As previously mentioned, it is believed that AR can induce sleep disorders by impacting both the quality and architecture of sleep, subsequently leading to depression, reduced libido, and organic sexual dysfunction (16, 19). In addition, it is thought that AR may directly cause sexual dysfunction through other mechanisms, including systemic inflammation. Some studies have shown that problems such as erectile dysfunction, orgasm problems, lower sexual desire and lower sexual satisfaction are more common in AR individuals than in healthy individuals (9, 16-18, 20). Accordingly, to organize these interrelated determinants and outcomes, the study is anchored in the Theory of Unpleasant Symptoms (TOUS).

The TOUS, an intermediate-level nursing theory, elucidates the nature of the symptom experience**.** It comprises three primary components: (1) the symptoms experienced by the patient; (2) the factors influencing the symptom experience; and (3) the outcomes resulting from the symptom experience (performance). Symptoms are perceived indicators of change in normal functioning. Symptoms can occur alone or often occur simultaneously with more than one symptom and interact with each other. When one symptom is not controlled, it can cause another symptom to be more severe.

According the theory the factors influencing symptom experience are classified as physiological (e.g., the presence of any diseases), psychological (e.g., mood state), and situational components (e.g., noise, or heat). The third component is based on the evaluation of functional performance (physical activity, influencing activities of daily living, etc.), role performance (social activities, activities related to work and other roles), cognitive performance (concentration, thinking, problem solving, etc.). It is explained that many or more severe symptoms will affect the functional, role and cognitive performances more negatively (21, 22).

In literature, no study on factors affecting sexual dysfunction in individuals with AR was found. To our knowledge, this is the first study to examine factors affecting sexual function in individuals with AR. Furthermore, although AR is a common chronic disease that negatively affects all aspects of quality of life, it has rarely been addressed in nursing studies. It is thought that determining factors affecting sexual function in individuals with AR will be a useful guide for nurses and other health professionals in determining interventions for the management of sexual dysfunction. The aim of this study was to compare sexual dysfunction in individuals with and without allergic rhinitis and to examine the effects of physical, psychological and situational factors according to the TOUS on their sexual dysfunction experiences. This study addresses the following research questions:

- Does sexual function differ between adults with allergic rhinitis and healthy adults?
- Within the framework of the Theory of Unpleasant Symptoms, how are physiological (e.g., sinonasal symptom severity; asthma), psychological (anxiety, depression), situational (smoking, alcohol use, employment, socioeconomic status), and symptomatic (sleep quality, fatigue) factors associated with sexual dysfunction in adults with allergic rhinitis?

2. METHODS

This was a descriptive and between-group comparative study.

2.1. Sample and Participants

The study included 249 Allergic Rhinitis (AR) patients who applied to the Ear, Nose and Throat (ENT) outpatient clinic of a training and research hospital in Antalya between September 2020 and September 2021, and 235 healthy individuals as the control group. The sample size was determined by using the known population sampling formula (N=700) and was defined as 249. Participants were selected using the purposive sampling method. The control group included healthy individuals who were aged between 18 and 55, were married, and did not have allergic rhinitis or any chronic diseases. Individuals aged 18-55 years who had been diagnosed with moderate to severe persistent allergic rhinitis (AR) through a skin prick test were enrolled in the study. Since the highest age limit was determined as 55 in studies conducted on sexuality in individuals with AR (9, 17, 23), 55 was accepted as the upper age limit in this study. Individuals with active mental illness, diabetes mellitus, chronic renal failure, and hypertension individuals with AR were excluded from the study, considering that it can affect sexual function.

2.2. The Framework of The Study

In this study, performance was determined as sexual function according to the TOUS, sexual function in individuals with AR affects physiological, psychological, and situational variables as well as other symptoms that they experience. Based on this theory, sleep problem was accepted as the primary symptom and fatigue as the secondary symptom in the study. Physiological factors in the study were defined as age and perceived health status of the patient, psychological factors as anxiety and depression, and situational factors as education, occupation, working status, economic status, using alcohol, smoking, presence of asthma, and type of treatment (Figure 1).

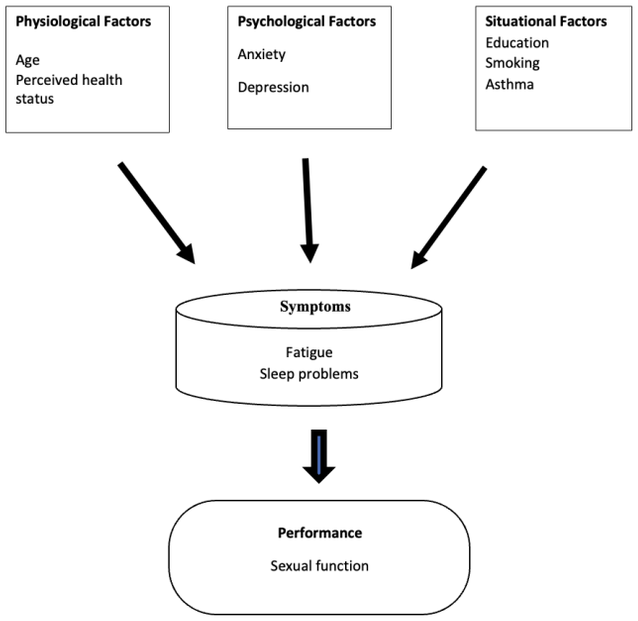

Figure 1. *Conceptual framework of this study*

2.3. Data Collection Tools

The Descriptive Information Form, consisting of 13 items, includes questions about individuals' characteristics (age, education, occupation, working status, economic status, alcohol use, smoking, presence of asthma, and type of treatment). The form was developed by the researchers based on previous literature (9, 10, 13).

The 22-item Sinonasal Outcome Test (SNOT-22) was developed by Hancı et al. in 2015. The SNOT-22 assesses the severity of both nasal and extranasal allergic rhinitis (AR) symptoms. It comprises 22 items categorized into five domains, namely rhinologic symptoms, extranasal rhinologic symptoms, ear/facial symptoms, psychological dysfunction, and sleep dysfunction. The total score that can be obtained on this scale ranges from 0 to 110 points. Higher scores on the scale indicate an increase in symptom severity and dysfunction. The Turkish version of the scale was adapted by Hancı et al. in 2015 (24, 25) . Cronbach's alpha value of the scale, which was adapted into Turkish, was 0.88, and it was determined as 0.81 in this study.

The Female Sexual Function Index (FSI) was developed by Rosen et al. (2000) to assess sexual functions in women. It consists of 19 items and 6 subdimensions (orgasm, pain, lubrication, desire, satisfaction, and arousal). It is a Likert-type scale, and each item is scored between 0 and 5. The cut-off point is 26.55. Those with a score below 26.55 are considered to have female sexual dysfunction (26). Cronbach's alpha value was calculated as 0.98 in the Turkish validity-reliability study of FSFI by Aygin and Aslan (2005) (27), and it was calculated as 0.88 in this study.

The International Index of Erectile Function (IIEF) was developed by Rosen et al. in 1997. It comprises 15 items and is designed to assess various aspects of male sexual function, including erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. Each item is scored on a scale of 0 to 5 points. The total score is then used to classify the severity of erectile dysfunction, with the following interpretations: 0-10 points indicate severe dysfunction, 11-16 points indicate moderate dysfunction, 17-21 points indicate mild to moderate dysfunction, 22-25 points indicate mild erectile dysfunction and 26-30 points indicate no erectile dysfunction.(28). In the original study, a high degree of internal consistency was reported for each of the five domains and for the total scale, with Cronbach's alpha values of 0.73 and above for the subscales, and 0.91 and above for the total scale. The Turkish adaptation of the IIEF was conducted by the Turkish Society of Andrology, and in this study, the Cronbach's alpha value for the scale was determined to be 0.85.

The Pittsburgh Sleep Quality Index (PSQI) was developed by Buysse et al. in 1989 [30]. It comprises 19 items and assesses seven subcomponents of sleep quality, including subjective sleep quality, sleep duration, habitual sleep efficiency, sleep latency, sleep disturbances, daytime dysfunction, and the use of sleeping pills. The total score is calculated by summing the scores from the seven subcomponents and can range from 0 to 21. A PSQI total score of ≤5 indicates good sleep quality, while a total score >5 suggests poor sleep quality (29). The Turkish adaptation of the PSQI was carried out by Agargün et al. in 1996, and they reported a Cronbach's alpha value of 0.80 for the scale in their study (30). In the current study, the Cronbach's alpha value was determined to be 0.78 for the PSQI.

The Fatigue Severity Scale (FSS) was developed by Lauren B. Krupp et al. in 1989. It is composed of 9 items and is used to assess the severity of fatigue. The FSS is a Likert-type scale where respondents rate their agreement with statements using a scale of 1 (strongly disagree) to 7 (strongly agree). The total score on the FSS can range from 9 to 63, with higher scores indicating greater severity of fatigue. (31). The Turkish adaptation study of the scale was conducted by Armutlu et al., and Cronbach's alpha value was calculated as 0.88 (32). The Cronbach's alpha value was calculated as 0.80 in this study.

The Beck Depression Inventory (BDI) was developed by Beck et al. in 1961. It consists of items covering 21 symptom categories, with each item offering four response options. Responses to each item are scored on a scale ranging from 0 to 3. The total score on the BDI is obtained by summing the scores of all the items. A higher total score on the BDI indicates a higher level of depression. The scores means as follows: 0-9, minimal; 10-16, mild; 17-29, moderate; 30-63, severe depression. (33) The Turkish adaptation study of the scale was conducted by Hisli in 1988. In the adaptation study, Cronbach's alpha value was calculated as 0.80 (34), and it was calculated as 0.76 in this study.

The Beck Anxiety Inventory (BAI) was developed by Beck et al. in 1993. This scale is designed to assess the frequency of anxiety symptoms experienced by an individual. It comprises 21 items, and each item is scored on a scale from 0 to 3. A higher score on the BAI indicates a higher level of anxiety. Scores on the scale means as follows: 0-17, low; 18-24, medium; ≥25, high anxiety levels (35, 36). The Turkish adaptation study of the scale was conducted by Ulusoy et al in 1998. Cronbach's alpha value of the scale was calculated as 0.92, and it was calculated as 0.84 in this study.

2.4. Data Collection

Data collection forms were applied by the researcher using face-to-face interview technique in an empty room in the outpatient clinic where the research was conducted. Filling the forms took approximately 20-25 minutes. The healthy control group consisted of parents of students at the school where the researchers' children attended. First, parents were informed about the study in school WhatsApp groups and the questionnaires were administered online to those who agreed to consent to participate in the study.

2.5. Data Analysis

SPSS 23 (Statistical Package of Social Science) software package was used for analyzing. The findings were statistically evaluated using a 95% confidence interval at a 5% significance level. Subject demographics and scale scores were reported using descriptive statistics. The determinants of sexual function in individuals with AR were assessed using logistic regression analysis. Variables identified by TOUS were analyzed in the logistic regression model. The forward selection method was used to decide which variables would be included in the model.

2.6. Ethical Considerations

The study received ethical approval from the Akdeniz University Non-Interventional Clinical Research Ethics Committee (Approval date:April 8, 2020; Number: KAEK-305). Individuals with allergic rhinitis and healthy controls were informed about the study, and both written and verbal informed consent was obtained prior to participation.

3. RESULTS

In this study, a total of 249 individuals with allergic rhinitis (AR) and 235 individuals in the healthy control (HC) group were included. The comparison of the descriptive characteristics of these two groups, as presented in Table 1, indicated that there were no statistically significant differences between the groups (p>.05).

A high-level, statistically significant difference was found between the mean scores of individuals in the AR group and HC group on the BDI, FSI, PSQI, and SNOT22 scales, between the total FSI and subgroup scores of women in both groups, and between the IIEF subgroup scores of men in both groups (p<.01). In further analysis, it was found that there was a statistically significant difference between the BAI scores of the individuals in the AR group and the BAI scores of the individuals in the HC group, and the difference was due to the individuals with moderate depression scores in the AR group (p<.05). Although individuals with AR were not treated for depression, most had moderate depression scores. In addition, individuals with AR had high rates of anxiety, poor sleep, fatigue, and sexual dysfunction (Table 2).

It was determined that male individuals with statistically highly significant AR smoked and consumed alcohol more than female individuals and that male individuals had another chronic disease (p<.01). It was found that 45.1% of female individuals and 34.6% of male individuals used leukotriene antagonists and that 7.9% of female individuals and 25.9% of male individuals received oral antihistamine treatment (p<.01) (Table 3).

Table 1. *Clinical characteristics of patients with allergic rhinitis and healthy controls*

Clinical characteristics		Allergic rhinitis (n=249) X±SD	Healthy controls (n=235) X±SD	p
Age		38.91±13.266	36.30±12.851	.934
		n (%)	**n (%)**	
Gender	Female Male	154 (51.5) 95 (51.4)	145(48.5) 90 (48.6)	.974
Educational level	Literate Primary education Secondary education Higher education	16 (69.6) 121 (51.9) 45 (47.49) 67 (50.4)	7 (30.4) 112 (48.1) 50 (52.6) 66 (49.6)	.291
Employment status	Not Working Working	163 (48.5) 86 (58.1)	173 (50.7) 62 (43.4)	.083
Occupation	Greenhouse Worker Officer Self Employed Housewife Retired Other	110 (55.8) 25 (50.0) 19 (52.8) 42 (50.6) 4 (44.4) 49 (45.0)	87 (44.2) 25 (50.0) 17 (47.2) 41 (49.4) 5 (55.6) 60 (55.0)	.604
Economic Status	Income expenditure Income= xpenditure Income expenditure	86 (50.6) 117 (53.9) 46 (47.4)	84 (49.4) 100 (46.1) 51 (52.6)	.546
Using Alcohol	Yes No	52 (51.5) 197 (51.4)	49 (48.5) 186 (48.6)	.543
Smoking	Yes No	41 (55.4) 208 (50.7)	338 (44.6) 202 (49.3)	.271

Student-t test and Ki-Kare test were used

Table 2. *Comparison of symptom and sexual function scores between patients with allergic rhinitis and healthy controls*

Measure (scale/subscale) |		Allergic rhinitis (n=249) X±SD	Healthy controls (n=235) X±SD	p
Beck Depression Inventory (BDI)		18.08±8.168	13.94±8.300	**.000**
Beck Anxiety Inventory (BAI)		22.08±8.168	20.03±9.468	**.011***
Fatigue Severity Inventory (FAI)		3.8063±1.53	2.79±.97	**.000****
Pittsburgh Sleep Quality Index (PSQI)		10.23±1.666	4.20±1.888	**.000****
Sinonasal Outcome Test (SNOT22) questionnaires		55.59±13.80	42.52±15.81	**.000****
FSFI sub-domains				
Desire		3.58±.94	4.43±1.25	**.000****
Arousal		3.67±.94	4.40±1.5	**.000****
Lubrication		3.01±1.38	3.62±1.97	**.002****
Orgasm		3.00±1.43	3.43±1.86	**.023***
Satisfaction		2.81±1.34	3.37±1.96	**.004****
Pain		2.40±.88	3.63±2.32	**.000****
FSFI **total**		18.56±3.8	22.91±6.64	**.000****
IIEF				
Erectile function		20.36±8.97	26.31±9.166	**.000****
Orgasmic function		7.59±2.56	14.42±2.71	**.000****
Sexual desire		7.07±2.12	13.17±2.11	**.000****
Intercourse satisfaction		7.76±5.12	13.80±5.18	**.000****
Overall satisfaction		7.16±2.32	13.12±2.449	**.000****
		n(%)	**n(%)**	
Depression	Minimal depression Mild Depression Moderate Depression Severe depression	99(49.0) 20(24.1) 113(66.1) 17(60.7)	103(51.0) 63(75.9) 58(33.9) 11(39.3)	**.000****
Anxiety	Mild Anxiety Moderate Anxiety Severe Anxiety	44 (39.6) 128 (55.9) 70 (56.9)	67(60.4) 101(44.1) 53(43.1)	**.009****
Fatigue	Not tired Tired	144 (40.3) 105 (82.7)	213(59.7) 22(17.3)	**.000****
Sleep Quality	Poor sleep Quality sleep	242 (84.6) 7 (3.5)	44(15.4) 191(96.5)	**.000****
FSFI (according to cut-off point ≤26.55)	Sexual dysfunction Normal sexual function	137 (73.3) 17 (15.2)	50(26.7) 95(84.8)	**.000****
IIEF (according to cut-off point ≤25)	Erectile dysfunction No erectile dysfunction	55 (57.3) 39 (39.8)	41 (42.7) 59(60.2)	**.003****

*Student-t test and Ki-Kare test were used *p<.05 ** p<.01*
FSFI: Female Sexual Function İndex, IIEF: International Index of Erectile Function

Table 3. *Distribution of smoking, alcohol use, comorbidities, and allergic rhinitis treatments by sex among patients with allergic rhinitis*

Distribution of Characteristics	Female n(%) 164 (66.9)	Male n(%) 81 (33.1)	p
Using alcohol Yes No	8(4.9) 156(95.1)	30(37.0) 51(63.0)	**.000****
Smoking Yes No	8(4.9) 156(95.1)	18(22.2) 63(77.8)	**.000****
Another chronic disease Yes No	56(34.1) 108(65.9)	7(8.6) 74(91.4)	**.000****
Presence of asthma Yes No	72(43.9) 92(56.1)	28(34.6) 53(65.4)	NS
Allergic Rhinitis Treatment Oral antihistamines Nasal corticosteroids Leukotriene Antagonists Others	13(7.9) 32(19.5) 74(45.1) 45(27.4)	21(25.9) 12(14.8) 28(34.6) 20(24.7)	**.000****

NS = nonsignificant, p values were determined using chi-squared test and t test.

The best model in logistic regression was formed in step 4 (Table 3). It was determined that women without asthma experienced 65.5% less sexual dysfunction than women with asthma. Female individuals experienced sexual dysfunction 1.070 times more as BAI scores increased, 1.066 times more as BDI scores increased, and 1.028 times more as SNOT 22 scores increased (Nagelkerke R^2: 0.345; Model: x^2= 18.759; p= .000) (Table 4).

The best model in logistic regression was formed in step 4 (Table 4). It was determined that non-smoking male individuals experienced 72.0% less sexual dysfunction than male individuals who smoked. It was found that male individuals experienced sexual dysfunction 1.014 times more as PSQI scores increased, 1.175 times more as BDA scores increased, 2.058 times more as SNOT 22 scores increased, and 2.858 times more as FAI scores increased (Nagelkerke R^2: 0.194; Model: x^2= 17.761 p= .023) (Table 5).

Table 4. *Determinants of factors affecting sexual dysfunction in women with allergic rhinitis*

Independent variables	β	SE	Wald	p	OR	95% CI	
Constant	-4.622	1.466	9.932	.002	0.072		
BAI	.068	.031	4.681	.030	1.070	1.006	1.138
BDI	.064	.027	5.846	.016	1.066	1.012	1.123
Asthma (None)	-1.035	.389	7.086	.008	.355	.166	.761
SNOT22	.079	.026	9.163	.002	1.082	1.028	1.139

*Variables included in the multivariable logistic regression model were age; education; occupation; employment status; socioeconomic status; alcohol use; smoking; physician-diagnosed asthma; type of allergic rhinitis treatment; and total scores on the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Female Sexual Function Index (FSFI), Pittsburgh Sleep Quality Index (PSQI), and the 22-item Sinonasal Outcome Test (SNOT-22).The most significant model was formed in step 5. Nagelkerke R2:0.345 Model: x2= 18.759 p= .000 *p<.05, **p<.01 CI: confidence interval. OR: odds ratio. SE: Standart Error*

Table 5. *Determinants of affecting sexual dysfunction in men with allergic rhinitis*

Independent variables	β	SE	Wald	p	OR	95% CI	
Constant	2.917	.725	16.206	.000	.054		
PSQI	.113	.050	5.025	.025	1.120	1.014	1.236
BDI	1.188	.524	5.141	.023	3.280	1.175	9.160
SNOT22	.722	.476	2.304	.129	2.058	.810	5.229
FSI	1.050	.425	6.105	.013	2.858	1.243	6.575
Smoking (No)	-1.273	.675	3.560	.059	.280	.075	1.051

*Variables included in the multivariable logistic regression model were age; education; occupation; employment status; socioeconomic status; alcohol use; smoking; physician-diagnosed asthma; type of allergic rhinitis treatment; and total scores on the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Female Sexual Function Index (FSFI), Pittsburgh Sleep Quality Index (PSQI), and the 22-item Sinonasal Outcome Test (SNOT-22). Forward selection method was used. The most significant model was formed in step 5. Nagelkerke R2:0.194, Model: x2= 17.761 p= .023 *p<.05, **p<.01 CI: confidence interval OR: odds ratio. SE: Standart Error*

4. DISCUSSION

In the study, the sexual functions of individuals with AR were compared with healthy individuals, and the factors affecting sexual function were examined based on the TOUS. In the study, sexual dysfunction was more common in both men and women in the AR group than in the healthy control group (Table 2). These findings are consistent with previous studies on individuals with AR (9, 16-18, 20). According to research findings, factors affecting sexual function were determined as the patient's health status (SNOT-22) (physiological factors), anxiety, depression (psychological factors), asthma, smoking (situational factors), fatigue, and poor sleep (symptomatic factors) (Tables 4 and 5). Therefore, factors affecting the sexual dysfunction of individuals with AR will be discussed in this section.

The patient's perceived health status, which is one of the physiological factors, was evaluated with SNOT-22 in the study. An increase in SNOT – 22 score means that both nasal and non-nasal physiological symptoms are experienced intensely. In the study, as the severity of nasal and non-nasal physiological symptoms experienced by both male and female individuals due to AR increased, their sexual dysfunctions were found to increase, as well (Tables 4 and 5). Consistent with our findings, two studies using SNOT-22 found a significant association between the severity of physiologic symptoms related to allergic rhinitis and sexual dysfunction in individuals with AR (9, 23) . In another study, it was noted that physiological symptoms related to AR, such as chronic nasal congestion, runny nose, and sneezing, may cause embarrassment and avoidance of close contact, thus affecting sexual activities and satisfaction (16). Therefore, in the management of sexual dysfunction, it is important to ensure individuals' compliance with AR treatment to control their physiological symptoms.

Among psychological factors, anxiety was found to be related to sexual dysfunction only in women (Table 4), whereas depression was found to be related to sexual dysfunction in both men and women (Tables 4 and 5). The literature review showed that there were no studies on the association between depression and anxiety and sexual function in individuals with AR. However, there were studies showing that mental health problems such as anxiety and depression were more common in individuals with AR than in healthy individuals, as in this study (37-39). According to many studies, decreased sexual desire and arousal in women are associated with depression and anxiety (40-43). For example, in one study, it was determined that depressed women were twice as likely to experience sexual problems, including lower levels of sexual desire, arousal, and orgasmic function, than women without depression (44). In the same study, it was determined that women with anxiety were 33-50% more likely to experience the same sexual problems than women without anxiety (44). Studies conducted on men showed that depression and anxiety were associated with sexual dysfunction (45-47). In this respect, it was important to evaluate individuals with AR experiencing sexual dysfunction in terms of anxiety and depression.

The presence of asthma, one of the situational factors, was found to be related to sexual dysfunction in women (Table 4), consistent with the literature. In a study, moderate and severe asthma and female gender were associated with sexual dysfunction (48). Another study found that asthma affected sexual desire and satisfaction in both sexes, affecting orgasm and self-esteem in women but not in men (49). Smoking, a situational factor, has been related to sexual dysfunction in men with AR (Table 5). In the literature, it was shown that both direct use and exposure to smoking were associated with erectile dysfunction (50).

Among symptomatic factors, fatigue and insufficient sleep were related to sexual dysfunction in men (Table 5), but not in women. A study of individuals with AR found a significant association between sleep quality, fatigue and sexual dysfunction in men. In the same study, no association was found between sleep quality and sexual dysfunction in women, while fatigue was found to be associated with sexual dysfunction (9). While certain studies have suggested a potential connection between sexual function and factors such as the severity of allergic rhinitis, medication side effects, reduced sleep quality, and fatigue, these studies have faced criticism due to their limited sample sizes, absence of control groups, and the absence of specific measurement tools (9, 16-18, 20). The study's findings are important in this regard.

Our study has several limitations. One of its major limitations is it relied on self-reported measures, which may be susceptible to bias. Another limitation is that it is a cross-sectional study. The strength of the study is that it is the first study to examine determination of factors affecting sexual function in individuals with AR and that it is based on the TOUS.

5. CONCLUSION

It was determined that individuals with AR experienced more anxiety, depression, fatigue, poor sleep, and sexual dysfunction than healthy individuals. As the Unpleasant Symptoms Theory suggests, individuals' sexual functioning was affected not only by the presence of direct symptoms (nasal symptoms) associated with allergic rhinitis, but also by the presence of other symptoms (psychological, physiological, and situational factors). These findings may help to raise awareness among nurses and other health professionals about the sexual dysfunction that individuals with AR experience and the factors affecting sexual dysfunction. Sexual dysfunction, sleep problems, fatigue, anxiety, and depression may affect the quality of life in individuals with AR. The use of standard measurement tools, as in this study, may help nurses to target specific symptoms and plan interventions for individuals with AR. Educating individuals with AR about the effects of fatigue, sleep problems, anxiety, and depression on sexual dysfunction can raise awareness about these problems and change health-seeking behaviors. Nurses should develop intervention programs that consider the physiological, psychological, and situational factors and other symptoms of the patient in the management of sexual dysfunction.

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Clinical and Experimental Health Sciences

Evaluation of the Impact of Post-Earthquake Trauma Levels on Children's Nutritional Processes in Parents Living in Earthquake Zones

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ABSTRACT

Objective: This study aims to evaluate the effect of post-earthquake trauma levels on children's nutritional processes in parents who live in the earthquake zone.

Methods: The study population in Hatay consists of individuals affected by the earthquakes on February 6 and 20, 2023. The study is cross-sectional and descriptive. Data were collected via face-to-face interviews. Parental trauma was assessed using the "Post-Earthquake Trauma Level Determination Scale," and attitudes towards children's nutrition were evaluated using the "Mother's Attitudes Towards the Feeding Process Scale."

Results: This study involved 144 parents of children aged 9–72 months living in Hatay province. Following the earthquake, loss of life and property, limited access to clean water, hygiene and sanitation, and restricted opportunities for food preparation and cooking increase the risk of malnutrition in children under five. The post-earthquake trauma level score among parents was 65.1±19.2; the attitude score towards children's nutrition was 76.8±27.7. There is a positive correlation between both scales. As trauma levels increase, negative affect during meals, issues related to insufficient and unbalanced nutrition, force-feeding, and reaction to others' viewpoints also rise.

Conclusions: Depending on the magnitude of the earthquake's impact, loss of life and property, limited access to clean water, food safety and security issues, and the stress and trauma it creates in parents may lead to malnutrition in children. In order to manage stress in the earthquake zone for parents and ensure adequate and balanced nutrition for children, psychosocial support, access to clean water, ensuring safe food production and consumption, conducting risk and benefit analyses of foods, and providing nutritional education should be among the main goals.

Keywords: Earthquake, nutrition, feeding, trauma, children

1. INTRODUCTION

Disasters, whether natural, anthropogenic, or technological, pose significant challenges to societal functions and public health on a global scale. These events can cause profound physical, economic, and social losses, disrupting routines, overwhelming local resources, and often necessitating international aid (1). Slowly developing disasters, such as prolonged cold spells, famine, and drought, may allow limited adaptation time for communities, yet their gradual impact can erode resilience over time. Conversely, sudden-onset disasters, including earthquakes, floods, and landslides, lead to immediate devastation, significantly increasing morbidity and mortality rates among affected populations (2-4). The health ramifications of disasters extend beyond initial injuries. Displacement often results in overcrowded and unsanitary living conditions, heightening the risk of communicable diseases due to restricted access to clean water, sanitation, and healthcare (4). The disruption of local food systems can lead to acute food insecurity, with initial scarcity followed by insufficient nutrient intake during recovery, exacerbating vulnerabilities (3, 4). Malnutrition, defined as an imbalance between nutrient intake and physiological needs, can manifest as undernutrition or, less frequently, overnutrition.

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Disasters exacerbate malnutrition through interrupted food access, poor infant feeding practices, maternal stress, and compromised healthcare (5, 6). The World Health reported that 149.2 million children under five are stunted, 45.4 million are underweight, and 38.9 million are overweight (7). Particularly vulnerable are infants and young children, whose growth and development can be severely impaired by inadequate nutrition, environmental stressors, and psychological trauma (8). Malnutrition is a leading contributor to post-disaster child mortality, with long-term consequences for cognitive and psychosocial development, especially in regions with pre-existing high malnutrition rates (9). Research on malnutrition determinants in disaster-hit areas is limited, primarily consisting of general guidelines with few region-specific studies (10, 11). The catastrophic earthquakes in Türkiye on February 6, 2023, affecting eleven provinces, highlight the need for targeted investigation. The impact of eartaqute varied, with Hatay being one of the most severely affected regions (12). Notably, no comprehensive study has yet explored the relationship between parental trauma post-earthquake and the nutritional status of children aged 9 to 72 months in Hatay. This research aims to fill this gap by assessing how parental trauma influences the nutritional well-being of young children, informing integrated interventions that address both caregiver mental health and child nutrition, thereby mitigating long-term health consequences and enhancing resilience against future crises.

2. METHOD

2.1. Research Type

This study is a cross-sectional descriptive study conducted in Hatay province, affected by two earthquakes (Mw7.7 and Mw7.6) on February 6, 2023, originating from the Pazarcık and Elbistan districts of Kahramanmaraş. Data collection began in November 10, 2023 after ethics committee approval and continued until April 31, 2024.

2.2. Population and Sample of the Study

Parents residing in Hatay province following the earthquakes and their children aged 9–72 months who consented to be included in the study's sample.

The Çukurova University Non-Interventional Ethics Commission gave their approval for this study in accordance with decision number 6 of the meeting that took place on 3/11/2023. The study did not include people who chose not to participate or who wanted out of the study for any reason.

2.3. Calculation of Sample Size

The sample size for the study was calculated by comparing the mean scores of the "Post-Earthquake Trauma Level Determination Scale," as utilized in Ediz and Gülbahçe's (2019) examination of post-traumatic stress disorder levels among university students affected by the Van earthquake, and the Feeding Process Maternal Attitudes Scale from Yılmaz's (2020) study on children's eating behavior and mothers' feeding attitudes (10, 13). An independent sample t-test was employed, with an effect size of 0.83 (Cohen's d), a margin of error of 0.05, and a power of 0.95, resulting in a minimum required sample size of 39 participants per group, totaling at least 78 individuals. Sample size estimation was conducted using the G*Power 3.1.9.4 software (14).

2.4. Inclusion Criteria

The study was meticulously planned and executed with the participation of individuals residing in Hatay province. Specifically, the participants included parents or guardians of children aged 9 to 72 months who consented to take part in the research. To ensure the integrity of the data collected, participants were required to meet specific inclusion criteria included that they must not have any chronic diseases that could potentially influence their responses or the study outcomes. This careful selection process aimed to create a homogenous group that could provide reliable insights into the effects of the earthquake on their children's nutritional status and the associated trauma levels. By focusing on this demographic, the study sought to gather relevant and context-specific data that could inform future interventions and support for families affected by the disaster.

2.5. Exclusion Criteria

Participants in the study were subject to specific exclusion criteria to ensure the validity of the findings. Parents or guardians residing outside Hatay province, those with children younger than 9 months or older than 72 months, and individuals who chose not to participate or withdrew from the study were excluded. Additionally, individuals with chronic diseases that could influence their responses (n= 5 participants that have major psychiatric disorder) were not considered. This thorough exclusion process was essential in ensuring that the data accurately reflected the experiences of those directly impacted by the earthquake, thereby enhancing the quality of the research findings.

2.6. General Mother's Attitudes Towards the Feeding Process Scale

Dilsiz and Dağ (2018) developed the Mother's Attitudes Towards Feeding Process Scale to assess mothers' perspectives and methods for feeding children aged 9-72 months (15) MATFPS allowed scores from 27 to 135. MATFPS scores, both individually and overall, have increased, suggesting feeding issues among mothers have also increased. The parents completed the questionnaire together for the study. In scoring, 17 negative items are encoded inversely (1=7, 2=6, 3=5, 4=4, 5=3, 6=2, 7=1). Overall, Cronbach's alpha is 0.769. The subscale "attitudes in developing positive habits" has a Cronbach's alpha of 0.818, while the subscale "attitudes in developing negative habits" has 0.822. Mothers with higher

scores are "positive" about developing their children's eating habits, while those with lower scores are "negative".

2.7. Post-Earthquake Trauma Level Determination Scale

Tanhan and Kayri (2013) created the Post-Earthquake Trauma Level Determination Scale to easily and rapidly evaluate PTSD symptoms in individuals following an earthquake (16). "I do not agree at all," "I slightly agree," "I moderately agree," "I very much agree," and "I completely agree" were the options on this twenty-item, five-point likert-type scale. Behavioral issues, emotional limitations, affective difficulties, cognitive structural issues, and sleep problems make up the five sub-factors that make up the scale. On the scale, a score of 20 was considered very low and a score of 100 was considered very high. Individuals were more severely impacted by the earthquake as their scores on the scale increased. All of the PETLDS items had an internal reliability coefficient (Cronbach's alpha) of 0.87.

2.8. Plan of Research

To gather comprehensive information about the participants in the study, a questionnaire was administered using a face-to-face interview method. This questionnaire was designed to collect sociodemographic data from disaster victims, including age, gender, education level, and socioeconomic status, while also assessing the impact of the earthquake on their overall nutritional status, such as changes in dietary habits and food availability. Additionally, it featured two specific scales that the "Post-Earthquake Trauma Level Determination Scale," which measures trauma levels experienced by individuals following the disaster, and the "Mother's Attitudes Towards the Feeding Process Scale," evaluating maternal attitudes and behaviors related to children's feeding practices. Prior to administering these scales, permissions were obtained from the original authors via email, ensuring compliance with ethical standards and respect for intellectual property.

2.9. Statistical Analysis

Statistical analyses were conducted using SPSS 22.0. Normality of the dependent and independent variables was assessed using the Kolmogorov-Smirnov/Shapiro-Wilk tests, along with visual tools like probability plots and histograms. Parametric tests (Independent Groups T-test, ANOVA) were applied to normally distributed quantitative variables, while non-parametric tests (Mann-Whitney U test, Kruskal-Wallis test) were used for non-normally distributed variables. Descriptive analyses for normally distributed data employed mean and standard deviation, while median and interquartile range were used for non-normally distributed data. The Pearson test was used for normally distributed variables, and the Spearman test for non-normally distributed ones to determine correlation coefficients. The p value of less than .05 was considered statistically significant (17).

3. RESULTS

This study was completed by 144 parents who had children aged 9–72 months and lived in Hatay province, which was affected by the biggest magnitude from the 6 February Kahramanmaraş epi-center earthquake.

This study involved a total of 144 parents, comprising 113 mothers and 31 fathers. The educational attainment of the study participants predominantly comprised high school and university qualifications. The participants in the study had an average income level ranging from 0 to 12,000 TL (Table 1).

Table 1. *Distribution of individuals according to their sociodemographic characteristics*

Sociodemographic characteristics	Total Number		%
Mother	113		78.5
Father	31		21.5
Parent Education			
Primary School	8		5.6
Middle School	50		34.7
High School	16		11.1
University	70		48.6
Income Level			
0-12.000 TL	82		56.9
12.000-30000 TL	38		26.4
30000-50000 TL	20		13.9
50000 and above	4		2.8
Variables	**Mean±SD**	**Median (IQR)**	**Minimum-Maximum**
Parental age (years)	31.9±0.5	32.0 (9.0)	19-48
Average age of children (months)	40.9±1.6	40.0 (31.0)	9-72
Children's body weight (kg)	15.2±0.4	14.0 (8.0)	6-30

SD:Standart deviation , IQR: Interquartile range

The average age of the parents involved in the study was 31.9±0.5 years, whereas the average age of the children was 40.9±1.6 months. The average body weight of the children was 15.2±0.4 kg (Table 1).

The total score on the post-earthquake trauma level scale ranges from 28 to 92, with a mean score of 65.1±19.2. The sub-factors that most significantly contributed to the total mean score of the scale were emotional limitation (15.9±7.0) and cognitive structuring (15.2±5.2) (Table 2).

The mean post-earthquake trauma level score of those who answered "yes" to earthquake-related parameters ranged from 68.4±18.4 to 74.6±16.3, which was higher than the total mean score of the scale (65.1±19.2). The total post-earthquake trauma scores of individuals who suffered loss of life and property, were compelled to migrate, and faced challenges in accessing clean water and food preparation were significantly higher than those who did not experience these hardships (Table 3; p<.05).

Table 2. *Evaluation of the trauma level of individuals after the earthquake*

Post-earthquake trauma level sub-factors and total score	Mean±SD	Minimum-Maximum
Behavioral problems	12.8±5.1	4-20
Excitemental Limitation	15.9±7.0	5-25
Affective	11.4±2.2	5-20
Cognitive structuring	15.2±5.2	4-20
Sleep problems	9.6±3.3	3-15
Total score	65.1±19.2	28-92

SD: Standart deviation

Table 3. *Evaluation of the effects of earthquake-related factors on trauma level*

	Post-earthquake trauma level score		
Earthquake related parameters	Yes Mean±SD	No Mean±SD	p value*
Did you experience any loss of life in the earthquake?	74.6±16.3	61.9±19.1	<.001
Did you experience any property loss in the earthquake?	68.4±18.4	59.1±19.2	.005
Did you have to migrate from the area you lived in after the earthquake?	71.7±18.3	60.7±18.6	.001
Did you ever have trouble accessing clean water?	70.3±19.8	61.8±18.1	.01
Did you have any trouble when preparing food?	72.4±19.7	61.5±18.0	.001

*SD: Standart deviation, *P<0,05; Independent sample t test*

The total mean score of the maternal attitudes towards the feeding process scale was 76.8±27.7, with minimum and maximum values ranging from 27 to 135. The sub-factors that contribute the most to the total score of the scale were "Attitudes Towards Inadequate/Unbalanced Nutrition (27.6±8.4)" and "Negative Emotional State During Meals (17.1±7.2)" (Table 4).

Table 4. *Evaluation of maternal attitudes related to the feeding process*

Sub-factors and total scale score of the maternal attitudes regarding the feeding process	Mean±SD	Minimum-Maximum
Negative Emotional State During Meal	17.1±7.2	6-30
Attitudes Towards Inadequate/ Unbalanced Nutrition	27.6±8.4	8-40
Negative Feeding Strategies	13.0±6.1	5-25
Force Feeding	7.8±5.4	4-20
Reaction to Others' Opinions	11.1±5.3	4-20
Total scale score of the maternal attitudes regarding the feeding process	76.8±27.7	27-135

SD: Standart deviation

Parents generally felt nervous while feeding their children (86.8±26.4). In addition, parents didn't know how much food their children should consume at one meal (93.8±33.1). Parents had no idea when their children were full (104.7±25.9). Parents thought that they didn't feed their children well enough (81.3±26.7) (Table 5).

Table 5. *Evaluation of attitudes and behaviors related to child nutrition after the earthquake*

	Mother's Attitudes Towards the Feeding Process Scale		
Parameters associated with parents' attitudes and behaviors related feeding process	Yes Mean±SD	No Mean±SD	P value*
Did you feel nervous when feeding your child?	86.8±26.4	69.7±26.5	<.001
Did you know how much food your child should consume in one meal according to her age?	72.5±24.5	93.8±33,1	<.001
Did you believe that you were not feeding your child well enough?	81.3±26,7	72.0±28.1	.04
Did you think your child is thinner than her peers?	82.4±24.7	74.0±28.8	.08
Did you have any idea when your child was full?	72.0±25.1	104.7±25.9	<.001

*SD: Standart deviation, *P<0,05; *Independent sample t test*

A significant positive correlation was identified between post-earthquake trauma level scores and maternal attitudes scale scores during the feeding process among parents affected by the earthquake (r=0.65, p<.001, Table 6). As the trauma level escalated, the average scores of the sub-factors pertaining to negative mood during meals, circumstances involving insufficient and unbalanced nutrition, force-feeding, and responses to others' opinions also rose (Table 6, p<.05).

Table 6. *Relationship between Parents' Post-Earthquake Trauma Level and Feeding Processes*

	Post-Earthquake Trauma Level Determination Scale	
	Correlation coefficient**	P value*
Mother's Attitudes Towards the Feeding Process Scale	r:0.65	p<.001
Negative Affect During Meal	r:0.61	p<.001
Attitudes about Insufficient/ Unbalanced Feeding	r:0.66	p<.001
Force Feeding	r:0.40	p<.001
Reaction to the Viewpoints of Others	r:0.50	p<.001

r: Correlation coefficient, p<005. ** Pearson correlation*

4. DISCUSSION

Natural disasters profoundly affect human life, resulting in fatalities, injuries, habitat destruction, and the inability to fulfill fundamental needs such as food and water. Vulnerable populations in society, such as children, pregnant women,

lactating mothers, and the elderly, exhibit increased susceptibility to malnutrition and psychosocial distress following natural disasters (18). Malnutrition, encompassing both undernutrition and overnutrition, represents a disparity between nutritional needs and consumption, marked by the cumulative deficiency of energy, protein, and macro or micronutrients (5, 19). Following an earthquake, fatalities and property damage, restricted access to potable water, hygiene and sanitation, as well as limited food preparation and cooking facilities may elevate the risk of malnutrition in children under five years old. Restricted access to potable water can result in a rise in infectious diseases and primary cholera due to insufficient hygiene and sanitation (20, 21). Substandard nutrition and housing conditions in low-income families correlate with malnutrition status (22). It was found that the majority of individuals participating in this study had low income levels. In our country, this income level is below the 2024 net minimum wage (23). Health expenditures of low-income families are constrained, potentially impacting their budget for sufficient and balanced nutrition as well as hygiene and sanitation conditions (20). The Türkiye Demographic and Health Survey-2018 data indicates that some children under five years were classified as stunted, underweight, or overweight. Stunting predominantly occurs in children of mothers lacking any formal education or those who have not finished primary school (24) This study examines parents with educational attainment at the high school and university levels. The average age of the children is noted, and their body weights are mentioned. However, a portion of the parents were unaware of their children's heights, which prevented the calculation of average heights and BMIs.The earthquakes originating from the Pazarcık district of Kahramanmaraş province are the most significant seismic events documented in Turkey since 1939. Significant earthquakes can induce psychological trauma and adversely impact individuals' overall health and mental well-being. Individuals who endure earthquakes may exhibit various psychological symptoms, particularly post-traumatic stress disorder, depression, and anxiety (25). Traumatic events typically have adverse effects on individuals psychologically, physically, and socioeconomically (26). In a study conducted with individuals affected by the Van district earthquake that occurred in 2012, a threshold value was determined at which individuals were traumatized (16). In this study, the trauma levels of the parents were found to be higher than the threshold value determined in the 2012 Van earthquake. When the sub-factors of the scale are evaluated, it is determined that the emotional limitation and cognitive structuring scores are higher than the other sub-factor items. Emotional limitations mainly include the items of losing confidence in the future, feeling like life has no meaning, decreasing desire to live after what one has experienced, increasing regrets after the earthquake, and feeling very helpless/powerless. The cognitive structuring sub-factor includes items such as worrying about acquaintances, feeling like an earthquake could happen at any moment, seeing images of an earthquake in front of one's mind, and worrying about the future. The high impact and intensity of the February 6 earthquake centered in Kahramanmaraş, the helplessness experienced in the face of the events witnessed, and the inability to cope with the current situation may affect individuals psychologically (27). In this study, the main factors affecting the trauma level of individuals can be evaluated as loss of life and property in the earthquake, migration after the earthquake, difficulty in accessing clean water, and problems in food preparation and cooking. Malnutrition constitutes a significant public health issue among children under five years of age, placing them at heightened risk of mortality and morbidity. The risk of malnutrition in children under five years old can be assessed through nutritional history, anthropometric measurements, biochemical indicators, clinical evaluation, and validated reliable scales (28). A study involving mothers with children under five years of age determined the mean score of the Mother's Attitudes Towards the Feeding Process Scale (MATFPS) (29). In a separate study, the total score of MATFPS was reported (13). The overall mean score of MATFPS is established. The sub-dimensions that contributed the most to the total score of the scale are "Attitudes Towards Inadequate/Unbalanced Nutrition" and "Negative Mood During Meals." Parents often feel nervous while feeding their children. In addition, parents generally do not know how much food their children should consume at one meal. Parents have no idea when their children are full. Parents often think that they do not feed their children well enough. A systematic review and meta-analysis indicated a heightened risk of malnutrition following an earthquake for individuals who were underweight, stunted, had low body weight, and suffered from anemia (11). In order to prevent an increase in the risk of malnutrition after an earthquake, it is important to screen and evaluate nutritional status and to prevent and improve identified problems (30). Parental exposure to chronic stress may negatively affect children's nutritional status and diet quality (31). This study identifies a robust and positive correlation between post-earthquake trauma levels and parental nutritional attitudes and behaviors. As individuals' trauma levels increase, the mean scores of the sub-factors of negative mood during meals, situations related to inadequate and unbalanced nutrition, force-feeding, and reacting to the opinions of others also increase. As parents' trauma levels increase, children may develop eating disorders (32). The occurrence of an eating disorder may trigger the risk of malnutrition in children (33). Therefore, especially after natural disasters such as earthquakes, where psychological vulnerability increases, nutritional education for children and parents, and psychosocial support can constitute the basic steps of stress and malnutrition management (27, 30)

5. CONCLUSION

Following the devastating earthquakes in Hatay, Turkey, on February 6, 2023, the region experienced significant losses in life and property, as well as widespread disruptions to essential infrastructure. These conditions heightened parental stress and trauma, particularly due to fatalities

and restricted access to clean water and safe food. In the aftermath, malnutrition rates, especially among infants and children under five, were observed to increase, raising the risk of illness and mortality within vulnerable groups. Elevated trauma levels among parents were found to negatively influence their attitudes and behaviors related to child nutrition. To address these complex challenges, psychosocial support for affected families should be prioritized to help restore mental well-being. It is also critical to guarantee continuous access to clean water and the safe production of food, alongside thorough nutritional risk-benefit assessments to ensure the health of children. Based on the available data, the implementation of tailored nutrition and psychosocial education programs for parents in affected areas is essential. Additionally, developing and standardizing specific nutrition guidelines for earthquake-prone regions will help address the unique needs of these communities and improve overall child health outcomes.

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Clinical and Experimental Health Sciences

The Effects of Mini-Implant Assisted Rapid Palatal Expansion on Intrabony Canals in The Anterior Maxilla

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ABSTRACT

Objective:This study aims to evaluate the impact of mini-implant assisted rapid maxillary expansion (MARPE) on intrabony canals in the anterior maxilla.

Methods:The cone beam computed tomography (CBCT) scans obtained before and after treatment from 24 patients who underwent mini-implant assisted rapid maxillary expansion (MARPE) were analyzed retrospectively. In the sagittal plane, three levels of the incisive foramen (lower, middle, and upper) were assessed. Mediolateral and buccolingual widths were measured on axial sections, and the widest diameter of the canalis sinuosus was measured in the coronal section. Pre-treatment and post-treatment measurements were statistically compared.

Results:The mediolateral width of the incisive foramen showed a statistically significant increase at the lower and middle levels during the T1 stage compared with the T0 stage (p=.0001). In the buccolingual width of the incisive foramen, measurements at the lower level in the T1 stage also exhibited a statistically significant increase compared to the T0 stage (p=.0049). However, no statistically significant difference was found in the measurements at the middle level (p=.365). Additionally, the measurements on the right side of the canalis sinuosus demonstrated a statistically significant increase in the T1 stage compared to the T0 stage (p=.0004).

Conclusion:MARPE causes expansion of the intrabony canals in the anterior maxilla. The expansion of these canals may be attributed to the need for increased vascularization and innervation accompanying the increased bone volume.

Keywords: Mini-implant assisted rapid maxillary expansion, incisive canal, canalis sinuosus, cone beam computed tomography, maxillary expansion

1. INTRODUCTION

Maxillary constriction is defined as the insufficient transverse width of the maxilla relative to the mandible (1). It is considered a significant issue requiring correction of transverse skeletal relationships between the maxilla and mandible upon diagnosis. Various appliances have been developed for maxillary expansion, techniques include slow palatal expansion, semi-rapid palatal expansion, rapid maxillary expansion (RME), surgically assisted rapid palatal expansion (SARPE), and in recent years, mini-implant assisted rapid palatal expansion (MARPE) (2).

Traditionally, maxillary constriction has been treated using the RME method. However, the effectiveness of RME is limited in adolescents approaching the end of their growth phase and in adults. Studies have shown reduced efficacy in these age groups due to limited skeletal effects, such as dental tipping, decreased buccal bone thickness, dehiscences, and adverse periodontal outcomes (3–5).

In adults, the progressive ossification of the midpalatal and circummaxillary sutures necessitates greater forces to achieve effective transverse maxillary expansion (6). To address this challenge, the Surgically Assisted Rapid Palatal Expansion (SARPE) technique was developed for skeletally mature individuals. While SARPE is highly effective in overcoming skeletal resistance by surgically facilitating suture separation, it involves a complex treatment protocol with inherent risks, including severe bleeding, damage to branches of the maxillary nerve, tooth devitalization, infection, postoperative pain, and increased treatment costs (7).

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In response to the limitations of SARPE, the Mini-Implant Assisted Rapid Palatal Expansion (MARPE) technique has emerged as a less invasive alternative, particularly suitable for late adolescents and young adults where partial suture patency remains. By utilizing mini-implants anchored in the palatal bone, MARPE ensures a more uniform distribution of expansion forces directly to the skeletal structures, thereby minimizing undesirable dental side effects such as buccal tipping and periodontal compromise (8,9). Additionally, MARPE avoids surgical intervention, reduces patient morbidity, and is more cost-effective. However, its success is contingent upon patient-specific anatomical factors, and in cases of complete suture ossification, its efficacy may be limited (10).

Therefore, both SARPE and MARPE present distinct advantages and disadvantages. The choice between these techniques should be individualized based on skeletal maturity, the degree of suture ossification, anatomical considerations, and patient preference regarding invasiveness and treatment complexity (11).

The maxilla is richly vascularized and innervated. The dominant blood supply is provided by the pterygopalatine segment of the maxillary artery, with branches such as the posterior superior alveolar, infraorbital, greater palatine, and sphenopalatine arteries. The greater palatine nerve, accompanied by the greater palatine artery, passes through the pterygopalatine canal to the hard palate. The posterior superior nasal branch of the maxillary nerve emerges via the nasopalatine branch through the incisive canal, innervating the palate and roots of the maxillary anterior teeth. The infraorbital foramen, located on the anterior aspect of the orbital surface of the maxilla, carries the infraorbital nerve and vessels. The anterior superior alveolar nerve, which innervates the incisors, canines, and surrounding soft tissues, resides in a bony canal known as the canalis sinuosus, approximately 2 mm in diameter, lateral to the infraorbital canal. This canal may contain accessory channels terminating in the alveolar bone near the palatal roots of the maxillary incisors and canines (12–14).

Traditional radiographic methods often fail to accurately sufficient these anatomical structures. Conventional imaging techniques are limited in visualizing the intrabony courses of neurovascular structures (15,16). As a result, cone-beam computed tomography (CBCT) has emerged as an essential method for identifying anatomical variations prior to surgery, enabling comprehensive three-dimensional assessments. Accessory foramina and canals in the anterior maxilla occur in over 15% of the population and are clinically significant due to the potential for severe complications if these neurovascular structures are damaged (13).

Choi et al. (17) reported that MARPE induces increases in the mediolateral and buccolingual dimensions as well as the incisive canal dimensional volume. Although many studies have examined the skeletal and dental effects of MARPE (9,10,18), limited research exists on its effects on the canals within the maxilla. To our knowledge, no prior studies have examined the canalis sinuosus. Therefore, the aim of this study is to evaluate changes in the canalis sinuosus and nasopalatine canal in the anterior maxilla, as observed in CBCT images of patients who underwent MARPE, which induces sagittal and transverse expansion of the maxilla. The null hypothesis (H0) of this study is that "Mini-implant-assisted palatal expansion has no effect on the canals in the anterior maxilla." This hypothesis was formulated based on the assumption that, despite MARPE's known skeletal and dental effects, the rigid anatomical nature and potential resistance of neurovascular canals—such as the nasopalatine canal and canalis sinuosus—might prevent significant morphological changes. Since limited evidence exists regarding the response of these intrabony structures to orthopedic forces, a null hypothesis was adopted to objectively evaluate whether MARPE induces measurable alterations in these canals.

2. METHODS

2.1. Subjects

This retrospective study received approval from the Non-Interventional Clinical Research Ethics Committee of Aydın Adnan Menderes University Faculty of Dentistry (protocol number 2024/04). A G*Power analysis calculated that at least 22 participants were needed to achieve 90% statistical power with a 95% confidence level. Based on this, the records of individuals who underwent MARPE at Aydın Adnan Menderes University Faculty of Dentistry between January 2015 and April 2024 were reviewed. Ultimately, 24 participants who met the inclusion criteria were selected for the study. The participants' ages ranged from 14 to 28 years. The final sample consisted of 24 individuals, including 16 females with a mean age of 18.18 years and 8 males with a mean age of 16.25 years. The inclusion criteria were defined as follows:

1. Skeletal maxillary constriction;
2. Use of the Maxillary Skeletal Expander (MSE, Biomaterials/Korea) as the expansion device;
3. Availability of pre – and post-MARPE CBCT images;
4. Absence of artifacts in CBCT images;
5. Cervical vertebral maturation stage CVM4 or greater, according to the modified Bacetti classification (19);
6. No craniofacial syndromes or deformities.

Exclusion criteria included CBCT data with insufficient quality, history of orthognathic surgery, and anterior trauma.

2.2. Materials and Treatment Protocol

The MSE device used in this study consisted of bands placed anchored to the maxillary first molars and integrated with an expansion screw through soldered connections. The mini-implants had diameters of 1.8 mm and lengths ranging from 11 to 13 mm to ensure bicortical engagement. The palatal mini-implants were symmetrically positioned parallel to the midpalatal suture (Figure 1).

Figure 1. *Maxillary Skeletal Expander (MSE; Biomaterials/Korea) appliance*

The treatment protocol for MSE involves attaching bands to the maxillary first molars and taking an impression to create a working model. The MSE device is adapted as closely as possible to the palate on this model. Activation rates are determined based on the patient's age: one turn per day for early adolescents (under 15 years), two turns per day for late adolescents (15–20 years), and four turns per day for adults (over 20 years). When diastema formation is observed, the activation rate is halved, and expansion continues until sufficient transverse correction is achieved. Patients are instructed to visit the clinic every 7 to 10 days to evaluate diastema formation.

After active expansion, which lasts approximately one month, the MSE device is retained in situ to allow bone formation at the expansion site. Bone formation in the midpalatal suture is typically observed within 3 to 6 months, with complete osseous consolidation expected within this timeframe. Accordingly, the MSE device is retained for 6 months to 1 year (17).

All CBCT images were obtained using a Planmeca Promax 3D Mid device (Helsinki, Finland) at 90 kV, 8 mA, and a 12-second scan time. The field of view (FOV) was set to 8.0 x 5.0 cm (401 x 401 x 251), and the voxel size was 0.200 mm. Measurements were analyzed using Planmeca Romexis Viewer Launcher 4.6.0.R (Helsinki, Finland) by a single investigator.

2.3. CBCT Reference Points, Planes, and Measurements

Calibration and reorientation protocols were manually applied to standardize pre-treatment (T0) and post-expansion (T1) CBCT images using Planmeca Romexis software. The images were aligned parallel to the palatal plane along the midpalatal suture, with the sagittal and axial sections oriented through the anterior nasal spine (ANS) and posterior nasal spine (PNS) (Figure 2).

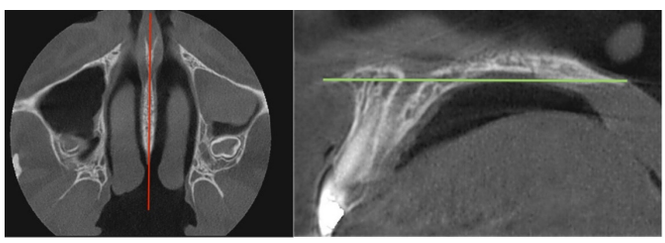

Figure 2. *Reorientation protocols*

In CBCT images, mediolateral and buccolingual dimensions of the incisive canal were measured at three vertical levels: upper, middle, and lower. The maximum mediolateral width of the canalis sinuosus was assessed in the coronal view. Measurements taken before and after treatment were statistically compared (Figure 3).

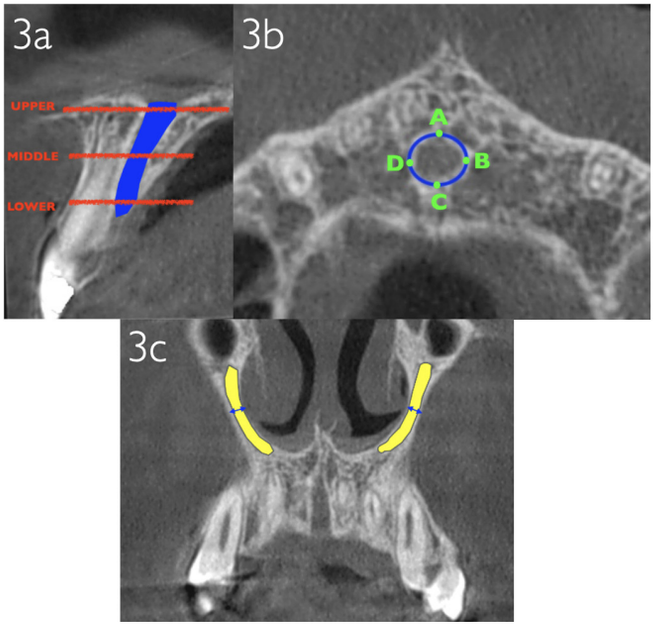

Figure 3. *References and measurements related to the incisive canal and canalis sinuosus*
3a. The 3 vertical levels of the incisive canal. (upper-middle-lower), 3b. A most anterior border of the incisive canal; B, most lateral left side of the incisive canal; C, most posterior border of the incisive canal; D, most lateral right side of the incisive canal; AC, buccolingual width of the incisive canal; BD, mediolateral width of the incisive canal, 3c. Maximum mediolateral width of the canalis sinuosus

2.4. Statistical Analysis

Data analysis was conducted using SPSS 25.0 software, while sample size calculations were carried out with G*Power 3.1 software. The Kolmogorov-Smirnov test was used to evaluate the normality of quantitative variables. Descriptive statistics, including mean and standard deviation, were calculated. Mann-Whitney U and Kruskal-Wallis tests were used to analyze differences in measurement values across study groups. Relationships between measurement values were examined using Spearman correlation analysis. Intra-group comparisons of measurements were conducted using Wilcoxon and Friedman tests. A p-value of $< .05$ was deemed statistically significant.

3. RESULTS

The study included 24 participants, comprising 16 females and 8 males. The mean age of the female participants was 18.18 years (range: 14–28), while the mean age of the male participants was 16.25 years (range: 14–22). The overall mean age of the study population was 17.54 years (Table 1).

Table 1. *Demographic characteristics of the patients in the study*

Gender n	**Male**	8
	Female	16
Age Mean	**Male**	16.25
	Female	18.18
	Total	17.54

No statistically significant differences were found between male and female participants in any of the measured parameters at either T0 or T1 time points (p > .05) (Table 2).

Table 2. *Comparison of Measurement Outcomes Between Male and Female Participants at Different Time Points*

T0/T1	Measurements		Male	Female	p*
T0	IC ML		3.56	3.41	.602
	IC BL		3.27	3.03	.370
	Canalis sinuosus	Right	1.19	1.20	.952
		Left	1.08	1.23	.265
T1	IC ML		4.58	4.34	.349
	IC BL		3.60	3.49	.631
	Canalis sinuosus	Right	1.29	1.49	.204
		Left	1.26	1.23	.735

**: Paired-samples t-test*

Descriptive statistics are presented as mean ± standard deviation.

Mediolateral (ML) measurements of the incisive canal (IC) at the lower level (T1) were significantly higher compared to pre-expansion measurements (T0) (p=.001). Likewise, middle-level measurements showed significant increases (p=.001). However, no significant differences were observed at the upper level (p=.257). Buccolingual (BL) dimensions at the lower and upper levels also increased significantly (p=.049 and p=.026, respectively), while middle-level changes were not significant (p=.365) (Table 3).

Table 3. *Comparison results of dependent measurements for IC ML and BL*

		T0	T1	p*
IC ML	Lower	3.45±0.90	4.74±1.62	**.001**
	Middle	3.19±0.88	4.34±1.56	**.001**
	Upper	3.76±1.38	4.19±1.61	.257
IC BL	Lower	3.41±1.01	3.83±0.78	**.049**
	Middle	2.97±0.80	3.24±1.31	.365
	Upper	2.94±1.05	3.51±1.48	**.026**

**: Paired-samples t-test*

Descriptive statistics are presented as mean ± standard deviation.

IC: Incisive Canal ML: Mediolateral BL: Buccolingual. T0:pre-MARPE. T1:post-MARPE

The T1 right measurements of the canalis sinuosus were significantly higher than the T0 right measurements (p=.004). However, no statistically relevant difference was detected between the left measurements at T0 and T1. (p=.343) (Table 4).

Table 4. *Comparison results of dependent measurements for the canalis sinuosus.*

	T0	T1	p*
Right	1.19±0.32	1.42±0.40	**.004**
Left	1.18±0.36	1.26±0.44	.343

**: Paired-samples t-test*

Descriptive statistics are presented as mean ± standard deviation

4. DISCUSSION

MARPE has been shown to promote transverse expansion of the maxilla while also causing three-dimensional alterations in the zygomaticomaxillary complex (20). This study aimed to investigate how the volume increase of the canalis sinuosus, which provides vascularization and innervation to the anterior region and is adjacent to the midpalatal suture, is affected by the expansion.

Various radiological methods have been employed for the diagnosis of maxillary constriction and the evaluation of post-expansion outcomes. Maxillary occlusal radiographs have been considered a fundamental tool for assessing the opening and ossification of the midpalatal suture. However, it has been noted that the posterior region of the suture is inadequately visualized and overlapping of other bony structures on the suture results in unclear images (21,22). The use of posterior-anterior cephalograms is regarded as the most appropriate and reliable method for diagnosing transverse maxillary constriction (23). Vanarsdall (24) recommended posterior-anterior cephalograms for identifying skeletal transverse discrepancies. However, two-dimensional radiographs may not accurately represent actual hard tissues (25).

In the absence of a definitive gold standard, transverse maxillary constriction is commonly diagnosed by orthodontists using clinical evaluations, model analyses, and two-dimensional radiographic assessments. With the advent of CBCT, it has been demonstrated that CBCT-based measurements or reconstructed posterior-anterior cephalograms are more reliable. Furthermore, CBCT allows orthodontists to comprehensively assess the condition by observing various slices of the images. For this reason, CBCT was used in this study to analyze pre – and post-treatment changes associated with MARPE (26,27). CBCT offers significant advantages in maxillofacial imaging, particularly due to its ability to provide high-resolution, three-dimensional visualization of anatomical structures without superimposition or distortion (13). It enables accurate assessment of complex intrabony canals, such as the nasopalatine canal and canalis sinuosus, which are often difficult to evaluate using conventional two-dimensional radiography (16). Additionally, CBCT involves relatively low radiation doses compared to medical CT, making it a safer option for dental and orthodontic applications (28). However, CBCT also presents certain limitations. Despite lower radiation than conventional CT, it still exposes patients to higher doses than standard panoramic or cephalometric radiographs (15). Furthermore, CBCT's limited soft tissue contrast reduces

its utility in evaluating non-osseous structures. Artifacts caused by metal objects, such as orthodontic appliances or restorations, can also impair image quality. Therefore, while CBCT is invaluable for detailed bony assessments, its use should be justified based on clinical necessity to adhere to the ALARA (As Low As Reasonably Achievable) principle (16). We preferred CBCT due to its ability to provide superimposition-free and magnification-free images.

In the literature, many studies involving MARPE report an average retention period of 3 months following expansion, with evaluations conducted using CBCT images taken 3 months post-expansion. While collecting archival records, CBCT images taken 3 months after the expansion procedure were selected for post-treatment evaluation. It was determined that the post-treatment images were captured before the removal of the expansion appliance (29–31).

CBCT images obtained three months post-expansion were compared to baseline (T0) scans to allow adequate time for the reshaping of the incisive canals and the osteogenic maturation of the midpalatal sutures. In certain cases, irregular and indistinct margins were noted in the anterior and posterior regions of the incisive canal, suggesting incomplete the osteogenetic maturation of the midpalatal sutures. For these instances, an imaginary ellipse was traced along the lateral walls of the incisive canal, marking anterior and posterior reference points. The slight alteration in the labiopalatal dimension of the incisive canal was attributed to cortical ossification as the incisive canals progressed (17,28).

In this study, when the mediolateral and buccolingual width measurements of the incisive canal were compared before and after treatment, a significant increase was observed in all values. Statistically significant skeletal increases were identified in the lower and middle measurements for mediolateral width and in the lower and upper measurements for buccolingual width. Similarly, Choi et al. (17) reported significant increases in the mediolateral and buccolingual widths, as well as the volume of the incisive canal, in all individuals treated with MARPE. These findings are in concordance with the outcomes of our study.

Previous study demonstrates that the increases in the mediolateral and buccolingual dimensions of the incisive canal are associated with the sagittal and transverse growth of the maxilla. Additionally, the expansion of the canalis sinuosus is linked to the vertical dimensional growth of the maxilla. These findings confirm that the maxilla undergoes three-dimensional growth (14). The orthopedic forces applied through MARPE are transmitted directly to the midpalatal suture and adjacent skeletal structures via mini-implants, leading to controlled suture separation and maxillary expansion. This mechanical separation generates tensile stresses that also affect nearby anatomical structures, such as the nasopalatine canal and canalis sinuosus. The observed enlargement of these canals can be explained by passive displacement and adaptive bone remodeling in response to mechanical loading, ensuring the maintenance of neurovascular function during skeletal expansion (8,14). Therefore, the null hypothesis of this study, “Mini-implant assisted palatal expansion has no effect on the canals in the anterior maxilla” was rejected.

The findings of this study have important clinical implications for orthodontic treatment planning involving MARPE. Understanding the morphological changes in intrabony canals, such as the nasopalatine canal and canalis sinuosus, is essential to minimize the risk of neurovascular complications during expansion procedures. Awareness of potential canal enlargement can help clinicians avoid inadvertent injury when placing mini-implants, performing surgical interventions, or planning future implant placements in the anterior maxilla. Moreover, these insights contribute to a better prediction of patient-specific responses to MARPE, enhancing the safety and efficacy of treatment. Incorporating detailed CBCT analysis into routine assessment protocols may improve individualized treatment planning and long-term outcomes in orthodontic patients requiring maxillary expansion.

This study has certain limitations. The small sample size may reduce the generalizability of the results. The wide age range of participants required different activation protocols, which could have influenced the extent of maxillary expansion and canal changes. As a retrospective study, there was limited control over variables such as appliance retention time and individual anatomical differences. Additionally, CBCT evaluations were performed at a single time point post-expansion, preventing assessment of long-term remodeling. Future studies with larger, homogeneous samples and longitudinal follow-up are needed to confirm these findings and evaluate the stability of canal adaptations over time.

5. CONCLUSION

The MARPE technique is an effective and minimally invasive method for correcting transverse maxillary constriction, promoting not only skeletal expansion but also three-dimensional remodeling of the maxilla. This study demonstrated that MARPE leads to significant enlargement of intrabony structures, including the nasopalatine canal and canalis sinuosus, likely reflecting adaptive responses to increased bone volume and the need for vascular and neural accommodation. These findings emphasize the importance of evaluating anatomical changes during orthodontic treatment planning to minimize potential neurovascular risks. Further research with larger, age-homogeneous samples and long-term follow-up is needed to assess the stability and clinical implications of these morphological changes. Additionally, investigating the impact of canal enlargement on procedures such as implant placement or surgical interventions in the anterior maxilla would provide valuable clinical guidance. Routine use of CBCT in MARPE cases is recommended to monitor these structural adaptations and enhance patient safety.

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Ethics Committee Approval: *This study was approved by Ethics Committee of Aydın Adnan Menderes University, Noninvasive Clinic Ethics Committee (Approval date: 24.07.2024.; Number:2024/04.)*

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Author Contributions:

Research idea: EK
Design of the study: EK
Acquisition of data for the study: OU
Analysis of data for the study: OU
Interpretation of data for the study: OU
Drafting the manuscript: EK, OU, YAU
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Clinical and Experimental Health Sciences

Evaluation of the Burden of Caregiving of Individuals Caring for Cancer Patients

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ABSTRACT

Objective: Cancer is a public health problem caused by the uncontrolled proliferation of cells in the body, which has increased especially in recent years. Caregivers are individuals who are involved in the diagnosis, treatment and termination of the disease and may encounter physical, social or emotional problems at this stage. In this study, it is aimed to evaluate the caregiving burden of caregivers and the factors related to it.

Methods: The study was designed in a cross-sectional study. The data related to the study were collected by face-to-face interview method with 362 people who gave verbal consent to participate in the study between 5-25 March 2024 at a University Hospital. For the study, a data collection form consisting of 27 questions was used by the researchers by reviewing the literature.

Results: The median total score obtained from the scale was 31.0 (23.0-41.3). Accordingly, the participants had a moderate level of caregiving burden. According to the study, the burden of caregiving was found to be higher in women, those living in extended families, those residing in villages and those with chronic diseases compared to others (p<.05).

Conclusion: It has been determined that caregivers have a certain level of burden according to various situations. Mental health trainings can be given to individuals on this subject. It can be integrated with home health services to ensure that those who stay with the people they care for can spare time for themselves to reduce the burden of care.

Keywords: Patient Care, Cancer, Caregiver Burden

1. INTRODUCTION

Cancer is a public health problem caused by the uncontrolled proliferation of cells in the body, which has increased especially in recent years (1). Cancer is one of the leading causes of death in the world. It approximately 10 million deaths worldwide in 2020. The most common types of cancer in the world are breast, lung, colorectal, and lung and colorectal cancers are the most common causes of death (2). According to 2024 data in Turkey, cancer-related deaths ranked second among the causes of death after circulatory system diseases (3).

Due to increased exposure to environmental carcinogens, development of diagnostic facilities, more and more cancers are diagnosed every year, and the average life expectancy of patients is increasing with the increase in treatment possibilities (4). The prolonged duration of cancer diagnosis and treatment , uncertainties in the outcome of the disease, and prolonged life expectancy cause many physical and psychological problems in patients with cancer (5).

Caregivers are defined as individuals who provide necessary care, usually in the home, which includes tasks that may be physically, emotionally, socially or financially demanding, requiring a significant amount of time and energy (6). The caregiver is often a member of the patient's immediate family, such as a spouse, son, daughter, close friend or neighbor (7). Caregivers are involved in many different ways in the diagnosis, treatment and end of treatment (8). Given the increasingly complex symptoms, lack of self-care, the emergence of new needs and major lifestyle changes, caregivers of cancer patients face many challenges (9,10).

The concept of "burden" has emerged to describe the negative effects of the caregiving process on caregivers (11). Caregiver burden is defined as the degree to which individuals perceive damage to their physical and psychological health, daily routines, social lives and finances due to caregiving (8). Caregivers face physical, social or emotional problems and responsibilities individually or in combination (12). Especially

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in advanced cancer, patients experience poorer physical functioning, more severe pain and higher levels of anxiety and depression, resulting in a lower quality of life for them and a heavier burden on caregivers (5).

Considering all these situations, it is important to determine the burden perceived by caregivers and to identify the factors that may be associated with this burden. In this study, it is aimed to evaluate the caregiving burden of caregivers and the factors associated with it.

2. METHODS

The study was designed as a cross-sectional study. Approval for the study was obtained from the local Ethics Committee (date/number: 2024/4914). The population of the study consisted of individuals providing care to cancer patients who applied to the oncology clinic of a university hospital between March 5-25. The sample size was calculated as 320 people with a 10% difference, 5% margin of error and 95% confidence interval based on the mean score (36.6±7.6) found for the same scale in a previous study with the G-power program. The data of the study were collected with 362 people who gave verbal consent to participate in the study at a University Hospital between 5-25 March 2024. The questionnaire was administered by face-to-face interviews to the relatives of cancer patients who were hospitalised in the oncology service of the hospital and who applied to the oncology outpatient clinic. Each questionnaire was completed in 20 minutes.

For the study, a data collection form consisting of 26 questions was prepared by the researchers by reviewing the literature. The first part of the data collection form consisted of 11 questions about sociodemographic characteristics, the second part of the data collection form consisted of 9 questions about the caregiving process, the third part of the data collection form consisted of 6 questions about the patient being cared for and the last part of the data collection form consisted of the Caregiving Burden Scale.

2.1. Caregiving Burden Scale

The scale was developed by Zarit, Reever and Bach-Peterson in 1980 and its Turkish validity and reliability was conducted by İnci and Erdem in 2008. The scale consists of 22 statements that determine the impact on the life of the caregivers of the individual in need. The scale has a five-point Likert-type evaluation ranging from 0 to 4 as “never”, “rarely”, “sometimes”, “quite often”, “almost always”. A minimum score of 0 and a maximum score of 88 can be obtained from the scale. A high scale score indicates that the distress experienced is high. Scores obtained from the scale can be divided into four categories by grading them as (0-20) little/no burden, (21-40) moderate burden, (41-60) severe burden and (61-88) excessive burden (13).

2.2. Statistical Analysis

Statistical analysis of the data was performed using SPSS 22.0 package program. The conformity of the data to normal distribution was evaluated by Kolmogorov-Smirnov test and Histogram graph. Since the data were not normally distributed, median ± 1st Quarter-3rd Quarter values were used to summarize the numerical data, and numbers and percentages were used to summarize the categorical data. Mann Whitney U and Kruskal Wallis tests were used to compare the numerical data because the data were not normally distributed. Mann Whitney U test with Bonferroni correction was used as post hoc. P<.05 was accepted as the cut-off value for statistical significance.

3. RESULTS

The mean age of the participants was 45.8±14.2 years. 63.8% of the participants were female and 77.1% were married. Sociodemographic characteristics of the participants are given in Table 1. Of the caregivers, 76.5% of the participants lived in nuclear families, 49.5% lived in the city center, and 32.6% were primary school graduates. 40.1% of the participants had a chronic disease (Table 1).

Table 1. *Sociodemographic characteristics of the participants*

Features		n	%
Gender (n=362)	Female	231	63.8
	Male	131	36.2
Marital status (n=362)	Single	83	22.9
	Married	279	77.1
Education status (n=362)	İlliterate	17	4.7
	Primary School	118	32.6
	Middle School	57	15.7
	High Schoolı	106	29.3
	University and above	64	17.7
Income status (n=362)	My income is less than my expenses	111	30.7
	My income is equal to my expenses	176	48.6
	My income is more than my expenses	75	20.7
Social security (n=362)	No	50	13.8
	Yes	312	86.2
Family type (n=362)	Living alone	24	6.6
	Nuclear family	277	76.5
	Extended family	61	16.9
Place of residence (n=362)	Village	57	15.7
	District	126	34.8
	City center	179	49.5
Presence of chronic disease (n=362)	No	217	59.9
	Yes	145	40.1
Type of chronic disease (n=145)	Diabetes Mellitus (DM)	31	21.4
	Hypertension (HT)	42	29.0
	DM+HT	25	17.2
	Other	47	32.4

Of the caregivers 72.9% of the caregivers lived with the patient and 72.3% of these participants had been living in the same house with the patient for more than 3 years. The rate of caregiving by someone other than the patient being cared for was 11.3%, and the rate of caregiver assistance was 56.1%. Of the participants, 9.9% stated that they had received training in

caregiving and 18.0% had experience in caregiving. 93.6% of the participants had a relationship with the caregiver. Of the individuals who were close to the patient, 38.3% stated that they were the spouse of the patient (Table 2).

Table 2. *Characteristics related to the care giving process*

Features		n	%
Staying with the patient (n=362)	No	98	27.1
	Yes	264	72.9
Length of stay with the patient (n=264)	0-6 months	15	5.7
	6 months-1 year	19	7.2
	1-2 years	28	10.6
	2-3 years	11	4.2
	More than 3 years	191	72.3
Caring for someone else (n=362)	No	321	88.7
	Yes	41	11.3
Presence of caregiver helpers (n=362)	No	159	43.9
	Yes	203	56.1
Training in caregiving (n=362)	No	326	90.1
	Yes	36	9.9
Caregiving experience (n=362)	No	297	82.0
	Yes	65	18.0
Presence of closeness with the patient (n=362)	No	23	6.4
	Yes	339	93.6
Degree of closeness (n=339)	Mother	73	21.5
	Father	68	20.1
	Wife	130	38.3
	Other (uncle, aunt etc.)	68	20.1

The mean age of the patients receiving care was 61.7±15.2 years. 55.5% of the patients were male, 82.9% were receiving active treatment, and 12.7% had a disability. Of those with disabilities, 58.6% were unable to walk. Breast cancer was the most common type of cancer in the patients receiving care with 14.6% (Table 3).

Table 3. *Characteristics of the patient under care*

Features		n	%
Gender (n=362)	Female	161	44.5
	Male	201	55.5
Treatment status (n=362)	Does not take	62	17.1
	Receiving	300	82.9
Presence of physical or mental disability (n=362)	No	316	87.3
	Yes	46	12.7
Type of disability (n=46)	Alzheimer	12	26.1
	Inability to walk	27	58.6
	Other (vision, hearing, etc.)	7	15.3
Cancer type of the patient receiving care (n=362)	Breast	53	14.6
	Lung	50	13.8
	Colorectal	46	12.7
	Prostate	37	10.2
	Stomach	31	8.6
	Leukemia-lymphoma	29	8.0
	Unspecified	28	7.7
	Gynecological	26	7.2
	Liver-pancreas	21	5.8
	Urinary system	13	3.6
	Other	28	7.8

The median total score was 31.0 (23.0-41.3). Accordingly, the participants had a moderate level of caregiving burden. The median scale score of women was higher than that of men (p=.045). A statistically significant difference was found between the median scale scores of caregivers according to family type (p<.001). The median scale score of caregivers living in extended families was higher than that of caregivers living in single and nuclear families (p=.003, p<.001). The median scale score of those with chronic diseases was higher than those without chronic diseases (p=.032). A statistically significant difference was found between the scale score medians according to the place of residence of the caregiver participants (p=.008). The median scale score of caregivers living in the village was found to be higher than caregivers living in the province and district (p=.006, p=.003). No statistically significant difference was found in the total scale score of the caregiver participants according to educational status, marital status, social security and income status (Table 4).

Table 4. *Comparison of the median values of the total score obtained from the scale according to the sociodemographic characteristics of the participants*

		Scale total score Median (1st-3rd quartile)	p	Post-hoc
Gender[a]	Female	33.0 (24.0-42.0)	**.045**	
	Male	29.0 (21.0-40.0)		
Education status[b]	İlliterate	27.0 (22.5-52.5)	.835	
	Primary School	31.5 (25.0-41.3)		
	Middle School	33.0 (20.5-41.5)		
	High School	30.5 (23.0-42.0)		
	University and above	31.0 (21.3-40.8)		
Marital status[a]	Single	31.0 (21.0-42.0)	.551	
	Married	31.0 (23.0-41.0)		
Social security[a]	No	29.5 (20.8-47.3)	.693	
	Yes	31.0 (23.0-41.0)		
Income status[b]	My income is less than my expenses	32.0 (23.0-44.0)	.434	
	My income is equal to my expenses	29.5 (23.0-40.0)		
	My income is more than my expenses	31.0 (23.0-42.0)		
Family type[b]	Living alone[1]	32.5 (18.5-40.0)	**<.001**	1-3* 2-3**
	Nuclear family[2]	29.0 (21.5-40.0)		
	Extended family[3]	38.0 (28.5-46.0)		
Presence of chronic disease[a]	No	30.0 (22.0-40.0)	**.032**	
	Yes	33.0 (24.0-44.0)		
Place of residence[b]	Village[1]	37.0 (26.5-47.0)	**.008**	1-2[a] 1-3[b]
	District[2]	31.0 (20.0-41.0)		
	City center[3]	30.0 (23.0-40.0)		

** p=.003 **p<.001 a-> p=.006 b-> p=.003. [a] Mann-Whitney U test [b] Kruskal-Wallis test*

The total scale score of caregivers who stayed in the same house with the patient was found to be higher than those

who did not stay with the patient (p=.002). When the median total scale score was compared according to the degree of closeness between the patient and the caregiver, a statistically significant difference was found (p=.037). The total scale score of those who cared for their fathers was lower than those who cared for other relatives (p=.007). The total scale score of caregivers of patients with disabilities was higher than caregivers of patients without disabilities (p=.014). A statistically significant difference was found when the median total scale score of patients with disabilities was compared according to the type of disability (p=.003). The total scale score of caregivers of patients with Alzheimer's disease was higher than that of caregivers of patients with other disabilities (p=.003) (Table 5).

Table 5. *Comparison of the median values of the total score obtained from the scale according to the characteristics of the caregiving process and the patient being cared for*

Features		Scale total score Median (1st-3rd quartile)	p	Post-hoc
Staying with the patient[a]	No	29.0 (18.8-37.0)	.002	
	Yes	32.0 (24.0-43.8)		
Degree of proximity[b]	Mother[1]	34.0 (24.5-42.0)	.037	2-4*
	Father[2]	27.5 (18.0-37.8)		
	Wife[3]	30.0 (23.0-42.3)		
	Other[4](uncle, aunt etc.)	36.0 (25.5-43.8)		
Presence of physical or mental disability[a]	No	30.0 (22.0-41.0)	.014	
	Yes	37.0 (26.5-45.5)		
Type of obstacle[b]	Alzheimer[1]s	43.5 (37.5-53.8)	.003	1-3**
	Inability to walk[2]	34.0 (28.0-42.0)		
	Other[3] (vision, hearing, etc.)	25.0 (19.0-30.0)		

**p=.007 **p=.003 Mann-Whitney U test Kruskal-Wallis test*

4. DISCUSSION

In this study, which aimed to evaluate the care burden of caregivers and related factors, it was found that more than half of the caregivers were women and the mean age of all participants was 45.8 years. In a study conducted in Korea, the average age of caregivers was 46.2 years and the majority of them were women, and in similar studies in America, it was determined that more than half of the caregivers were women and their average age was similar (14-16). Cancer is a major public health problem affecting all countrie (17). It has been defined that 61.9% of cancer-related deaths and 56.4% of newly diagnosed cancer cases are seen in individuals aged 65 years and older (18). Accordingly, considering that caregivers are usually relatives of patients, it is predictable that caregivers are around a certain age. In addition, considering that women are generally more interested in the work at home due to the social structure of our country, it can be thought that the fact that the individuals who care for the sick person at home are women is related to this.

In this study, it was found that the most common type of cancer in the patients receiving care was breast cancer, followed by lung cancer. Similarly, it has been reported in previous studies that the most common cancer types of patients receiving care are lung, then colorectal, and breast cancer (14,19). In Turkey, the most common cancers in men are trachea, bronchus and lung cancer, while breast and thyroid cancer in women. In this context, the results of our study are consistent with the data of our country (20).

In our study, it was determined that the participants had moderate caregiving burden. In similar studies, it was determined that the participants had a moderate level of caregiving burden (21,22). This result suggests that caregivers have similar difficulties even in different countries and with different diseases. Even if the socio-cultural structure is different in each country and society, this burden on caregivers should not be ignored.

In this study, it was found that women had a higher caregiving burden than men. In studies conducted in different countries, it has been reported that women have a higher burden of caregiving than men (22,23). This may be due to reasons such as the fact that the majority of caregivers are women, patients are usually family members, women are naturally more emotional and sensitive, society's expectations from women are high, and women's work at home is not given due importance.

In our study, it was found that caregivers living in extended families had higher caregiving burden than those living in single and nuclear families. In other studies conducted with caregivers, no difference was found in the burden of caregiving according to the family type of the participants (24,25). The reason for this difference may be due to the type of cancer of the patients, the different patients cared for by the research subjects, and the different sample of the study. It is expected that those who live in extended families will have a high burden of caregiving due to the high number of individuals who need help other than the patient they care for.

In this study, it was found that those with chronic diseases had higher caregiving burden than those without chronic diseases. Similarly, in different studies, it has been determined that those with health problems have higher caregiving burden (24,26). The time requirement, treatment processes and complications related to the disease may cause a burden on individuals with a chronic disease due to their own illness. Caring for another person in addition to caring for their own illness adds another burden to this burden.

In this study, it was determined that caregivers living in the village had higher care burden than those living in the province and district. In different studies, no difference was found in the care burden of the participants according to the settlement (22,27). This situation may vary depending on the differences and difficulties in the access of individuals

to health services in the settlements where the study was conducted. In this context, individuals in some settlements may have more burden.

In this study, it was found that caregivers who stayed in the same house with the patient had higher care burden than those who did not stay with the patient. In a study, it was determined that the burden of caregiving was higher in those living in the same house with the patient (28). It is possible that caregivers who stay in the same house with the patient have difficulty in finding the opportunity to spare time for themselves and therefore feel more burdened.

In this study, it was found that caregivers of fathers had lower caregiving burden than caregivers of other relatives. In different studies, it was determined that caregivers who care for their spouses had lower caregiving burden than caregivers who care for other relatives (29,30). This difference may depend on the type and severity of the disease of the individuals being cared for, the place and duties of the caregivers in the family, and the age and disease status of the caregivers.

In our study, it was found that caregivers of patients with disabilities had higher caregiving burden than caregivers of patients without disabilities, and caregivers of patients with Alzheimer's disease had higher caregiving burden than caregivers of patients with other disabilities. In studies, it has been determined that caregivers of elderly individuals who need help in daily living activities have a higher burden of care (31,32). In addition to the difficulties in caring for sick individuals, it is expected that the presence of other disabilities and problems will cause an increase in the current burden of caregivers. In addition, when the type of disability is mental, it can be thought that the patient's compliance with the treatment is also affected, so the difficulties faced by caregivers increase even more.

5. CONCLUSION

According to the study, the burden of caregiving was found to be higher in women, those living in extended families, those residing in villages and those with chronic diseases. It was found that the burden of caregiving was higher in those who stayed with the patients they care for when the patient had a disability in addition to the disease.

Mental health trainings can be given to individuals who are found to have a high caregiving burden in order to overcome this situation. In this regard, trainings and studies can be conducted in cooperation with provincial directorates of health and provincial directorates of family and social services. Instead of caregivers in extended families helping family members alone with a patriarchal understanding, equal rest time and space can be provided for everyone. Assistance services can be provided by local governments in this regard. Provincial health directorates can list those with caregiving burdens and prioritize them in hospital priority protocols. Those who stay with their caregivers can be supported by the provincial directorates of family and social services for short periods of time by the provincial directorates of family and social services with personnel who can provide elderly care at certain times and can work in an integrated manner with home health services in order to enable them to spare time for themselves to reduce their caregiving burden. Suggestions can be presented to health policy makers in order to emphasize the importance of the issue by conducting more studies of this kind.

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Clinical and Experimental Health Sciences

Can Systemic Inflammation Be Predicted in Cervical Discopathy Patients Using Hemogram Data?

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ABSTRACT

Objective: To compare hemogram data of patients with single-level discopathy, multi-level discopathy and no discopathy on cervical MRI.

Methods: The patients' clinical history, MRI images and hemogram data were retrospectively accessed from our hospital data system. The subjects' values of hemogram parameters including neutrophil count, lymphocyte count, white blood cell (WBC) count, platelet (PLT) count, neutrophil /lymphocyte ratio (NLR), PLT/lymphocyte ratio, MPV and RDW were recorded. In addition, cervical MRI images and radiological reports were accessed from the hospital data system.

Results: In the study, data from a total of 416 patients were evaluated in 3 groups. The first was the single-level cervical discopathy (CDH) group (n=79), the second was multilevel CDH (n=186), the third was the control group (n=151). Statistically, there was no difference between MPV, RDW, WBC, neutrophil and PLT counts in the three groups. While there was no difference between patients without discopathy detected on cervical MRI and patients with single-level discopathy, there was a statistical difference in the PLT/lymphocyte ratio and lymphocyte count compared to the multi-level CDH group. While the lymphocyte count was lower in the patient group without discopathy (p=0.012), the Plt/lymphocyte ratio was higher (p=0.02).

Conclusion: This study, which we conducted using the hemogram data of patients with and without cervical discopathy detected on cervical MRI, shows that we cannot predict systemic inflammation using only the hemogram data.

Keywords: Cervical discopathy, hemogram, mean platelet volume, inflammation, neck pain

1. INTRODUCTION

When we look at the etiology of musculoskeletal pain, back pain comes first and neck pain comes second (1). One of the important etiological causes of neck pain is intervertebral disc diseases (2,3). Cervical discopathy is a condition that occurs when a degenerative disc compresses the cervical spinal nerve root, usually seen in people between 30-40 ages (1). There is no strong evidence that the intervertebral discs (with degenerative or other changes) are the cause of discogenic pain (4). On the otherhand, it is known that cervical disc degeneration causes inflammation and likewise inflammation causes degeneration, and inflammation triggers the secretion of inflammatory cytokines from disc cells and the migration of immunocyte cells (5).

Although cervical disc herniation is associated with biomechanical changes in segmental motion, disc mechanism, and foramen morphology, dynamic studies on the motion or anatomical features of the cervical spine that would provide a better understanding of cervical biomechanics in patients with disc herniation are still lacking. The shape, size, and spatial relationship of the intervertebral foramen to the nerve root are important. Symptoms resulting from nerve root irritation are usually related to changes in the size or shape of the foramina through which the nerve passes. Changes in the structure of the spinal foramen are often used to understand the pathoanatomy in terms of corresponding changes in the clinical signs, symptoms, or pathophysiology of certain spinal disorders (6).

Platelets are the smallest blood components but are highly reactive. They are primarily involved in the maintenance of normal hemostasis and especially in fibrosis processes. Abundant evidence of their multifunctional nature has been demonstrated in many studies in recent years. Mean platelet volume (MPV) is an correct measure of the size of a platelet according to the volume distribution calculated by

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hematological analyzers during routine blood morphology testing. The normal value of MPV is 7.5-12.0 fl. The percentage of large platelets should be 0.2-5.0% of all platelets. Studies have found that mean platelet volume (MPV) can provide some information about both the course and prognosis of many diseases such as respiratory diseases, cardiovascular diseases, rheumatoid arthritis, juvenile systemic lupus erythematosus, Crohn's disease, diabetes and neoplastic diseases (7).

Frequently evaluated hemogram parameters are; red blood cell (RBC), red blood cell distribution width (RDW), white blood cell (WBC), lymphocyte, hemoglobin (Hb), neutrophil, mean corpuscular volume (MCV), hematocrit (HCT), HCT: mean platelet volume (HCT), etc.

Many studies have been conducted to show the role of some hematological parameters in some diseases. For example, the potential value of the ratio of platelet count to lymphocyte count or the ratio of neutrophil count to lymphocyte count in the diagnosis of ankylosing spondylitis, rheumatoid arthritis, psoriasis and Behcet's disease has been revealed by studies (8).

In this retrospective study, we looked for an answer to the question of whether there is a difference between the hemogram data of patients with single-level discopathy, multi-level discopathy and non-discopathy in cervical MRI.

2. METHOD

This study was conducted retrospectively using the recorded data of patients who applied to the Physical Therapy and Rehabilitation outpatient clinics of Alaaddin Keykubat University Training and Research Hospital and Sıtkı Koçman University Training and Research Hospital. Ethics committee approval for this study was given by the Alanya Alaaddin Keykubat University Medical Faculty Hospital Clinical Research Ethics Committee. Date and decision number are indicated. (23/10/2024-23-03) Patient data were collected between November 2024 and December 2024. A total of 466 patients from the hospital system were retrospectively screened. Hemogram data were checked and patients with active infection findings, leukopenia and severe anemia were excluded. In addition, patients with chronic heart disease, diabetes mellitus, hypertension and those using a medication that could change platelet counts were identified and their data were not recorded. Hemogram data of 416 patients eligible for the study were divided into 3 groups, taking into account cervical MRI findings (79 patients with single-level discopathy on cervical MRI, 186 patients with multi-level discopathy, 151 patients without a serious discopathy image, i.e. accepted as the control group). Patients with cervical bulging and cervical lordosis flattening were included in the control group. The median ages of the single level CDH, multi-level CDH, and control groups were 43 (20-69), 48 (23–78), and 33 (18–61) years, respectively. There were 26 men and 53 women in the single level CDH group, 63 men and 123 women in the multi-level CDH group, and 24 men and 127 women in the control group. The patients' clinical history, MRI images and hemogram data were retrospectively accessed from our hospital data system. The subjects' values of hemogram parameters including neutrophil count, lymphocyte count, white blood cell (WBC) count, platelet (PLT) count, neutrophil /lymphocyte ratio (NLR), PLT/lymphocyte ratio, MPV and RDW were recorded. In addition, cervical MRI images and radiological reports were accessed from the hospital data system.

2.1. Statistical analysis

No similar study was found in the literature examining the independent variables in our study (single-level disc herniation, multi-level disc herniation, control group). Therefore, we used the G power 3.1 program to calculate the sample size of our study. In the calculation, a medium effect size (f= 0.25), 5% margin of error (alpha = 0.05) and 80% power (1-beta= 0.80) were taken, and the total sample size was calculated as 159. SPSS 25.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Categorical variables in the study were compared using the Chi-square test. The Kolmogorov – Smirnov test was used to evaluate the normality of continuous variables. Continuous variables were expressed using mean±SD, median value, and minimum-maximum values. When comparing independent groups, one-way ANOVA was used for data that were normally distributed, and the non-parametric equivalent Kruskal-Wallis test was used for data that were not normally distributed. Statistical significance level was accepted as p<.05.

Table 1. *Demographic data of participants*

	Single level (n=79)		Multi-level (n=186)		Control (n=151)			
	n	Mean±SD	n	Mean±SD	n	Mean±SD	p	Posthoc test
Age (year)		41.±11.2 43 (20-69)[a]		47.6±10.8 48 (23-78)[a]		34±10.7 33 (18-61)[a]	<.001*	Control<single level<multi-level
Sex								
Male	26		63		24		<.001[β]	
Female	53		123		127			

*amedian (minimum-maximum), *Kruskal-Wallis test, β Chi – square test*

Table 2. *Hemogram data*

	Single level (n=79)	Multi-level (n=186)	Control (n=151)		
Dependent variables	Mean±SD	Mean±SD	Mean±SD	p	Posthoc test
White blood cell	7.2 ± 1.5 7.2 (4.5-11.5)*	7.3 ± 1.7 7.1 (4.4-14.6) *	6.9 ± 1.5 6.9 (4.1-12.4) *	.085[a]	
Neutrophil	4.2±1.1 4.2 (2.0-7.0) *	4.1±1.2 4.0 (1.8-9.4) *	4.0±1.1 3.8 (2.0-8.7) *	.480[a]	
Lymphocyte	2.3±0.5 2.2 (1.2-3.9) *	2.4±0.6 2.3 (1.2-5.4) *	2.2±0.6 2.1 (1.1-4.1) *	.012[a]	Multi-level >control (p=.01)
Neutrophil / lymphocyte ratio (NLR)	1.9±0.7 1.8 (0.8-5.0) *	1.8±0.7 1.7 (0.8-5.1) *	2.0±0.8 1.9 (0.7-5.1) *	.167[a]	
Platelet (PLT)	267.1±51.7	262.2±53.8	271.5±59.5	.308[b]	
RLT/ lymphocyte ratio	123.1±37.0 117.0 (60.3-244.1) *	117.3±34.3 112.9 (45.5-245.7) *	132.5±41.5 128.3 (53.5-259.6) *	.002[a]	Control >multi-level (p=.02)
MPV	10.8±0.9 10.7 (8.7-13.1) *	10.6±0.9 10.5 (7.8-13.1) *	10.6±0.9 10.6 (9.1-13.4) *	.365[a]	
RDW	13.0±0.9 12.9 (11.6-17.2) *	13.2±1.1 13.0 (11.5-19.6) *	13.3±1.4 13.1 (11.1-19.8) *	.461[a]	

**median (minimum-maximum), a Kruskal-Wallis test, b One-way ANOVA test*

3. RESULTS

According to the data we obtained from the study; there was a significant difference in age between the 3 groups (p < .001) (average age control group< single level group< multi-level group). When neutrophil (p = .480), WBC (p = .085) and PLT (p = .308) values were examined, no significant difference was found among the patients included in the study. The lymphocyte count was found to be lower in the group without cervical discopathy (p = .012). The mean MPV value was 10.8±0.9 fL in the single-level CDH group, 10.6±0.9 fL in the multi-level CDH group and 10.6±0.9 fL in the control group. However, when all 3 groups were compared statistically, the difference between the MPV values was not significant (p = .365). The mean RDW value was found to be 13±0.9 in the single-level CDH group, 13.2±1.1 in the multi – level CDH group, and 13.3±1.4 in the control group. However, when the above data were compared statistically, no significant difference was found in terms of RDW values (p = .461). In addition, the neutrophil/lymphocyte ratio was similar in both the discopathy (single-multiple levels) and no discopathy groups (p = .167). When the control group was compared with the multilevel CDH group in terms of PLT/ lymphocyte ratio, a significant difference was observed, higher in the control group (p = .02) (Table 2).

Participants' age and gender data are given in Table 1, and hemogram data and the rates obtained from these data are given in Table 2.

4. DISCUSSION

Spondylosis and cervical disc herniation may trigger inflammation and ischemia caused by biochemical and immunological factors involved in the pathophysiology of radiculopathy. A relationship has been shown between high interleukin-6 levels and slower healing rate in patients with lumbar radicular pain (9). The hypothesis of this study was whether it is possible to evaluate systemic inflammation using hemogram data in patients diagnosed with cervical discopathy. However, according to our study, no significant relationship was found between cervical discopathy and hemogram data. It is complicated to classify neck pain in people presenting to health institutions. Degeneration is also common in asymptomatic populations, making it difficult to interpret images. For example, in a systematic review, 29% of asymptomatic 20-year-olds had disc protrusion and 4% had facet degeneration, while these rates were reported as 43% and 83%, respectively, in 80-year-olds (9). Considering this data, we did not include patients without neck pain in our study. Consistent with these data, in our study, the mean age of the patients without discopathy according to cervical MRI findings was lower than the mean age of the patients with discopathy.

In a study investigating the relationships between C reactive protein levels, leukocyte count, erythrocyte sedimentation rate, MPV, RDW, PLR and NLR and their high – frequency hearing thresholds in patients with ankylosing spondylitis; significant increases in NLR, leukocyte count, ESR and CRP were detected in patients with ankylosing spondylitis (10). We also compared NLR and leukocyte count values between each group, but we did not find any significant difference.

A systematic review found a correlation between VAS scores and levels of TNF alpha, TNFR1, IL6, IL8, and interferon-gamma. Marshall et al identified autoantibodies against nucleus pulposus herniated into the epidural space (11). The idea that IL8, IL6, IL15 and type 1 interferon initiate pathological processes in discopathies was also supported in a similar study (12). Considering that the count of neutrophils increases and the count of lymphocytes decreases as a physiological response to stress in the body; NLR can be used to predict the increase in inflammation in the body (13). We compared the groups, thinking that the neutrophil/

lymphocyte ratio might have increased due to increased systemic inflammation in patients with cervical discopathy, but we found NLR to be similar. However, according to the data of our study, the lymphocyte count was found to be lower in patients without discopathy in cervical MRI, while the plt/lymphocyte ratio was found to be higher.

In a retrospective study including 20 patients with sciatic pain, 73 patients with lumbar disc herniation (LDH) and 57 patients as a control group; MPV values were found to be higher in the lumbar discopathy group; RDW values were also found to be higher in this group compared to the sciatic and control groups (14). In our study, when we compared patients with single-level and multi-level cervical discopathy with the control group, we found similar MPV and RDW values.

In a study, the type and extent of disc herniation at presentation in 108 patients who underwent anterior discectomy for cervical radiculopathy was analyzed on MRI using a four-point scale. These were divided into disc protrusion and disc herniation groups. Study outcomes were assessed at baseline and two years after surgery using the visual analog scale for pain, 36-Question Short Form Survey and Neck Disability Index for quality of life. Patient-perceived improvement was also assessed at this time. In patients with cervical radiculopathy, the type and extent of disc herniation measured on MRI before surgery was not associated with symptom severity at presentation or clinical outcome two years after surgery (15). Since the design of our study was retrospective, we could only evaluate cervical MRI findings and hemogram data, but we could not use any scale to evaluate the pain level. Future studies are needed that evaluate prospectively not only the MRI images of the patients but also their hemogram parameters together with their pain levels.

According to a recent study, it was determined that the absolute lymphocyte count before treatment was directly related to the survival rate in patients diagnosed with esophageal cancer treated with chemotherapy, radiotherapy and immunotherapy (16). The data of our study showed that the absolute lymphocyte count was lower and the plt/lymphocyte ratio was higher in the group with normal MRI findings. However, the neutrophil/lymphocyte ratio was similar between the groups.

226 patients diagnosed with insulin-dependent diabetes mellitus and 79 adult patients with latent autoimmune diabetes were retrospectively examined and diabetic nephropathy risk factors were investigated. When the lymphocyte count of patients with insulin-dependent diabetes was examined, it was found that it was higher than the latent autoimmune diabetes group. However, the opposite was seen, the neutrophil/lymphocyte ratio was lower (17). Similar to this study, we found that the lymphocyte count was significantly higher in patients with cervical discopathy than in the group without discopathy; on the other hand, the ratio of the neutrophil count to the lymphocyte count was not different.

If we look at the shortcomings of our study, we can count the following; not using any other inflammation parameter other than hemogram and not evaluating nerve root compression in cervical MRI. In addition, patients' pain and quality of life levels can be evaluated in a prospectively designed study.

5. CONCLUSION

In our study, we wanted to answer the questions of whether we can use the previously defined rates that can be indicators of inflammation using hemogram data in patients with cervical discopathy and if so, is there a relationship with the level of discopathy. According to the data of this study in patients with cervical discopathy; we can say that we cannot predict systemic inflammation using only hemogram data. However, prospectively designed studies with larger sample sizes and using more inflammation parameters are needed.

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Design of the study: DB
Acquisition of data for the study: DB, HA
Analysis of data for the study: HA
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Clinical and Experimental Health Sciences

Prevalence of Hypomineralized Second Primary Molars and Associated Factors in Preschool Children in Trabzon, Türkiye: A cross-sectional study

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ABSTRACT

Objective: To determine the prevalence of hypomineralized second primary molar (HSPM) along with its related factors in a sample group of Turkish preschool children.

Methods: A group of 513 Turkish preschool children (29-84 months) from Ortahisar district of Trabzon was selected randomly. Information regarding potential risk indicators was collected through a questionnaire containing questions about sociodemographic status, prenatal, perinatal and postnatal conditions. The HSPM was scored using the European Academy of Paediatric Dentistry (EAPD) criteria. Decayed, missing, and filled teeth (dmft) and P–Pulpal involvement, U-Ulceration, F-Fistula and A-abscess (pufa) index were evaluated. Descriptive analysis and multivariate logistic regression analysis were used for statistical evaluation. A p-value below 0.05 was considered as significant.

Results: The prevalence of HSPM was found to be 6.8% for children and 4.6% for teeth. The most common type of HSPM defect in teeth was white creamy demarcated opacities. In this study, a significant association was found between child age and HSPM (B= – 1.074; $p < .05$). Among the postnatal factors, breastfeeding duration of more than 6 months (B= 1.307; $p < .05$); in the first year of life no lower respiratory tract infection (B= – 0.866; $p < .05$), having a history of otitis (B= 1.105; $p < .05$), and renal disease were found to be factors associated with HSPM (B= 2.773; $p < .05$).

Conclusion: HSPM prevalence observed in this study is consistent with prior research, and its associations with early-life conditions—including renal disease, otitis, and extended breastfeeding—reinforce the etiological relevance of postnatal systemic stressors in enamel hypomineralization. These findings warrant further etiopathogenic investigations.

Keywords: Hypomineralized second primary molar, Prevalence, Preschool Children, Kidney disease

1. INTRODUCTION

Developmental enamel defects refer to quantitative or qualitative abnormalities that occur in both primary and permanent teeth as a result of damage to the enamel organ (1). These developmental disturbances of enamel may result from genetic mutations and familial factors, as well as acquired systemic or local environmental influences (e.g., amelogenesis imperfecta, fluorosis), and are primarily classified as hypoplasia and hypomineralization (2,3). Hypomineralization is a qualitative defect of enamel characterized by well-demarcated opacities in which the translucency of enamel is reduced (1).

Enamel hypomineralization is a common developmental defect of enamel in both permanent and primary teeth (4). When these defects occur in one to four permanent first molars, often involving the permanent incisors, the condition is referred to as molar incisor hypomineralization (MIH) (5). This can involve one or more permanent first molars and, in some cases, permanent incisors (6). During the primary dentition, a condition similar to MIH has been noticed. It mainly affects the second primary molars and has been named hypomineralized second primary molar (HSPM) (5,6). While some studies have reported prevalence rates of 4.9% in the Netherlands, 5.6% in India, 18.9% in Spain, the global prevalence of HSPM varies from 0% to 41% (5,7-10).

The clinical presentation of hypomineralized second molars is similar to that of MIH, with the following observed characteristics: demarcated opacities, post-eruptive enamel breakdown, atypical caries, atypical restorations, and

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extraction due to HSPM (11). In children with these enamel defects, hypersensitivity can be observed when consuming cold food and drinks, even when breathing, beginning after the eruption of the teeth. This sensitivity could be cause children to avoid brushing their teeth, leading to poor oral hygiene and cavities (12). Elfrink et al. (13) reported increased risk of MIH in children with primary molar hypomineralization. The results of a meta-analysis conducted by Garot indicate that the presence of HSPM is a precursor for MIH (14).

The etiology of hypomineralized second molars is considered to be multifactorial, although no definitive conclusion has yet been reached. Prenatal risk factors for HSPM include maternal illnesses, the use of antibiotics and other medications, as well as tobacco and alcohol consumption. Additionally, perinatal factors such as delivery type, complications, prematurity, Apgar scores, and low birth weight are frequently investigated, while postnatal considerations commonly include breastfeeding duration, respiratory illnesses, infections, antibiotic use, and fever (15).

To the best of our knowledge, only one study from Türkiye has investigated the prevalence and aetiology of HSPM (16). The aim of this study was to determine the prevalence of HSPM in a sample of Turkish children attending preschool education and to evaluate the associated etiological factors. The null hypothesis of the study; the prevalence of HSPM in Turkish preschool children is not significantly influenced by prenatal, perinatal, or postnatal etiological factors.

2. METHODS

2.1. Ethical Aspects

The present observational cross-sectional study was approved by the Ortahisar district Directorate of National Education Department of Education and the Ethical Committee of the Dentistry Faculty of Karadeniz Technical University (Protocol No: 2023/4, March 2023). This study is in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. The research was conducted from September to November 2023 in the city of Trabzon in Türkiye.

2.2. Study Design and Sample Selection

The number of children who should be included in the study was calculated as 467 according to the binomial distribution with a prevalence of 14.1% and a sensitivity of 3% at 95% confidence interval based on the study by Owen et al. (17). Also, in the literature is stated a minimum of 300 individuals is required for the conduct of prevalence studies on MIH/HSPM (18). According to this reference, a total of 467 children would have been sufficient. However, to account for potential data losses, the decision was made to include at least 500 children in the study.

The study, concentrated on the Ortahisar district, the largest in Trabzon, which encompasses 25 public and 20 private schools. The schools were selected using a computer-assisted program called Research Randomizer (Version 4.0) to ensure a balanced gender distribution and adequate representation of both public and private schools (19).

2.3. Calibration Training and Exercise

Before the fieldwork and the dental examination period, three pediatric dental specialists (specializing in the relevant field for 2 years) underwent calibration courses for scoring the HSPM, including theoretical – practical training and differential diagnosis of different clinical conditions based on clinical case photographs and books on the subject (20). The accuracy of their diagnoses was determined by consensus between two experts in the field of pediatric dentistry (Assoc. Prof. Dr. and Prof. Dr.) using annotated photographs from books and articles describing HSPM.

After the training, the three investigators who would take part in the study evaluated the images for HSPM according to the EAPD criteria in two separate sessions 1 week apart, using the article by Ghanim et al. (21) in which there were different cases from the photographs used during the training. Lesions were graded as mild, moderate and severe for each surface separately in different situations in accordance with the EAPD criteria (18).

2.4. Collection of Clinical and Questionnaire Data

The study was conducted on preschool children registered in private and public kindergartens in the Ortahisar district of the Trabzon province. One week prior to the intraoral examinations, the relevant schools were visited and cooperation was established with the school administration. A questionnaire form comprising questions pertaining to the aetiology of hypomineralisation and an informed consent form outlining the content of the study were delivered to the parents one week prior to the examination day (Contact numbers are available to communicate on questions that are not understood in the questionnaire). The informed consent form and questionnaires were collected on the day of the examination. Children of parents who had consented to the study and completed the questionnaire in its entirety were included in the study. This study excluded children who did not attend the relevant kindergartens, children whose legal parents did not give consent or completed the questionnaire incompletely, children who could not adapt to the study, children with intellectual and developmental disabilities or syndromes, parents whose native language was not Turkish, children with fixed orthodontic appliances, and children with severely damaged or missing primary second molars where the cause of breakage or tooth loss cannot be determined.

A questionnaire was developed based on a literature review to identify potential etiological factors associated with HSPM (22-25). The questionnaire covers a variety of aspects, including demographic information (such as the child's gender, mother's level of education and the family's socioeconomic status, etc.), details of the child's systemic health, and questions about the child's prenatal, perinatal

and postnatal experiences (such as the mother's illnesses and medications during pregnancy, type and timing of delivery, duration of breastfeeding and the child's illnesses etc.).

2.5. Dental Examination

The intraoral examinations of the children were conducted in classrooms designated by the school administration as appropriate for this purpose. For each researcher, an area consisting of a table and a chair was allocated in these classrooms. The intraoral examinations of the children included in the study were conducted in the classroom setting with the use of a sterile mouth mirror and the World Health Organization (WHO) periodontal probe, illuminated by an artificial light source (Winlite lamp). Following the standard infection control protocol, disposable gloves and a personalised sterile examination set were used. The investigators (ENT, MNO, HST) conducted the examinations in a face-to-face positioning (26).

During the examination, the teeth were not dried, and excess saliva and plaque were removed from the surface of the teeth with the help of a sterile cotton roll, in accordance with the recommendations of Ghanim et al. (21).

During the examination, the diagnosis of HSPM was made according to EAPD criteria (18). In accordance with the EAPD criteria, lesions in teeth diagnosed with HSPM were assigned scores between 2 and 6 based on their clinical appearance. Limited opacities were given a score of 2 (21 if white or creamy, 22 if yellow or brown), post-eruptive enamel breakdown (PEB) 3, atypical restoration 4, atypical caries 5 and missing teeth due to MIH/HSPM 6. Lesion extension criteria was also graded as 'I' if the lesion covered less than one-third of the affected tooth surface, II' if at least one-third but less than two-thirds of the affected surface, and 'III' if the lesion covered at least two-thirds of the affected tooth surface. Children with hypomineralisation in at least one of the second molars were diagnosed with HSPM. Clinical status, degree of defect, and tooth eruption status were scored separately for each surface of the teeth using the long version of the MIH/HSPM clinical data record form. For the diagnosis of second molar hypomineralisation, all surfaces of four second molars were evaluated separately. Defects smaller than 1 mm are not considered (18). In addition to the second primary molars, which are index teeth, primary canines and also any other teeth were evaluated for the presence of hypomineralisation.

Each child was examined for oral hygiene, and the presence of dental plaque was recorded. Dental status was evaluated using the DMFT/dmft index recommended by the World Health Organization (WHO) for epidemiological studies, with scores calculated by summing the number of decayed (d/D), missing (m/M), and filled (f/F) teeth (27). In addition, the PUFA index—representing Pulpal involvement (P), Ulceration (U), Fistula (F), and Abscess (A)—was applied to assess the clinical consequences of untreated dental caries in primary molars and canines (28). In the study, the PUFA score was recorded as present if any primary molar or canine exhibited signs of pulpal involvement, ulceration, fistula, or abscess.

2.6. Statistical Analysis

The data analysis was performed using SPSS for Windows 22.00 (Statistical Package for the Social Sciences, SPSS Inc. Chicago, IL, USA). To assess the interobserver agreement, Fleiss Kappa test was used to calculate the interobserver agreement coefficients. Descriptive statistics were presented as mean ± standard deviation (sd), median, minimum-maximum values, and frequencies, depending on the type of data (continuous or categorical parameters). The normality of the data was checked using the Kolmogorov-Smirnov test.

In this study, multivariate logistic regression analysis models were used to determine the predictive effect of independent variables on the presence/absence of HSPM (dependent variable). In this study, five different regression models were applied. The first model included specific independent variables such as gender, age, maternal education level, age at birth, family income level, and school type. The second model included the independent variables plaque presence/absence, dmft, and pufa. The third model included additional variables such as prenatal factors (maternal illness during pregnancy, medication use). The fourth model included variables related to the perinatal period (type of delivery, time of delivery, birth weight, duration of breastfeeding). Finally, the fifth model included variables related to the postnatal period (systemic disease, asthma, sinusitis/rinitis, antibiotic use, kidney disease, otitis, frequent fever, etc.). In regression models with categorical predictors, dummy variables were created as needed. A p-value below than 0.05 was considered as significant.

3. RESULTS

Fleiss Kappa values showed almost perfect agreement between observers (1st session:0.825-0.993, 2nd session:0.861-0.970). Our study was performed on a total of 513 children, 247 (51.9%) boys and 266 (48.1%) girls, aged between 29 and 84 months. Of the children, 135 (26.3%) were 29-56 months old and 378 (73.7%) were 57-84 months old. HSPM was found in 35 out of 513 children (6.8%), while it was not observed in 478 children (93.2%). Therefore, the prevalence of HSPM was determined to be 6.8% in the study sample (Table 1).

A total of 2052 second primary molars were examined in the study, of which 94 (4.6%) had HSPM. This resulted in a tooth-based prevalence of HSPM of 4.6%. When the distribution of defects in hypomineralized second molars was examined, the most common type of defect was white or creamy limited opacities (63.8%). This was followed by atypical caries (13.8%). Hypomineralization defects mostly covered less than 1/3 of the tooth surface (50%). The number of tooth surfaces affected by second molar hypomineralization was 171. The hypomineralization defects were mostly located on the lingual/palatinal surface (35.1%). When the index teeth with hypomineralized second molars were examined, it was seen that the right and left upper second molars (numbers 55 and 65) were mostly affected. No hypoplasia or amelogenesis imperfecta was found in any of the index teeth (Table 2).

Table 1. *Independent variables included in the regression models*

Variables		N	(%)
Child gender	Male	247	(51.9)
	Female	266	(48.1)
Child age (month)	29-56 months	135	(26.3)
	57-84 months	378	(73.7)
School	Public	364	(71)
	Private	149	(29)
Mother's age at birth	16-30 years	254	(49.5)
	31-44 years	259	(50.5)
Mother's education	Primary school	43	(8.4)
	Middle school	44	(8.6)
	High school	144	(28.1)
	University	248	(48.3)
	PhD	34	(6.6)
Mother's occupation	Working	232	(45.2)
	Not working	281	(54.8)
Level of income	Low	74	(14.4)
	Medium	179	(34.9)
	Higher	260	(50.7)
dmft	=0	215	(41.9)
	>0	298	(58.1)
Plaque	Present	468	(91.2)
	Absent	45	(8.8)
pufa (index teeth and primary canines)	Present	11	(2.1)
	Absent	502	(97.9)
HSPM	Present	35	(6.8)
	Absent	478	(93.2)
Duration of breastfeeding	Never	26	(5.1)
	6 months and less than 6 months	34	(26.1)
	More than 6 months	351	(68.4)
	Don't know/don't remember	2	(0.4)
Lower respiratory tract infections (bronchitis, etc.) in the first year of life	Yes	128	(25.0)
	No	374	(72.9)
	Don't know/don't remember	11	(2.1)
Otitis in the first year of life	Yes	43	(8.4)
	No	452	(88.1)
	Don't know/don't remember	18	(3.5)
Kidney disease in the first year of life	Yes	9	(1.8)
	No	503	(98.1)
	Don't know/don't remember	1	(0.2)
Total		513	(100)

In addition, hypomineralization defects without HSPM were the most common (2.3%) in primary canines, followed by the presence of diffuse opacities (1.1%). For primary canines, hypoplasia was detected in 2 (0.3%) children, while amelogenesis imperfecta was not detected at all. Of the 513 children included in the study, 13 had enamel defects in teeth other than the second primary molars and canines (first primary molars, primary incisors, and lateral incisors).

In the multivariate logistic regression first model analysis, which included sociodemographic factors (gender of the child, age of the child, school type of the child, age at birth, mother's education level, mother's occupation, family income level) were determined statistically significant relationship between that the age category and HSPM (B= – 1.074, p = .047; p < .05) (Table 3). HSPM was reported in the teeth of 4 (3%) children aged 29-56 months and 31 (8.2%) children aged 57-84 months (Table 4).

While 215 (41.9%) of the children in the study had a dmft score of "0", 298 (58.1%) had a dmft score above "0". Plaque was observed on the teeth of 468 (91.2%) of the children. None of the index teeth and primary canines of 502 (97.9%) children had a PUFA score (Table 1). The multivariate logistic regression analysis second model in our study included the presence of dmft, pufa and plaque. There was no statistically significant association between these factors and HSPM (p > .05).

Table 2. *Distribution of HSPM lesions in teeth*

	Tooth 55	Tooth 65	Tooth 75	Tooth 85	Total teeth
Variables	**n(%)**	**n(%)**	**n(%)**	**n(%)**	**n(%)**
HSPM					
Present	26 (5.1)	26 (5.0)	21 (4.1)	21 (4.1)	94 (4.6)
Absent	486 (94.7)	487 (95.0)	488 (95.1)	489 (95.3)	1950 (95.0)
Tooth not visible or less than 1/3 of the crown length visible	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)	2 (0.1)
Cannot be scored	0 (0.0)	0 (0.0)	4 (0.8)	2 (0.4)	6 (0.3)
Colour and type of defect					
White or creamy demarcated opacities	19 (73.0)	16 (61.4)	15 (71.3)	10 (47.6)	60 (63.8)
Yellow or brown demarcated opacities	2 (7.8)	2 (7.8)	1 (4.8)	1 (4.8)	6 (6.4)
Post-eruptive enamel breakdown (PEB)	1 (3.9)	2 (7.8)	1 (4.8)	3 (14.3)	7 (7.5)
Atypical restoration	1 (3.9)	1 (3.9)	2 (9.5)	2 (9.5)	6 (6.4)
Atypical caries	3 (11.4)	5 (19.1)	1 (4.8)	4 (19.0)	13 (13.8)
Missing due to MIH/HSPM	0 (0.0)	0 (0.0)	1 (4.8)	1 (4.8)	2 (2.1)
Lesion extension					
Less than 1/3 of the tooth surface	15 (57.7)	9 (34.6)	11 (52.4)	12 (57.1)	47 (50.0)
At least 1/3 but less than 2/3 of the surface	7 (27.0)	10 (38.4)	5 (23.8)	3 (14.3)	25 (26.6)
At least 2/3 of the tooth surface	4 (15.3)	7 (27.0)	5 (23.8)	6 (28.6)	22 (23.4)
Localization of the lesion[a]					
Buccal/Labial	10 (22.2)	12 (26.1)	19 (45.2)	16 (42.1)	57 (33.3)
Occlusal/İncisal	14 (31.1)	13 (28.3)	12 (28.6)	15 (39.5)	54 (31.6)
Lingual/Palatal	21 (46.7)	21 (45.6)	11 (26.2)	7 (18.4)	60 (35.1)

aTotal surface affected by HSPM = 171

Table 3. *Multiple regression analysis between HSPM (dependent variable) and predictor variables*

Model 1 Constant	**B**	**95% CI for EXP(B)**		**p**
		Lower	**Upper**	
Age category *29-56 months*	-1.074	0.118	0.987	.047*
Others (57-84 months)[a]				
Model 3 Constant	**B**	**95% CI for EXP(B)**		**p**
		Lower	**Upper**	
Duration of breastfeeding *More than 6 months*	1.307	1.261	10.841	.017*
Others (Never, 6 months and less than 6 months, Don't know/don't remember)[a]				
Lower respiratory tract infection (bronchitis etc.) in the first year of life *No*	-0.866	0.202	0.873	.020*
Others (Yes, Don't know/don't remember)[a]				
Otitis in the first year of life *Yes*	1.105	1.107	8.232	.031*
Others (Yes, Don't know/don't remember)[a]				
Kidney disease in the first year of life *Yes*	2.773	2.144	119.384	.007**
Others (Yes, Don't know/don't remember)[a]				

*a (reference category); confidence interval (CI); *p < .05; **p < .01*

Table 4. *Distribution of presence/absence of HSPM according to independent variables*

	HSPM	
	PRESENT n (%)	ABSENT n (%)
Age category		
29-56 months	4 (3.0)	131 (97.0)
57-84 months	31 (8.2)	347 (91.8)
Duration of breastfeeding		
Never	0 (0)	26 (100)
6 months and less than 6 months	6 (4.5)	128 (95.5)
More than 6 months	29 (8.3)	322 (91.7)
Don't know/don't remember	0 (0)	2 (100)
Lower respiratory tract infection (bronchitis etc.)		
Yes	13 (10.2)	115 (89.8)
No	20 (5.8)	354 (94.7)
Don't know/don't remember	2 (18.2)	9 (81.8)
Middle ear infection (otitis)		
Yes	6 (14.0)	37 (86.0)
No	28 (6.2)	424 (93.8)
Don't know/don't remember	1 (5.6)	17 (94.4)
Having a kidney disease		
Yes	2 (22.2)	7 (77.8)
No	33 (6.6)	470 (93.4)
Don't know/don't remember	0 (0)	1 (100)

The multivariate logistic regression third model analysis, which included prenatal factors (maternal illness during pregnancy, fever/infection, and antibiotic use), determined that there was no statistically significant relationship between the variables and the presence or absence of HSPM ($p > .05$). Furthermore, no statistically significant correlation was found in fourth model between perinatal period factors such as the type of delivery, the time of delivery, any complications that may have occurred during delivery, and the infant's birth weight and HSPM ($p > .05$). Among the postnatal etiologic factors, breastfeeding duration of more than 6 months, no lower respiratory tract infection in the first year of life, otitis in the first year of life, and kidney disease in the first year of life were found to be factors associated with HSPM in fifth model ($B= 1.307$, $p = .017$; $B= – 0.866$, $p = .020$; $B= 1.105$, $p = .031$; $B= 2.773$, $p = .007$; $p < .05$) (Table 3). However, there was no statistically significant relationship between HSPM and the variables of having any systemic disease, asthma, pneumonia/pneumonia, sinusitis/rinitis, urinary tract infection, stomach disorder, severe diarrhea, frequent fever, prolonged hospitalization, and antibiotic use in the first year of life ($p > .05$).

4. DISCUSSION

The reported prevalence of HSPM in the literature varies considerably, ranging from 0% to 41% (10). The present study focused on the prevalence and aetiological factors of HSPM in preschool children. Although the association between HSPM and MIH has been previously explored, to the best of our knowledge, only one study from Aydın, Türkiye has reported comparable prevalence data, identifying a 4.4% rate among children aged 3–5 years (16,29). Elfrink et al. (5) similarly reported a 4.9% prevalence in 5-year-old Dutch children, which is consistent with our findings. Comparable rates have also been reported in Delhi (5%), Nigeria (5.8%), Saudi Arabia (5.4%), and Iraq (6.6%) (30-33). These differences in prevalence may result from variations in age groups studied, population characteristics, geographic and environmental factors, as well as the diagnostic criteria and indices used (10,18).

Due to the variability and inconsistency in findings related to the aetiology and prevalence of MIH and HSPM, a standardized index for recording these defects was deemed necessary (34). In response, Ghanim et al. (18) developed a data recording form aligned with the EAPD diagnostic criteria, which has since been recommended for research use. Although HSPM is typically diagnosed around the age of 5 years (34), studies have also included broader age ranges—from 30 months to 10 years (8,17,32,35). Accordingly, in the present study, HSPM lesions were evaluated in preschool children aged 29–84 months using the EAPD-based recording form (18).

In a study conducted in Türkiye, the prevalence of tooth-based HSPM was found to be 2.31% (16). The tooth-based prevalence of HSPM detected in 2052 second molars in our study was 4.6%, which is consistent with global rates (0-29.8%) (10). Similar to an Australian study (17), our findings show that the prevalence of HSPM at the tooth level (4.6%) is lower than the prevalence at the child level (6.8%), possibly because this condition does not affect all teeth in affected children (10).

Our study identified limited white or creamy opacities as the most common HSPM defect in second primary molars (63.8%), typically covering less than one-third of the tooth and mainly on palatal/lingual surfaces (35.1%). A recent meta-analysis also reported limited opacities as the most common defect type (10). Similarly, Lima et al.(22) and Sidhu et al.(36) found that HSPM lesions mostly covered less than one-third of tooth surfaces. In contrast, Goyal et al. found lesions predominantly on buccal/lingual surfaces (35). This result is thought to be favourable in terms of the fact that limited opacities, which are relatively the mildest form of defects, require only follow-up and preventive care (10).

Since it is known that the development of the second primary molars approximately coincides with the development of the primary canine teeth, the primary canine teeth were also analysed in our study (37). The most common defects in the primary canine teeth were hypomineralisation without HSPM (2.3%), followed by diffuse opacities (1.1%). In their study, Goyal et al. (35) also found limited opacities, mostly in the primary canines after the second primary molars, and similar opacities in 4.6% of first primary molars and 1.5% of primary incisors. Similarly, non-HSPM hypomineralisation defects were also recorded in teeth (first primary molars, primary incisors and lateral incisors) other than index teeth and primary canines in our study.

In the literature, there is one study that found a significant association between maternal gestational age and HSPM (38), some studies, found no statistically significant association between the child's gender, maternal education level, family income level, and maternal age at birth and HSPM (9,22,32,39). In the multivariate logistic regression analysis model created by including demographic variables in our study, there was no significant relationship between some factors (child's gender, mother's age at birth, school type, etc.) and HSPM, while the only variable found to be significantly associated with HSPM was the child's age between 29-56 months. Upon analysis of the distributions, it was observed that the incidence of HSPM was 3% in children aged 29-56 months and 8.2% in children aged 57-84 months. Similarly, Masumo et al. (40) reported in their study that used a different index with; children in older age groups were more likely to have Developmental Defects of Enamel (DDE). It is thought that the result found in our study may be associated with the cross-sectional nature of our study and the fact that the children in our study were more in the older age group. According to some studies, a significant positive relationship was found between the prevalence and severity of dental caries in children with MIH-HSPM compared to unaffected children. (41,42). In our study, no statistically significant relationship was found between the relevant factors and HSPM in the multivariate logistic regression analysis model created by including plaque, dmft and pufa scores of the patients. Similarly, Owen et al. (17) did not find more caries experience in children with HSPM, but they found a significant relationship between the severity of HSPM defects and increased caries lesion severity.

Ghanim et al.'s (43) study noted that multiple factors work together to contribute to the cause of hypomineralized second molars. For instance, while the presence of maternal hypertension was identified as a significant factor associated with HSPM, a systematic literature review found no clear evidence of a correlation between drug use and infection during pregnancy and HSPM, and more comprehensive studies were recommended to determine their effect (44). The results of the multivariate logistic regression analysis model including prenatal factors in our study supported these results and no statistically significant relationship was found between HSPM and maternal illness, fever/infection, antibiotic use during pregnancy.

One study reported significant associations between HSPM and preterm birth, low birth weight, and delivery complications, but not with type of birth (15). Similarly, Lima et al. (22) found a significant association with preterm birth, but not with birth weight, delivery complications, or type of birth. In contrast, Lopes-Fatturi et al. (45) reported an association with delivery complications, but not with preterm birth. In our study, no statistically significant association was found between perinatal factors and HSPM. It has been suggested that the reporting of different results related to these factors in the literature may be due to the evaluation of populations with different regional and general geographical characteristics in different types of studies or the use of different indices (15,39,46).

In a meta-analysis published in 2021 by Lima et al. (15) and in the study by Lima et al. (22), among postnatal factors while no association was found between pneumonia and HSPM, asthma was found to be significantly associated with higher rates of HSPM. On the contrary, in a prospective twin study, no significant association was found between having asthma in the first 18 months of life and HSPM (46). Similarly, no association was found between asthma and pneumonia and HSPM in our study, and it is thought that this result, which is partially inconsistent with the literature, may be due to the fact that the data were obtained through a questionnaire and based on memory-based results and that the rate of patients who did not have asthma in the first year of life was 95.5% in our study.

While Lopes-Fatturi et al. (45) found no association between breastfeeding or its duration and HSPM, a meta-analysis by Lima et al. (15) reported a higher prevalence of HSPM in children who were not breastfed. Similarly, in our study, breastfeeding for more than 6 months was found to be a factor significantly associated with HSPM and absence of HSPM was found in 91.7% of children who were breastfed for more than 6 months. It is also reported that exclusive breastfeeding for the first 6 months is beneficial for both mother and child, protecting against infectious diseases and childhood malnutrition (47).

Lopes-Fatturi et al. (45) found that otitis media in the first year of life was significantly associated with a higher prevalence of HSPM, a finding supported by Ghanim et al. (43), who reported that the risk of developing HSPM is significantly higher in children with otitis media. As a result of our study, a statistically

significant relationship was found between otitis in the first year of life and HSPM. In the literature, bronchitis among lower respiratory tract infections has been associated with MIH (12). In our study, a statistically significant association was found between the absence of lower respiratory tract infection and HSPM. The rate of absence of HSPM was found to be 94.7% in those who had not had lower respiratory tract infection (bronchitis etc.). In the literature, in their study, Ghanim et al. (33) found that upper respiratory tract infection increased the risk of developing HSPM fourfold, while Lima et al. (22) found no significant association between upper respiratory tract infections such as sinusitis/rinitis and HSPM. The present study did not identify a statistically significant correlation between the occurrence of sinusitis or rhinitis during the first year of life and HSPM.

In a meta-analysis study, antibiotic use and frequent fever in children were associated with HSPM (15). In addition that, similarly our findings, there are studies in the literature reporting that there is no significant relationship between antibiotic use and frequent fever and HSPM (22,45). In the literature, Devi et al. (39) found a significant correlation between the history of hospitalisation in the first week of life and primary molar hypomineralisation, whereas Elfrink et al. (48) did not find a statistically significant relationship in their cohort study. Similarly, our study did not find a significant association between the history of hospitalisation with intravenous medication use due to long-term infection in the first year of life and HSPM.

According to the results of our study, a significant association was found between the presence of kidney disease in the first year of life and HSPM. Accordingly, 22.2% of the children who had kidney disease in the first year of life had HSPM, whereas 77.8% did not have HSPM. In our analysis, kidney disease had the highest impact on the presence or absence of HSPM, with a beta coefficient of B=2.773. Similarly, Koch et al. (49) reported in their study that chronic renal failure may lead to enamel defects in the primary dentition by affecting the enamel development of the primary teeth immediately after birth. Given the study conducted by Koch et al. (49), along with variations in the index used and age range, it is recommended that future prospective studies further investigate this relationship.

In the literature, Lopes-Fatturi et al. (45) found no significant association between urinary tract infection and HSPM, Devi K. et al. (39) reported a significant association between acute gastroenteritis and HSPM in their study. Elfrink et al. (48) found no significant association between vomiting and diarrhoea and hypomineralisation of primary molars in their prospective cohort study. Similarly, in our study, no significant association was found between postnatal factors such as urinary tract infection, upset stomach, severe diarrhea and measles and HSPM.

Since most of the existing studies are retrospective, factors such as data collection based on parents' memory and difficulty in recalling past illnesses, especially in families with more than one child, are also among the limitations. Finally, the fact that the study was conducted only in the Ortahisar district of Trabzon limits the validity of the findings across Türkiye. However, the present study also has several strengths enhance the reliability and validity of the findings. It was conducted with a relatively large sample, clinical examinations were performed by calibrated pediatric dentists, schools were selected through randomized allocation, and standardized data collection tools were used. Multicenter studies are needed to generalize the findings to the entire Turkish population.

5. CONCLUSION

The prevalence of HSPM was determined to be 6.8% in 513 Turkish preschool children enrolled in the study, which is consistent with prevalence rates reported in the literature. This study showed that breastfeeding for more than 6 months, absence of lower respiratory tract infection in the first year of life, otitis media in the first year of life, and kidney disease during the first year were associated with HSPM. Given these findings, it is essential that pediatric dentists closely monitor children for the identified risk factors to raise awareness among parents, thereby facilitating early diagnosis and timely implementation of preventive measures.

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Clinical and Experimental Health Sciences

Validity and Reliability Study of The Turkish Version of The Child Primary Care Adherence Scale

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ABSTRACT

Objective: This study was aimed at adapting the Child Primary Care Adherence Scale, developed to assess parents' compliance with the recommendations made by primary health care professionals, into Turkish and to conduct its validity and reliability study.

Methods: This methodological study was conducted in a family health center between February 2024-July 2024. In the sample of the study, 325 mothers who were over 18 years old and who had children between the ages of 2 and 6 years were included. The Descriptive Information Form and Child Primary Care Adherence Scale were used to collect data.

Results: The results of the validity analyses demonstrated that the scale items measured the targeted conceptual structure. According to the exploratory factor analysis, the scale consisted of two dimensions. According to the reliability analysis, stability was sufficient and internal consistency was quite reliable. The mean score the parents obtained from the overall Child Primary Care Adherence Scale was 53.35±10.89.

Conclusion: The results indicated that the Child Primary Care Adherence Scale could be administered to parents of children aged between 2-6 years in Turkish society to determine their compliance with the care recommendations given by health professionals who checked whether the items were comprehensible.

Keywords: Child, Childcare, Reliability, Validity

1. INTRODUCTION

If public health is to be improved and protected, protection and improvement of children's health is of extreme importance.

The primary responsibility for the protection and improvement of children's health first lies with the family, then with health workers and the social environment (1).

Supporting the health of a child, preventing diseases by detecting them at an early stage, providing vaccination services, and providing health counseling to families constitute the basis of healthy child monitoring (2).

Healthy child monitoring in Turkey is carried out according to the "Baby, Child and Adolescent Monitoring Protocol" published by the Ministry of Health. According to this protocol, a record should be kept for every baby and the baby's monitoring should be initiated as soon as the baby is born. Each registered child should be followed up at birth, and after the birth, within the first 48 hours, on the 15th and 41st days, once a month in the 2nd, 3rd and 4th months, once every three months in the 6th, 9th and 12th months, once every six months between the ages of 1 and 3, once a year between the ages of 3 and 21and each follow-up should be recorded (3). One of the most important steps in infant and child follow-ups is the provision of counseling to parents. Provision of detailed information to parents is of great importance if they are to adopt and implement the recommendations given to them, to comply with the follow-up program, and to improve their health literacy. It is known that parent education increases the success of healthy infant and child follow-ups and ensures the provision of higher quality service (4). Healthy child follow-ups offer the best opportunities for providing evidence-based preventive health services such as monitoring the growth

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and development of the child, administering childhood vaccinations, determining some behavioral problems of the child and the family, and informing families about accidents and nutrition (5). In the primary healthcare, both the family physician and the family health nurse follow-up the growth and development of the child, and provide guidance and counseling to parents so that they can raise healthy children. Primary health care workers provide guidance and counseling to parents on the important stages of child development such as teething, transition to complementary feeding, toilet training, and on problems such as accident/injury risk, sleep disorders, and nutritional problems that may arise during the growth and development process and on how they can manage the process correctly. Monitoring parental compliance with the counseling and guidance services provided for them is very important. Parents' adherence to the health advice given by primary health care workers minimizes unnecessary contact with health services, strengthens families' parental competence, and most importantly, allows children to grow and develop healthily. In the Turkish literature, there is a gap regarding measurement tools that can be administered to determine parents' compliance behaviors with the basic care recommendations made for their children. Thus, the present study was carried out to adapt the Child Primary Care Adherence Scale (CPC-Adherence Scale) (6) developed by Mourao et al. (2022) to Turkish and to conduct its validity and reliability study to assess parents' compliance with the recommendations made by primary health care professionals.

2. METHODS

This methodological study was conducted with mothers registered in a family health center in Menemen, a district of Izmir, a province in western Turkey, between February and July 2024. Inclusion criteria were as follows: being over the age of 18, having children between the ages of 2 and 6 years, being primary caregiver of the child, volunteering to participate in the study, and having no obstacles to communicate. Of the mothers who presented to the family health center for any reason, those who met the inclusion criteria were assigned to the study sample using the random sampling method. It is recommended that in validity and reliability studies performed to adapt a scale to Turkish (another) language and culture, the sample size should be five-fold or ideally ten-fold the number of the items in the scale in order to perform factor analysis (7). In another method regarding the sample size, if the sample includes 200 participants, it considered as "moderate", if 300 as "good", if 500 as "very good", and if 1000 as "excellent" (8). Thus, by taking the two methods into account, it was decided to include 325 individuals in the sample to adapt the 20-item scale into Turkish.

2.1. Data Collection

Data were collected using the face-to-face interview technique during weekday working hours at the family health center. The tools used for data collection were as follows:

2.1.1. Descriptive Information Form

The form prepared by the researchers included items questioning the participants' socio-demographic characteristics such as age, education level, income, the number of children in the family, the height and weight of the youngest child, and having or not a chronic health problem.

2.1.2. Child Primary Care Adherence Scale (CPC-Adherence Scale)

The scale developed in Portugal has 20 items and the following 2 dimensions: Adherence to a Safe Psychomotor Development dimension (items 1, 4, 5, 7-9, 11-13, 15-17, 19, 20) and Adherence to Nutritional Counselling (items 2, 3, 6, 10, 14, 18).

The respondent's level of adherence to recommendations in the scale is rated on a five-point Likert type scale ranging from 1 (never) to 5 (always), for each item, the respondent is also asked to choose the options "not recommended" and "not applicable" if the item is not recommended or not applicable. If the respondent chooses either the "not recommended" or "not applicable" option for an item, this item is not included in the scoring. In the original study of the scale, the Cronbach's alpha value was 0.894 for the Adherence to a safe psychomotor development dimension and 0.608 for the Adherence to Nutritional Counselling Dimension (6).

2.2. Validity-Reliability Studies

The language and scope validity studies of the scale were conducted by taking into account the methods and processes recommended by the World Health Organization for the translation and adaptation of instruments to other languages (9). Although the original language of the scale is Portuguese, the developer of the scale sent the English version of the scale to us. Thus, the English version of the scale was translated into Turkish by two linguists independently of each other. The two Turkish forms were merged into one form, which was then translated back to English by an academician who has a good command of both Turkish and English. After the back translation, the items in the original form and the back-translated form were compared and the items whose translations were different from the original ones were revised. The Turkish draft form was then sent to eight health professionals specialized in Public Health and Child Health to obtain their opinions to find out whether the items were comprehensible. They were asked to rate each item as follows: "the item is appropriate", "the item should be slightly revised", "the item should be seriously revised" and "the item is not appropriate". After the opinions of the eight health professionals were obtained, the Davis technique (1992) was used to calculate the scope validity index for the scale items. According to the Davis technique, the number of health professionals who selected options (a) and (b) was divided by the total number of the health professionals to obtain the "content validity index" for the item. The construct validity of the scale was first tested with the Confirmatory Factor Analysis (CFA) using the model created in accordance with

the original structure. However, it was noticed that the model was not compatible due to the low goodness of fit indexes and low item factor loadings. Therefore, the validity of the new two-factor structure was tested by applying Exploratory Factor Analysis (EFA). The reliability of the scale was tested considering its stability over time, internal consistency (Cronbach's Alpha, Spearman Brown internal consistency coefficients) and item total correlation. In addition, split-half and discriminant analyses were performed. After the Turkish version of the scale took its final form, it was again translated back to English. The back-translated form was then sent to the developer of the scale to receive her approval. After her approval was obtained, other analyses were performed.

2.3. Data Analysis

The SPSS (Statistical Package for the Social Sciences) 25 program, and SPSS AMOS (Analysis of Moment Structures) 22.0 version were used in the analysis of the data. Kolmogorov-Smirnov test was used to determine whether the data were normally distributed ($p< .05$). One-way and multi-way extreme value analyses were performed on the data set, and the four extreme values were not included in the analysis. The suitability of the sample size for factor analysis was tested with Kaiser-Meyer-Olkin (KMO) analysis. Content Validity Index was used for language validity. Exploratory factor analysis (EFA) was performed to analyze the factor structure of the scale. In the Principal Components Analysis, Orthogonal Varimax rotation was used to determine the factor structure. While stability over time was tested with the test-retest correlation, internal consistencies was checked with the Cronbach's Alpha, Spearman-Brown, Guttman internal consistency coefficients and item total correlation.

For discrimination, 27% upper and lower group comparisons were performed with the independent samples t test. Hotelling's T – squared test was performed for item means.

3. RESULTS

In the study, 325 mothers were included. Their mean age was 31.3±6.38 (min: 19, max: 54) years. They were all married. The median number of children in the family was two. The mean age of the children was 4.06±1.39 (min: 2, max: 6) years, and 52.3% of them were boys. None of the children was diagnosed with a chronic disease (Table 1). The mean score the mothers obtained from the overall Child Primary Care Adherence Scale was 53.35±10.89 (min: 27, max: 85). The mean values, standard deviation, lowest and highest scores obtained from the overall scale, and its items and dimensions are presented in Table 2. There was a very high positive correlation between the scores obtained from the overall scale and its Adherence to Psychomotor Development and Health Care Recommendations dimension (r=0.976), and a high positive correlation between the scores obtained from the overall scale and its Adherence to Safety Precautions Recommendations dimension (r=0.744) (Table 3).

Table 1. *Socio-Demographic Characteristics of the Participating Mothers and Anthropometric Characteristics of their Children*

Mothers			
Age	Mean±SD	31.3±6.38	
	Minimum-maximum	19-54	
Education	Illiterate	18 (5.5%)	
	Literate but not a graduate of any school	45 (13.8%)	
	Four-year primary school	143 (44.0%)	
	Four-year junior high school	47 (14.5 %)	
	Four-year senior high school	51 (15.7 %)	
	University	21 (6.5 %)	
Employment status	Employed	106 (32.6%)	
	Not employed	219 (67.4%)	
Income status	Income less than expenses	76 (23.4%)	
	Income equal to expenses	187 (57.5%)	
	Income more than expenses	62 (19.1%)	
The number of the children in the family	Median	2	
	Minimum-maximum	1-5	
Children			
Age	Mean±SD	4.06±1.39	
	Minimum-maximum	2-6	
Sex	Girls	155 (47.7%)	
	Boys	170 (52.3%)	
		Girls	**Boys**
Height (cm)	Mean±SD	103±12.5	105±11.1
	Minimum-maximum	76-154	73-133
Weight (kg)	Mean±SD	16.3±4.09	17.4±4.26
	Minimum-maximum	8.80-35.0	8.30-38.0

3.1. Validity Findings

The Content Validity Index (CVI) calculated using the Davis technique was 0.925, which was considered as appropriate. Minor revisions were made in ten of the items in line with suggestions made by health professionals who checked whether the items were comprehensible, and content adjustment was made in one item "*From the 4th month onwards, I introduced new foods to my baby in the recommended order*", The phrase "4th month" in the item was replaced with "6th month"). This change was asked to the author who developed the scale via e-mail whether it was appropriate and her approval was obtained. The results of the analysis of the content validity assessment scores performed with the Kendall's W test demonstrated that the opinions of the health professionals who checked whether the items were comprehensible were consistent (Kendall's W=0.190; p= .299). The results of the Exploratory Factor Analysis (EFA) and Principal Components Analysis performed for the construct validity of the scale demonstrated that the sample size was sufficient for EFA (KMO=0.908; Bartlett's Test of Sphericity=2967.08; p=

.001). Three factors' eigenvalue was above 1. Since there was only one item in the third factor, they were reduced to two dimensions and the EFA was performed.

Tablo 2. *Results regarding the Child Primary Care Adherence Scale (n=325)*

Items (I)	Mean	Standard Deviation	Minimum	Maximum
I1	2.43	1.22	1.00	5.00
I2	2.66	1.08	1.00	5.00
I3	3.56	0.98	1.00	5.00
I4	1.88	1.21	1.00	5.00
I5	4.50	0.73	1.00	5.00
I6	3.29	0.86	1.00	5.00
I7	3.81	0.92	1.00	5.00
I9	4.85	0.43	1.00	5.00
I10	3.47	1.11	1.00	5.00
I11	1.09	0.36	1.00	4.00
I12	2.83	1.10	1.00	5.00
I13	1.05	0.22	1.00	2.00
I14	2.28	1.15	1.00	5.00
I15	1.56	0.89	1.00	5.00
I16	3.31	0.88	1.00	5.00
I17	2.68	1.35	1.00	5.00
I18	3.30	0.86	1.00	5.00
I19	3.26	0.97	1.00	5.00
I20	1.52	1.12	1.00	5.00
Adherence to Psychomotor Development and Health Care Recommendations dimension *(Items 1, 2, 4, 10, 11, 12, 14, 15, 17, 18, 19, 20)*	28.97	8.86	14.0	56.0
Adherence to Safety Precautions Recommendations dimension *(items 3, 5, 6, 7, 9, 13, 16., 17)*	27.06	3.93	14.0	36.0
Child Primary Care Adherence Scale	**53.35**	**10.89**	**27.0**	**85.0**

Table 3. *Correlation between the mean scores obtained from the Child Primary Care Adherence Scale and its dimensions*

		1	2	3
1. Adherence to Psychomotor Development and Health Care Recommendations dimension	r	1		
	p			
2. Adherence to Safety Precautions Recommendations dimension	r	0.580	1	
	p	.001		
3. Child Primary Care Adherence Scale	r	**0.976**	**0.744**	**1**
	p	**.001**	**.001**	

The two-factor structure explained 47.66% of the total variance. There were 13 items in the first factor (items 1, 4, 5, 7, 8, 9, 11, 12, 13, 15, 16, 17, 19, 20) and 7 items in the second factor (items 3, 5, 6, 7, 9, 13, 16). However, the eighth item in the first factor was removed from the scale because it overlapped (factor loading was 0.488 for the dimension 1 and 0.487 dimension 2). The 17th item in the first factor, "I sit my baby/child in a car seat appropriate for his or her age and height during car trips", was moved to the second dimension because it was related to safety precautions and the "not necessary" option was marked too often in the responses received from the participants because the number of the participants who did not own a car was high. As a result, there were 11 items in the first factor (items 1, 2, 4, 10, 11, 12, 14, 15, 18, 19, 20) and 8 items in the second factor (items 3, 5, 6, 7, 9, 13, 16, 17). Factor loadings of all the items were above 0.30. The two-factor structure obtained was renamed as "Factor 1: Adherence to Psychomotor Development and Health Care Recommendations" and "Factor 2: Compliance with Safety Precaution Recommendations" by taking the item contents into account. Response bias was assessed with the Hotelling's T-squared test. The participants answered the items of the scale according to their own opinions and there was no response bias (Hotelling's T^2 =19455.068; p= .001).

3.2. Reliability Findings

The internal consistency of the scale was assessed with the Cronbach's Alpha. The calculated Cronbach's Alpha value was 0.905 for the overall scale, 0.897 for the Adherence to Psychomotor Development and Health Care Recommendations dimension, and 0.705 for the Adherence to Safety Precautions Recommendations dimension. The item-total score correlation value was below 0.30 for three items (items 5, 9, and 11), and below 0.20 for one item. However, since the factor loadings were above 0.30, the items were not removed from the scale (Table 4). The scale was administered twice to 30 people who were included in the sample at a fifteen-day interval, and it was observed that the scale met the criterion of stability over time (r=0.932; p< .001). The mean scores obtained from the first and second applications were compared with the paired samples t-test and no statistically significant difference was determined between the means (p> .05). In order to determine the distinctiveness of the scale items, the raw scores obtained from the scale were ranked from the highest to the smallest and the upper and lower 27% were compared using the independent samples t-test. A significant difference was determined between the upper and lower group score averages of all the items, indicating that the scale measured the feature it was intended to measure distinctively (Table 5). The scale items were divided into two as even and odd numbers using the split-half method, and a high correlation was determined between the two halves (correlation between the two halves: 0.718; Spearman Brown: 0.836; Guttman: 0.824).

Table 4. *Internal Consistency Findings Regarding the Child Primary Care Adherence Scale*

Factor 1: Adherence to Psychomotor Development and Health Care Recommendations dimension	Factor loadings	Item total score correlation	Cronbach's Alfa when the item is deleted
1. I encourage my child to play.	0.761	0.735	0.894
2. From the 6th month onwards, I introduced new foods to my baby in the recommended order (e.g. first meat, then fish).	0.753	0.767	0.894
4. I teach my child to speak correctly in his/her native language (e.g. telling stories, reading books).	0.845	0.746	0.894
10. Until my child was 12 months old, I only gave the foods recommended by the doctor.	0.510	0.505	0.902
11. I take my child to another physician or healthcare facility (e.g. specialist) if necessary/ recommended.	0.459	0.225	0.906
12. Starting at around 15 months old, I taught my child good sleep habits (e.g. going to bed early or going to bed at the same time every day).	0.658	0.682	0.896
14. I avoided giving my child cow's milk before he/she was 12 months old.	0.825	0.727	0.895
15. I brushed my child's teeth as soon as he/she started teething.	0.748	0.569	0.900
18. From the age of 12 months, I gave my child the food we ate in our family.	0.493	0.573	0.900
19. When my child was two years old, I encouraged him/her to stop using a pacifier.	0.495	0.539	0.900
20. I avoid giving my child food/drinks that contain a lot of salt, sugar or fat.	0.814	0.612	0.898
Factor 2: Adherence to Safety Precautions Recommendations dimension			
3. When my child has fever or symptoms of colic (mild symptoms), I give him/her the recommended medications.	0.502	0.594	0.899
5. I avoided giving small objects (e.g. marbles, necklaces) that could cause choking when my children were under the age of 3 years.	0.633	0.290	0.906
6. I only gave my baby one new food a week until he/she was 12 months old.	0.473	0.421	0.903
7. When my child shows mild symptoms of an illness, I take measures other than medication (for example, lukewarm bath to reduce fever, massaging the belly to relieve gas pain).	0.652	0.499	0.901
9. I protect my child from electrical outlets to protect him/her from electric shocks and burns.	0.659	0.230	0.906
13. I keep my child away from poisonous substances such as medicine and detergent that he or she can drink or eat.	0.461	0.010	0.908
16. I started toilet training when my child was around 18 months.	0.453	0.348	0.905
17. I sit my baby/child in a car seat appropriate for his or her age and height during car trips	0.622	0.642	0.898

Table 5. *Findings on Distinctiveness of the Child Primary Care Adherence Scale*

	Group	Mean	Standard Deviation	t	p
Factor1: Adherence to Psychomotor Development and Health Care Recommendations dimension					
Item (I)1	Upper	3.85	0.80	21.313	.001
	Lower	1.45	0.67		
I2	Upper	3.90	0.72	21.419	.001
	Lower	1.74	0.61		
I4	Upper	3.21	1.36	13.271	.001
	Lower	1.17	0.45		
I10	Upper	4.25	0.79	12.228	.001
	Lower	2.65	0.93		
I11	Upper	1.20	0.43	3.402	.001
	Lower	1.03	0.18		
I12	Upper	3.89	0.86	16.270	.001
	Lower	1.91	0.74		
I14	Upper	3.55	1.24	13.217	.001
	Lower	1.59	0.63		
I15	Upper	2.27	1.19	7.752	.001
	Lower	1.20	0.49		
I18	Upper	4.02	0.74	12.971	.001
	Lower	2.64	0.66		
I19	Upper	4.02	0.75	12.262	.001
	Lower	2.56	0.82		
I20	Upper	2.49	1.67	7.194	.001
	Lower	1.15	0.48		

Table 5. *(Continued)*

Factor 2: Adherence to Safety Precautions Recommendations dimension					
I3	Upper	4.33	0.71	14.525	.001
	Lower	2.70	0.77		
I5	Upper	4.79	0.57	6.435	.001
	Lower	4.07	0.87		
I6	Upper	3.75	0.69	8.744	.001
	Lower	2.73	0.85		
I7	Upper	4.39	0.65	11.418	.001
	Lower	3.07	0.86		
I9	Upper	4.95	0.20	4.226	.001
	Lower	4.64	0.64		
I13	Upper	1.06	0.25	0.555	.001
	Lower	1.09	0.28		
I16	Upper	3.79	0.89	7.216	.001
	Lower	2.86	0.80		
I17	Upper	4.15	1.05	16.641	.001
	Lower	1.77	0.82		

n:325,Upper: The 27% group with the highest scores (n1:88), Lower: The 27% group with the lowest scores (n2:88)

4. DISCUSSION

The Turkish adaptation, validity and reliability analyses of the Child Primary Care Adherence Scale, which was developed to determine the compliance of parents of children between the ages of 2 and 6 based on the recommendations given by primary health care workers, were conducted in the study. Validity is defined as the degree to which a measurement tool can accurately measure the feature what it is intended to measure without considering any other feature (10). Many methods can be used to test the validity of a scale. In the present study, content validity and construct validity were tested. Content validity is the extent to which the overall scale and each item in the scale serve the purpose. Since determining the scope of the subject requires a judgment, there should be common definitions between the health professionals who checked whether the items were comprehensible and the person who developed the scale in order to prevent evaluation according to different criteria (11). In the present study, the Davis Technique was used for the content validity (11). According to this technique, the content validity index (CVI) being above 0.80 indicates that there is content validity. The CVI value (0.92) obtained in the present study indicated that the tool could measure the targeted conceptual structure. The structure is a pattern formed by certain elements thought to be related to each other or by the relationships between elements (10). One of the methods used to define the clusters of related elements in the scale and to establish validity is factor analysis. Factor analysis is a multivariate statistical method aimed at finding, discovering, or testing the models that have been found by bringing together measurable or observable variables related to each other, and a small number of unrelated and conceptually meaningful new variables (factors, dimensions) (12). In the present study, Exploratory Factor Analysis (EFA) was conducted and Kaiser-Meyer Olkin (KMO) and Barlett's Test of Sphericity were conducted to test the data were suitable for factor analysis. The result of the KMO test should be at least 0.60 (13). In the present study, the KMO value was 0.90 indicating that the sample was perfectly adequate for factor analysis. The results of the Barlett's test of sphericity indicated that the matrix formed by the relationships between the variables was significant for factor analysis. Based on these values, it was concluded that factor analysis of the scale could be performed. One of the most critical questions to be answered when factor analysis is performed is the number of dimensions obtained. One of the criteria used to determine the number of factors is eigenvalues. Eigenvalues used in the analysis are obtained from the correlation matrix. Guttman-Kaiser's eigenvalue ≥1 rule is used to determine the number of factors. Only factors with eigenvalues greater than 1 are taken into account (13). In the present study, three factors with eigenvalues greater than 1 emerged, but since there was one item in the third factor, they were reduced to two factors and EFA was performed. Although the explained variance was over 50% in the three-factor structure, the explained variance in the structure reduced to two factors due to the presence of only one item in the third factor was 47.66%. Since the obtained value was close to 50%, it was considered acceptable. The factor loadings of all the items were over 0.30. The values obtained are expected to be higher than 0.30, and sometimes this value can be reduced to 0.10. If it is below 10%, it means that there may be a problem with that item (13,14). The 17th item in the scale, which includes the statement "*I sit my baby/child in a car seat appropriate for his or her age and height during car trips*", is within the scope of accident protection/safety precaution; thus, it was included in the first dimension during the analysis. Due to the small number of participants having a car in the sample, the option "not applicable" was marked too many times and therefore this item was moved

to the second dimension. Unlike the original scale, in the present study, since the 17th item was moved to the second dimension, the factor names were named as "factor 1: Adherence to Psychomotor Development and Health Care Recommendations" and "factor 2: Adherence to Safety Precautions Recommendations" considering the contents of the items. Responses similar to the expectations of society or the researcher are defined as response bias and they are analyzed with the Hotelling T2 test. The statistical significance obtained with the Hotelling T2 test indicates that there is no response bias. According to the results of the present study, the participating mothers answered the items according to their own opinions, indicating that there was no response bias. Reliability, one of the features that a scale must have, is an indicator of the stability of the measurement values obtained in repeated measurements under the same conditions with a measurement tool (10). Reliability means ensuring adequacy, equivalence, consistency, accuracy and stability (15). Stability and consistency in a measurement tool indicate the tool can give similar results in repeated measurements, while accuracy indicates it can determine the true measurement value. An unreliable measurement tool or a measurement tool with low reliability is also considered to have low scientific value (15). Internal consistency and stability methods were used to determine the reliability of the scale. Internal consistency is a reliability method that determines whether all aspects of the scale have the ability to measure. In order to be able to say that a scale has internal consistency reliability, it is necessary to prove that all dimensions of the scale can measure the same feature (15). One of the methods that measure internal consistency is the Cronbach's Alpha reliability coefficient. In the present study, since the scale is Likert type and contains more than three items, the Cronbach's Alpha reliability coefficient was used to measure internal consistency. In the present study, the Cronbach's Alpha value was 0.905 for the overall scale scale, 0.897 for the Adherence to Psychomotor Development and Health Care Recommendations dimension, and 0.705 for the Adherence to Safety Precautions Recommendations sub-dimension. According to these data, the internal consistency coefficient was above 0.70 for the overall scale and its dimensions. Another method used to determine internal consistency is the split-half method. In this method, if the correlation between two separate question groups is high, in other words, if they are consistent with each other, it is concluded that the whole instrument consists of questions that are closely related to each other, and this means that the measurement instrument has internal consistency (15). In the present study, the items of the scale were divided into two based on the even and odd numbers with the split-half method, and it was observed that there was a high correlation between the two halves, and therefore it was concluded that the internal consistency of the scale was good. Stability is the ability of a measurement tool to provide consistent results from one administration to another over time. Stability of the scale was determined using the test-retest method performed at intervals (15). With this test, the Pearson Product Moment correlation coefficient value was calculated (15). Coefficient values are expected to be above 0.70. The higher the value is, the more reliable the scores are (15). In the present study, the test-retest correlation value of the scale administered twice to 30 people at a fifteen-day interval was above 0.90 and met the stability criterion.

5. CONCLUSION

The results of the validity and reliability study obtained in the present study indicated that the Child Primary Care Adherence Scale could be used to determine the compliance behaviors of parents of children aged 2-6 in Turkish society to the care recommendations given by health professionals. The Child Primary Care Adherence Scale whose validity and reliability study in Turkish was conducted in the present study consists of 19 items and 2 dimensions. The 11th and 13th items of the scale are reverse scored in the analysis. The minimum and maximum possible scores that can be obtained from the scale are 27 and 87, respectively.

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Ethics Committee Approval: *Before the study was conducted, ethical approval was obtained from the Non-Interventional Research Ethics Committee of a state university (decision date: February 23, 2023, decision number: 0056) and institutional permission was obtained from the provincial health directorate to which the family health center is affiliated. Permission to adapt the scale into Turkish was obtained via e-mail from the author who developed the original scale.*

Author Contributions:
Research idea: BA
Design of the study: BA, GU, MY
Acquisition of data for the study: DA
Analysis of data for the study: GU
Interpretation of data for the study: BA, GU, DA, MY
Drafting the manuscript: BA, GU, DA, MY
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Clinical and Experimental Health Sciences

The Effects of Different Post Space Conditioning Procedures and Different Endodontic Sealers on the Push-Out Bond Strengths of Fiber Posts

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ABSTRACT

Objective: The bonding ability of intraradicular posts is important for successful restoration. Lasers have been used for this purpose. Both for post space conditioning and irrigation activation with lasers are interesting choices. The aim of this investigation was to evaluate the bond strength of fiber posts created via different irrigation and laser etching protocols on post spaces and different sealers.

Methods: Ninety-six mandibular premolar teeth were randomly assigned to two main groups. GuttaFlow Bioseal and MTA Fillapex root canal sealers were used for obturation. Different irrigation and laser etching protocols for post spaces were used to perform subgroup procedures. One main group was filled with GuttaFlow Bioseal, and the other was filled with MTA Fillapex. The main groups were then subdivided according to the irrigation and laser etching protocols on the post spaces. The fiber posts were cemented and then transversally sectioned. Push-out tests were performed. The push-out strength values were subjected to statistical analysis. Levene's homogeneity test was used to examine normally distributed variables. The group results were analyzed via two-way ANOVA to evaluate the effects of sealer type, post space preparation, irrigation system and their interactions.

Results: The impacts of various post space conditions on the bond strength were not observed. ($p > .05$). However, a significant difference was reported between the two different sealers ($p < .05$).

Conclusions: Post space conditioning procedures did not significantly affect the bond strength values. However, sealer alteration affected the bond strength values.

Keywords: Bond strength, Diode laser, Er: YAG laser, Fiber post, Push-out.

1. INTRODUCTION

Following adequate root canal obturation, strong restoration is needed to strengthen the remaining tooth structure. Given its weakened state, intraradicular posts are required to retain a crown (1), for which many clinicians have recommended adhesively luted fiber-reinforced composites (FRCs) because of their good physicochemical properties, biocompatibility, and ability to reduce the possibility of vertical root fracture (1-3). However, failure in the bonding of FRC posts is the most significant problem encountered, generally occurring between the root canal wall and resin cement (4), and the residues remaining after preparation of the post cavity consist of residual sealer, gutta-percha residues, and smear layer debris (5), necessitating removal of smear layer to clear the dentinal tubules, potentially improving the intertubular diffusion of the adhesive system and enhance the bonding ability of fiber posts (6). Furthermore, hybridization of the demineralized dentin surface, growth of lateral branches, and the formation of resin tags are important parameters for luting agents, which enable bonding between the post surface and the dentinal wall (2). In response, various irrigation and surface-conditioning protocols have been applied with these aims, many of which use laser devices (7-11), such as near-infrared lasers (9, 12), neodymium: yttrium-aluminum-garnet (Nd: YAG: 1064 nm) lasers(10, 13) and mid-infrared lasers (7, 8) to remove smear layers and debris. (7, 8, 14). Er: YAG laser etching of the post space has also been reported to improve the adhesion of fiberglass posts (15, 16), while an alternative application involves use of the post surface to strengthen the

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bond to resin cement. Meanwhile, for post surface treatment, previous studies have recommended laser irradiation, which allows favorable adhesion to resin cement (16-18).

Endodontic sealers are essential for filling the root canal's porous surface and sealing gaps between the root canal dentin and gutta-percha (19), with various options having been developed and used for root canal treatment (20). During post cementation, the endodontic sealer used for obturation may affect the contact between the root canal dentin and the resin cement, which may ultimately affect post retention (21). In response, mineral trioxide aggregate (MTA)-based sealers have recently been introduced to provide an adequate seal and biological properties, one such example of which is MTA Fillapex, supplied in the form of two pastes. This sealer includes MTAs, natural resin, salicylate resin, silica, and bismuth, which are mixed as instructed by the manufacturer, and according to previous research, the sealant releases calcium, creating an alkaline environment with whose materials help to mineralize hard tissues, in addition to having antimicrobial properties (22). Alternatively, GuttaFlow Bioseal (GFB), which consists of gutta-percha, bioactive ceramic glass, platinum catalyzer, polydimethylsiloxane, and zirconium dioxide, was introduced to increase bioactivity and promote the regeneration of apical tissues (23, 24), offering low solubility and porosity, low alkalization capacity (23), low dentin penetrability (25, 26) and cytocompatibility (24, 27) However, physicochemical studies are needed to confirm whether these features are present in clinical practice.

MTA Fillapex and GFB have been introduced so-called "bioactive" materials, which can form a biomineralized interface with the host tissue under optimal conditions, though different moisture conditions have similar effects on the dislocation resistance of the two root canal sealers (28). Therefore, the effects of laser application and different irrigation techniques on residual root canal debris and, consequently, on the bonding strength of fiber posts have not been studied. On this basis, a method was developed.

In responce, this study aimed to compare the adhesion of fiber posts under different irrigation and laser etching protocols and the effects of an MTA-based root canal sealer and a bioactive glass fiber sealer on the bond strength of fiber posts. In the present study, it was hypothesized that laser etching would affect the bond strength (h1 one way), while the null hypothesis was that the different sealers would have no effect on the push-out strength of the fiberglass posts (h0).

2. METHODS

2.1. Ethics committee approval and sample size calculation

The ethical committee of Ordu University approved this study (Approval date: 12.07.2024; Number 89), and the sample set size was calculated via G-Power (version 3.1.9.7; Universität Düsseldorf, Germany) with 95% power and a 0.5% margin of error (29). The push-out (MPa) parameter from a related article was used as the reference parameter, and to ensure the reliability of the results, the study is planned to be carried out on a total of 96 samples, with eight samples per group. The study organization is shown in Figure 1.

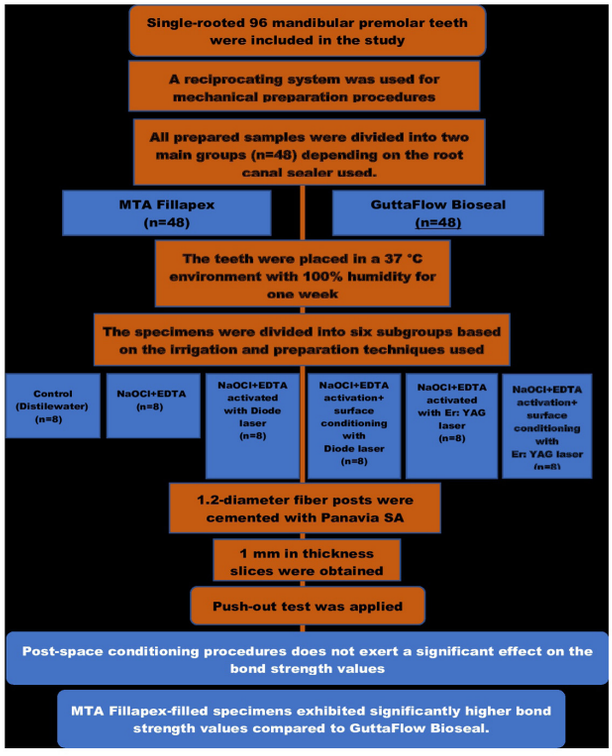

Figure 1. *Flowchart of the experimental design*

2.2. Sample preparation

Ninety-six mandibular premolar teeth with a single root and no internal resorption, as confirmed by periapical radiographs, were included. Teeth with any one of these features (external resorption, root caries, and an immature apex) were excluded from the research. Soft debris, ligament remnants, and calculus were removed via ultrasonic scaler tips and stored at 4 °C in a 0.5% chloramine–water solution until they were used for this study. A diamond disk (Superflex Diamond Disc 910S/220, North Bel, Italy) was used to remove the coronal part of the teeth under the cement–enamel interface at a root length of 15 mm, and this part was stored in distilled water. The working length was 1 mm from the length measured with a 10K file (Dentsply Maillefer, Ballaigues, Switzerland), and this measurement was taken at the apical foramen. A reciprocating system (REC; VDW, Munich, Germany) with an endodontic motor (Gold Reciproc; VDW, Munich, Germany) was used for mechanical preparation procedures, and apical preparation was initiated with 25/08 R25 and extended with 40/06 R40. The samples were rinsed with 5 ml of 5% NaOCl (Wizard, Rehber Kimya San. Tic. Aş., Istanbul, Türkiye) between instrument changes, then with 5 ml of 17% EDTA (Werax; Spot Dis Deposu AŞ, Izmir, Türkiye) solution for 1 min, then with 5 ml of NaOCl, and finally with 5 ml of distilled water. Afterward, the samples were dried with paper tips.

2.3. Groups' classification

All the prepared samples were divided into two main groups (n = 48) depending on the root canal sealer used. One group was filled with R40 Reciproc gutta-percha as the master cone and GFB (Coltene/Whaledent, Altstatten, Switzerland), whereas the other main group was filled with R40 gutta-percha as the master cone and MTA Fillapex (Angelus, Londrina, PR, Brazil) as the root canal sealer. The sealers were prepared as recommended by the manufacturers, after which the cold lateral compaction technique was used for root canal obturation and coronal cavity filling was completed with temporary filling material (Cavit G: 3 M ESPE). The teeth were placed in a 37 °C environment with 100% humidity for 1 week, and the root canal filling was subsequently removed with a Peeso Reamer #1 (Mani Inc.). Post space preparation was performed with drill #1 (Cytec Blanco Fiber Post System, Hahnenkratt, Konigsbach-Stein, Germany), with a length of 11 mm, with each drill being newly opened for use and discarded after a maximum of five uses. To ensure the complete removal of the obturation materials, the samples were checked via a stereomicroscope at 40X magnification, and residual materials were removed with H-files (Dentsply Mailfer, Ballaigues, Switzerland). Following these procedures, the samples were divided into six subgroups based on the irrigation and preparation technique used.

In subgroup 1 (the control group), irrigation was performed for 80 s using 6 ml of distilled water 1 mm behind the working length of a 31-gauge needle tip (Navi Tip, Ultradent, South Jordan, UT, USA). Meanwhile, in subgroup 2, the root canals were irrigated with 3 mL of 2.5% NaOCl for 40 s, followed by 3 mL of 17% EDTA for 40 s, and a 30-gauge needle tip was inserted 1 mm back from the working length.

In subgroup 3, the irrigation protocol was performed four times in 5-s periods (5 s of irrigation and 5 s of activation) with 2.5% NaOCl and 17% EDTA. Activation was conducted with a diode laser (Doctor Smile Erbium & Diode, Lambda Scientifica Srl., Vicenza, Italy) using a 600-µm fiber tip placed 1 mm back from the working length, and the laser was applied at power settings of 1.5 W, 15 Hz, and 100 mJ. A 600-µm fiber tip was used, which was gently retracted with helical movements from the apical to the coronal direction and reinserted at the apex (Figure 2). Next, subgroup 4 underwent all protocols performed in subgroup 3, and the surface was dried with paper points. Here, the same diode laser parameters were used for surface conditioning for 10 s in a dry environment, and a 600-µm fiber tip was used, which was gently retracted with helical movements from the apical to the coronal direction.

In subgroup 5, all protocols from subgroup 3 were performed, but the activation protocol was conducted using an Er: YAG laser (Fotona, Ljubljana, Slovenia) with an endodontic fiber tip (Precio 300/14; Fotona D.D.) in the medium–short pulse mode, with a 50-mJ pulse energy, a 20-Hz repetition rate, and 1-W average power. The laser tip was placed 1 mm back from the working length, and the irrigation and activation protocols were completed in 40 s per solution. Finally, in subgroup 6, all protocols from subgroup 5 were performed, and the surface was dried with paper points. The same Er: YAG laser parameters were used for surface conditioning for 10 s in a dry environment, but during the laser-etching process, the fiber tip was moved in a rotational direction toward the apex in the coronal direction.

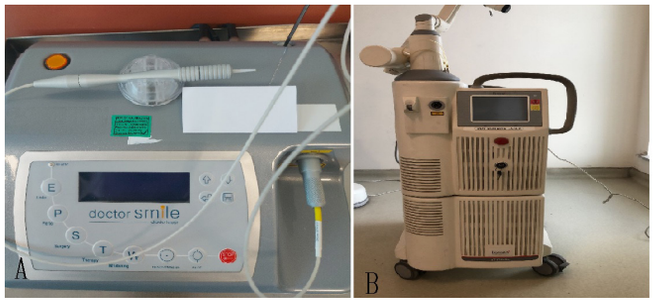

Figure 2. *A: shows Diode Laser (Doctor Smile Erbium & Diode, Lambda Scientifica Srl., Vicenza, Italy), B: shows Er: YAG laser (Fotona, Ljubljana, Slovenia) with an endodontic fibre tip (Precio 300/14; Fotona D.D.)*

In this study, a total irrigation volume of 6 ml was used over 80 s, the final irrigation was completed with 3 ml distilled water, and the excess irrigation solution with paper points was removed. We used 1.2-diameter fiber posts (Cytec Blanco Fiber Post System Hahnenkratt, Konigsbach-Stein, Germany) for the prepared post spaces, though samples incompatible with the fiber posts were excluded from this study, while samples of the same groups were reprepared. Seven days after completion of the endodontic procedures, Panavia SA Cement Plus (Kuraray Dental, Tokyo, Japan) was prepared manually by mixing (equal amounts of Pastes A and B) for 10 s, as instructed by the manufacturer. The resin cement was coated over the whole bonding surface of the post, after which the post was quickly inserted into the cavity. Gentle vibration was used to prevent air bubbles from entering the root canals, and after the paste was inserted into the cavity, a working time of 40 s was allowed. The coronal base and post head were cleaned of excess paste and cured for 20 s with a blue LED light (Elipar S10, 3 M ESPE, Neuss, Germany) at an intensity of 1,200 mW/cm2. Prior to the execution of the push-out tests, the samples were maintained for a period of 24 h at a humidity level of 100% and a temperature of 37 °C.

2.4. Push-out test

Following a 24-h incubation period, the samples were embedded in autopolymerizing acrylic resin (Orthoresin, Dentsply/DeTrey, Konstanz, Germany), and horizontal sections were taken with a water-cooled low-speed diamond disk (Megatome T180, Presi, France). Six serial slices, approximately 1-mm thick, were obtained, the first two of which were defined as coronal, the second two as middle, and the third two as apical. An extraction force of 0.5 mm/min was applied to disks 2, 4, and 6 from the apical to the coronal direction, and a metal plunger 1 mm in diameter was

used until displacement occurred. A universal test machine (Autograph A65x, Shimatzu, Japan) was used for this test, and the values of the maximum failure force (N) were recorded and then converted to MPa (Eq. [1]) by dividing by the measured bond area (Eq. [2]).

$$\text{Push-out bond strength (MPa)}: \frac{\text{Maximum load (N)}}{\text{Adhesion area (mm}^2\text{)}} \quad \text{Eq. (1)}$$

The bonding area was calculated for each slice using: $\pi (R+r) (h^2+(R-r)^2)^{0.5}$ Eq. (2)

where π is 3.14, R is the coronal root canal radius, r the apical root canal radius, and h the thickness of the slice.

The failure types on each section were evaluated via a stereomicroscope (Nikon SMZ25, Nikon Ltd., Tokyo, Japan) at 40X magnification; failure modes were examined and classified into three categories, including cohesive failure between the cement and post, adhesive failure between the cement and dentin, or mixed failure in both the dentin and cement; and percentages were calculated within each group.

2.5. Statistical Analysis

The push-out strength values were subjected to statistical analysis, and Levene's homogeneity test was used to examine normally distributed variables. The group results were analyzed via two-way analysis of variance (ANOVA) to evaluate the effects of the sealer type, post space preparation, and irrigation system, as well as their interactions. Subsequently, the results for the three root canal regions were analyzed via one-way ANOVA, and Tukey's multiple-comparison tests were used to compare the mean values for the push-out strength. All computational work was performed with statistical software (SPSS v 20.0; SPSS, Inc.).

3. RESULTS

The mean and standard deviation values of the groups are reported in Table 1, demonstrating that the bond strength values were not affected by the post space conditioning procedures (Figure 3). Meanwhile, Er: YAG laser irrigation activation and Er: YAG laser etching increased the bond strength values, although the differences were not statistically significant ($p > .05$).

Further, the application of MTA Fillapex resulted in significantly greater bond strength values than the application of GFB (intergroup df = 1, F = 8.868, p = .003; Table 2), and the resistance to dislodgment values in different root regions also differed significantly (Table 3). In addition, a statistically significant increase in bond strength values was observed in the coronal region in comparison with the middle and apical segments. ($p < .05$), though no difference was found between the other groups. Table 4 shows the failure types and percentages for each group, where the lowest percentage of cohesive failure occurred followed by mixed failure, and the adhesive failure mode exhibited the highest percentage of all failure modes.

Table 1. *Push-out bond strength values according to the sealer type and post space conditioning*

Dependent Variable: MPa				
Groups	SealerType	Mean	SD	N
Group 1	MTA	1,2736	1,15094	24
	GuttaFlow	,9070	,67102	24
	Total	1,0903	,95020	48
Group 2	MTA	1,2788	,88936	24
	GuttaFlow	,8971	,59843	24
	Total	1,0880	,77429	48
Group 3	MTA	,8153	,45445	24
	GuttaFlow	,7399	,43979	24
	Total	,7776	,44403	48
Group 4	MTA	,8790	,82145	24
	GuttaFlow	,8758	,82814	24
	Total	,8774	,81598	48
Group 5	MTA	1,3085	1,28437	24
	GuttaFlow	,8613	,96838	24
	Total	1,0849	1,14771	48
Group 6	MTA	1,2021	1,48331	24
	GuttaFlow	,5856	,44813	24
	Total	,8939	1,12783	48
Total	MTA	1,1262	1,06855	144
	GuttaFlow	,8111	,68400	144
	Total	,9687	,90936	288

SD: Standart deviation

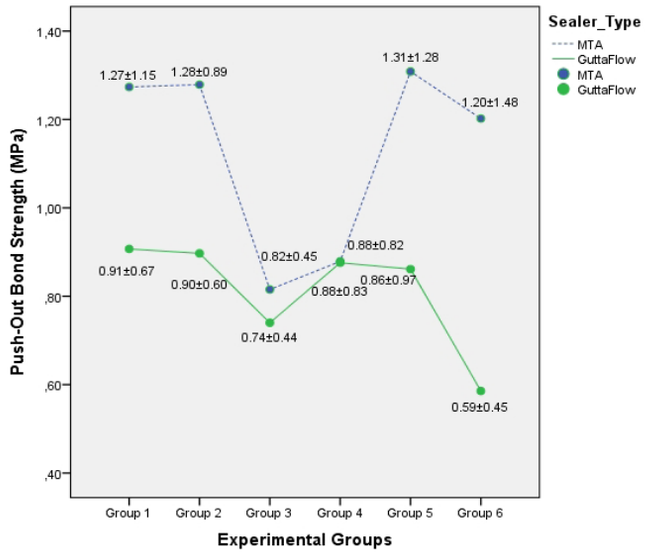

Figure 3. *Line graph representing push-out bond strength values (MPa) according to the sealer types and experimental groups.*

Table 2. *Push-out bond strength values of sealer groups*

Dependent Variable: MPa			(p = .003)	
Sealer Type	Mean	Std. Er	95% Confidence Interval	
			Lower Bound	Upper Bound
MTA	1,126	,075	,979	1,274
GFB	,811	,075	,664	,958

Table 3. *Mean (MPa) of push-out bond strength values in different groups and three regions of rootcanal*

		GROUPS											
Regions	N	1MTA	1GFB	2MTA	2GFB	3MTA	3GFB	4MTA	4GFB	5MTA	5GFB	6MTA	6GFB	**Total N=96**
Coronal	8	1.73^{Aa}	1.45^{Ab}	1.57^{Aa}	1.22^{Aa}	1.12^{Ab}	1.1^{Ab}	1.66^{Ab}	1.73^{Ab}	2.52^{Ab}	1.52^{Aa}	2.44^{Ab}	1.02^{Ab}	1.59^{b}
Middle	8	1.21^{Ba}	0.78^{ABab}	1.19^{ABa}	0.84^{ABa}	0.84^{ABab}	0.56^{ABa}	0.3^{ABa}	0.46^{ABa}	0.77^{ABa}	0.5^{ABa}	0.59^{ABa}	0.35^{Aa}	0.72^{a}
Apical	8	0.89^{ABa}	0.50^{ABa}	1.08^{Ba}	0.62^{ABa}	0.49^{ABa}	0.57^{ABa}	0.13^{Aa}	0.43^{Aa}	0.64^{ABa}	0.55^{ABa}	0.58^{ABa}	0.39^{Aa}	0.6^{a}
Total	24	1.27^{A}	0.91^{A}	1.28^{A}	0.90^{A}	0.82^{A}	0.74^{A}	0.88^{A}	0.88^{A}	1.31^{A}	0.86^{A}	1.2^{A}	0.59^{A}	

**Data with different superscript uppercase letters indicate significant differences within each row; data with different superscript lowercase letters indicate significant differences within each column ($p < .05$)*

Table 4. *Type of failure modes in groups*

Surface_ Treatment	Sealer_Type	Adhesive	Cohesive	Mix
Group 1	MTA	17(70,8%)	2(8,4%)	5(20,8%)
	GFB	19(79,2%)	1(4,2%)	4(16,6%)
Group 2	MTA	16(66,6%)	2(8,4%)	6(25%)
	GFB	18(75%)	5(20,8)	1(4,2%)
Group 3	MTA	20(8,3%)	1(4,2%)	3(12,5%)
	GFB	20(8,3%)	0	4(16,6%)
Group 4	MTA	19(79,2%)	0	5(20,8%)
	GFB	20(8,3%)	0	4(16,6%)
Group 5	MTA	16(66,6%)	3(12,5%)	5(20,8%)
	GFB	20(8,3%)	0	4(16,6%)
Group 6	MTA	17(70,8%)	6(25%)	1(4,2%)
	GFB	21(87,5%)	3(12,5%)	0

4. DISCUSSION

This study investigated the bond strength of fiberglass posts via different irrigation and laser-etching protocols and two root canal sealers on post spaces. The laser-etching process had no significant effect on the bond strength; therefore, this hypothesis was rejected. Similarly, the application of MTA Fillapex significantly increased the adhesion values when compared with GFB, so the null hypothesis was also rejected.

The success of adhesive systems and fiber posts in endodontic restoration lies in the removal of the smear layer and in the retention provided by resin tag formation and the demineralized surface (30). The smear layer includes hydroxyapatite crystallites and broken collagen fibers, meaning it may block tight dentin adhesion because of this spontaneous weakness (31). Preparing a post space requires removing the smear layer, which contains gutta-percha and sealer residuals that cover the dentin surface (32), after which the post space surface must be cleaned of these remnants for fiber post cementation to achieve tight junctions (33).

Several in vitro studies have been performed on this subject, some of which applied post surface conditioning (34), some applied post space conditioning (35), and others applied both of these procedures (36). In the present study, we elected to use post space conditioning methods to increase the adhesion values, as some researchers have suggested that irrigant activation techniques are efficient for enhancing post retention (37, 38), such as irrigation with NaOCl/EDTA, which is necessary to obtain a post space effectively free of a smear layer (39, 40).

Lasers have also been investigated in terms of enhancing the adhesion of fiber posts; for instance, Arslan et al (41) stated that Er:YAG laser etching and a self-etching adhesive system both increased the bond strength of fiber posts, while another study concluded that Er:YAG laser etching achieved the same outcome (15). In the present study, the application of Er: YAG laser irrigation and etching increased bond strength values, although the differences did not reach statistical significance, and these results may have been affected by sealer alteration.

Previous studies have shown that NaOCl interferes with bonding between the resin cement and dentin interface (42, 43), as NaOCl dissolves to yield sodium chloride (NaCl) and oxygen (O2). The polymerization process of adhesive systems can also be inhibited because of this oxygen release (43, 44). In the present study, we elected to use distilled water as the final irrigation solution to prevent the undesirable effects of the remaining solutions on the root canal walls. The total irrigation time for the irrigation activation cycles was 40 s for each solution, with a 5-s rest time in the laser groups, a timing protocol that can be beneficial in terms of avoiding severe peritubular erosion and the potentially severe effects of temperature increases on the root canal wall.

In earlier studies, laser-activated irrigation was utilized to eliminate the smear layer from root canals (8, 14). Özbay et al.(45) showing that the application of laser systems to activate NaOCl and EDTA irrigation solutions is an effective method for removing the smear layer. Regardless of the solution used, photon-induced photoacoustic streaming (PIPS) with an Er: YAG laser was more efficient than that with an Nd: YAG laser or an Er Cr: YSGG laser for smear layer removal. However, when a laser's energy is pulsed, it can form bubbles in irrigation solutions in the root canal and cavity (46). Meanwhile, Akyuz Ekim et al.(35) evaluated the effects of different final irrigation solutions and activation protocols on the bond strength of fiber posts, reporting that the PIPS laser activation irrigation method yielded higher bond strength values than did the Endo Vac negative apical pressure, a diode laser, Nd: YAG laser, and Er: YAG laser activation protocols. Some researchers have also evaluated the push-out bond strength values of fiber posts by applying laser systems to root canal dentin walls without irrigating solutions after post space preparation (15, 47). For instance, Arısu et al.(48) compared different post space treatments and reported that NaOCl/EDTA irrigation resulted in higher bond

strength values than did diode laser surface conditioning. In the present study, an Er: YAG laser with an endodontic fiber tip (Precio 300/14; Fotona D.D.) was used in medium–short pulse mode, and a diode laser was used for both root canal conditioning and irrigation activation. A comparison with these previous studies was thus ineffective because of the different application methods employed.

Several factors, such as the type of endodontic sealer, form of resin luting agent, configuration of the root canal, and organization of the dentinal tubules, affect the bond strength of the post cement–dentin interface (49). For example, an unsuitable type of endodontic sealer will create a substantial barrier to the adhesion of fiber posts. In addition, the formation of a smear layer, composed of sealer and gutta-percha remnants (32), following post space preparation can affect the bond strength (50). Similarly, Reyhani et al. (29) reported maximum bond strength values for fiber posts in a control (no sealer) group where sealer remnants could not be removed from the canal walls or from the entrance of the dentinal tubules, even with post space preparation and irrigation, altering the permeability and wettability of the root canal dentin surface and affecting the resin–dentin bond strength values (51).

Calcium silicate-based sealers have also been promoted as new root canal sealers with good biocompatibility, antimicrobial activity, and apatite-forming ability (52, 53), including MTA Fillapex, a twin-paste material containing silicon dioxide, bismuth oxide, and MTAs (52) and GFB, a polydimethyl siloxane with calcium silicate particles combined with gutta-percha powder introduced in late 2015 (54). No studies have previously been conducted on the effects of these sealers on the bond strength values of fiber posts; therefore, we employed these two root canal sealers in the present study. The results showed that MTA Fillapex-filled samples presented significantly greater bond strength values than the GFB-filled samples, and this difference may have been due to the gutta-percha powder in the GFB. Here, the irrigation protocols may be insufficient for the removal of the sealer remnants.

Previously, the effects of different root canal sealers and post cementation times on the bond strength values of fiber posts luted with RelyX TMARC were evaluated by Vilas-Boas et al. (50), who reported that the best endodontic sealer for obturation was AH Plus (epoxy and resin-based sealer), applied either immediately or 7 d after the completion of the endodontic procedures. The researchers also noted that caution is required with zinc oxide eugenol-based sealers and that bioceramic/calcium silicate-based sealers are incompatible options. The post cementation study time in the present research was 7 d after the completion of the endodontic procedures, and while a comparison of the results did not reveal significant differences, the bond strength values were lower than those in other studies, irrespective of the irrigation protocols applied. This may have been due to the different endodontic sealers used, but Reyhani et al. did not find a significant difference between MTA Fillapex and AH Plus in this regard (29).

Further, the values for the push-out bond strength were influenced by the root region of the sample, with the coronal samples exhibiting the highest bonding values, whereas the apical samples presented the lowest values, and this difference was statistically significant.

Meanwhile, a failure mode analysis indicated that for the two endodontic sealers used, most failure modes were adhesive at the cement–dentin interface, followed by mixed and cohesive (cement/post interface). This finding shows that Panavia F strongly bonded with the fiber post, and our findings were similar to those of previous studies (35, 55) .

This study has several limitations. While the lack of a control group with ‘no sealer’ could be a hindrance, the effect of sealer residues on dentin bonding is also an important topic, and the presence of a control group without a sealer could have been beneficial in making more meaningful interpretations when comparing the results. The study was conducted on extracted teeth, with a single type of fiber post and a single type of self-adhesive cement, which may not be representative of the clinical situation. As such, the long-term prognosis of the bond strength could not be evaluated, so further studies are needed to investigate the relationship between the effects of laser application and different root canal sealers on the adhesion of fiber posts.

5. CONCLUSION

The present study has demonstrated that the implementation of post space conditioning procedures does not significantly affect bond strength values, within the study’s defined parameters. Laser application did not affect the bond strength of the fiber posts, but the push-out strength values were affected by sealer alteration. Finally, compared with the GFB samples, the MTA Fillapex-filled samples presented significantly greater bond strength values.

The resistance to dislodgment values in different root regions also differed. A statistically significant increase in bond strength values was observed in the coronal region in comparison with the middle and apical segments.

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Design of the study: L.B.A.
Acquisition of data for the study: L.B.A., A.S.K., F.S., A.Ç.
Analysis of data for the study: A.S.K.
Interpretation of data for the study: L.B.A.
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Clinical and Experimental Health Sciences

The Inhibitory Effects of Resveratrol on Tumor Growth and Metastasis in Esophageal Adenocarcinoma

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ABSTRACT

Objective: Resveratrol is a small polyphenol that has been extensively researched across various therapeutic areas. Its inhibitory effect on esophageal cancer cell growth has also been demonstrated in a dose – and time-dependent manner. However, the underlying mechanism of this effect has not been fully elucidated yet. We aimed to investigate the anti-proliferative and anti-metastatic effects of resveratrol on esophageal adenocarcinoma (EAC) cells in vitro.

Methods: The EAC cells were treated with various doses of resveratrol, and cell viability was determined using the WST-1 assay. The apoptosis rate and the level of intracellular reactive oxygen species (ROS) were measured using flow cytometry. Adhesion, wound healing, and colony formation assays were carried out to evaluate the anti-metastatic effects of resveratrol.

Results: Resveratrol decreased the cell viability of OE33 cells in a dose-dependent manner. 150 µM resveratrol reduced cell viability by 49% compared to the control by increasing ROS levels. This inhibition was associated with the induction of apoptosis. The analysis revealed that resveratrol also suppressed the adhesion, migration, and colony forming capacity of the cells.

Conclusions: Resveratrol lower the viability and metastatic potential of EAC cells. These findings suggest resveratrol may be further evaluated clinically for this malignancy in humans.

Keywords: Resveratrol, esophageal adenocarcinoma, anti-tumor, anti-metastatic.

1. INTRODUCTION

Esophageal cancer is the seventh most prevalent cancer worldwide and the sixth leading cause of cancer-related deaths, with an overall poor prognosis. It is classified into two major histological subtypes: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). While ESCC is more common globally, the incidence of EAC has increased more rapidly than any other solid tumor, particularly in western countries (1,2). Chemotherapy and radiotherapy are the primary treatment regimens for EAC patients. However, the emergence of chemoresistance and radioresistance in an increasing number of tumors presents a significant challenge in cancer therapy. Furthermore, these treatments often cause adverse side effects, underscoring the urgent need for the discovery of natural products with effective anti-cancer properties (3).

Recent studies have demonstrated that diets high in vegetables and fruits are linked to a reduced incidence of esophageal cancer. It is hypothesized that flavonoids, a group of bioactive polyphenolic compounds found in fruits, vegetables, and plant-based beverages, may contribute to this reduced risk (4). Resveratrol, a natural polyphenol found in red grapes, wine, nuts, and various common garden plants, is the most widely studied among the many phytochemicals with potential health benefits. Additionally, its chemopreventive and chemotherapeutic properties have been demonstrated in various human carcinomas, such as colorectum, skin, liver, lung, pancreas, breast and prostate (5). Numerous reports highlight resveratrol's role as a cancer chemopreventive agent due to its ability to induce growth inhibition, cell cycle arrest, and apoptosis in various human cancer cell lines (3). Research on squamous cell carcinoma of the head and neck has revealed that resveratrol inhibits proliferation, invasion, as well as inducing cell cycle arrest and apoptosis (6), while studies on pancreatic cancer cells

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suggest that resveratrol shows significant potential as a complementary agent to chemotherapy by suppressing proliferation, angiogenesis, and metastasis (7). Previous studies have also suggested that a diet rich in resveratrol may reduce the risk of EAC (8) and resveratrol treatment has been shown to induce apoptosis in human esophageal carcinoma cells (9). However, the exact molecular mechanisms through which resveratrol exerts its anti-cancer effects remain unclear. Therefore, our study aims to further elucidate the underlying mechanisms of resveratrol treatment in EAC, ultimately contributing to the development of more effective therapeutic strategies for this malignancy.

2. METHODS

2.1. Cell Culture

OE33 human EAC cells (Sigma-Aldrich, Darmstadt, Germany) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (PAN Biotech GmbH, Aidenbach, Germany), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biochrom, Berlin, Germany) and 100 U/mL penicillin / 100 mg/mL streptomycin (PAN Biotech GmbH, Aidenbach, Germany). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Upon reaching 80% confluence, the cells were detached from the culture flask using a solution of 0.25% trypsin and 0.02% EDTA and subsequently transferred to a new culture flask.

2.2. Treatment with Resveratrol

Resveratrol (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) to prepare a 500 mM stock solution, which was stored at – 20 °C until use. For cell treatment, the stock solution was diluted in the growth medium. All treatments were conducted following overnight serum starvation. Each experiment was performed in duplicate, and all assays were repeated at least three times.

2.3. Cell Viability Assay

Cell viability was assessed using the WST-1 Cell Proliferation Assay (Roche, Basel, Switzerland). Briefly, OE33 cells were seeded into 96-well plates at a density of 6×10^3 cells/well and allowed to attach overnight. Following serum starvation in FBS-free medium, the cells were treated with resveratrol at varying concentrations (50 µM, 100 µM, 150 µM, and 200 µM). Control cells were treated with medium containing the vehicle. After a 24-hour treatment period, the medium was removed, and 10% WST-1 solution was added. Absorbance was measured at 440 nm after two hours using a 96-well plate reader (Molecular Devices, CA, USA). The viability of the treated cells was calculated as a percentage compared to the control cells.

2.4. Detection of Apoptosis Rate

The apoptosis rate was evaluated using the ApopNexin FITC Apoptosis Detection Kit (Chemicon International, Temecula, CA, USA) according to the manufacturer's protocol. Briefly, cells were seeded into 12-well plates at a density of 6×10^4 cells/well. Following 24 hours of serum starvation in FBS-free medium, cells were treated with 150 µM resveratrol for 24 hours. Subsequently, cells were dissociated, washed, and incubated in 500 µL of binding buffer containing 2 µL of annexin V-FITC and 2 µL of propidium iodide (PI) in the dark for 45 minutes at room temperature. The stained cells were analyzed using FACSCalibur flow cytometry (BD Biosciences, CA, USA), and the results were calculated with CellQuest software (BD Biosciences, CA, USA). Healthy cells were identified as Annexin-V-negative and PI-negative, early apoptotic cells as Annexin-V-positive and PI-negative, late apoptotic cells as Annexin-V-positive and PI-positive, and necrotic cells as Annexin-V-negative and PI-positive. The apoptosis ratio was calculated by summing the percentages of early apoptotic and late apoptotic cells.

2.5. Measurement of Intracellular ROS Levels

Intracellular ROS levels were detected by flow cytometry using 5-(and-6)-carboxy – 2′,7′-dichlorodihydrofluorescein-diacetate (H2DCFDA) (Molecular Probes Co. OR, USA) staining. as a probe. Briefly OE33 cells were seeded into 6-well plates at a density of 1.5×10^5 cells/well and starved overnight in the non-FBS medium. Following serum starvation, the cells were treated with 150 µM resveratrol for 24 hours. After treatment, the cells were collected by trypsinization and were washed with Phosphate-Buffered Saline (PBS). The cells were then stained with 10 µM H2DCFDA for 45 minutes at 37°C in a 5% CO_2 incubator. After incubation, the cells were washed and fluorescence intensity was measured using a flow cytometry (FACSCalibur, BD Biosciences, CA, USA).

2.6. Adhesion Assay

The effect of resveratrol on the adhesive ability of OE33 cells was evaluated using an adhesion assay. Briefly, cells were seeded into 6-well plates at a density of 1.5×10^5 cells/well and starved for 24 hours. After starvation, the cells were treated with 150 µM resveratrol for 24 hours. The cells were then collected by trypsinization, counted, and 5×10^4 cells/well were plated into a 96-well plate pre-coated with 1% fibronectin (Sigma, St. Louis, MO). After 1 hour incubation at 37°C in a 5% CO_2 atmosphere, the cells were washed with PBS, fixed with 3% paraformaldehyde for 10 minutes, and washed again with 2% methanol for 10 minutes. The cells were then stained with 0.5% crystal violet. The stained cells were measured at 540 nm using a 96-well plate reader (Enspire 2300, Perkin Elmer, MA, USA).

2.7. Wound-Healing Assay

Cell migration was assessed using a wound-healing assay. OE33 cells were seeded into a 12-well plate at a density of 2.5×10^4 cells/well and allowed to attach and grow overnight, followed by starvation for 24 hours. A small linear scratch was then gently created using a sterile pipette tip. After the cells were washed with PBS to remove cellular debris, they were treated with 150 µM resveratrol for 24 hours. The migration and wound closure ability, both before and after resveratrol treatment, were compared with microscopy (Carl Zeiss, Jena, Germany).

2.8. Colony Formation Assay

A colony formation assay was performed to assess the effects of resveratrol on the proliferative potential of OE33 cells. Briefly, the cells were seeded into a 6-well plate at a density of 1.5×10^5 cells/well. After 24 hours of starvation, the cells were treated with 150 µM resveratrol for 24 hours. Following treatment, the cells were collected by trypsinization and counted. The cells were then reseeded into a new 6-well plate at a density of 500 cells per well and allowed to continue growing for 1 week in a humidified incubator at 37°C with 5% CO_2. After 1 week, the medium was discarded, and the cells were stained with dimethylmethylene blue for 45 minutes. Excess stain was removed by washing with PBS, and the blue-black colonies were observed and counted using microscopy.

2.9. Statistical Analysis

All experiments were performed in at least three independent replicates. Statistical analyses were conducted using Jamovi 2.3.26 (Sydney, Australia). Student's t-test was used for comparisons between two groups, whereas one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for comparisons among three or more groups. All data are expressed as mean ± standard deviation (SD). A p-value of < .05 was considered statistically significant.

3. RESULTS

3.1. Resveratrol Reduced Viability of OE33 Cells.

The effect of resveratrol on the viability of OE33 EAC cells was determined using WST-1 assay. The cells were cultured in different concentrations of resveratrol (50–200 µM) for 24 hours. According to the assay, resveratrol decreased cell viability in a dose-dependent manner. A statistically significant decline was observed at concentrations above 100 µM, reaching 49% at 150 µM, while the percentage of viable cells dropped to 21% at 200 µM ($p < .05$). Based on this test result, 150 µM resveratrol concentration was used in subsequent experiments as it was close to the IC50 value (Figure 1).

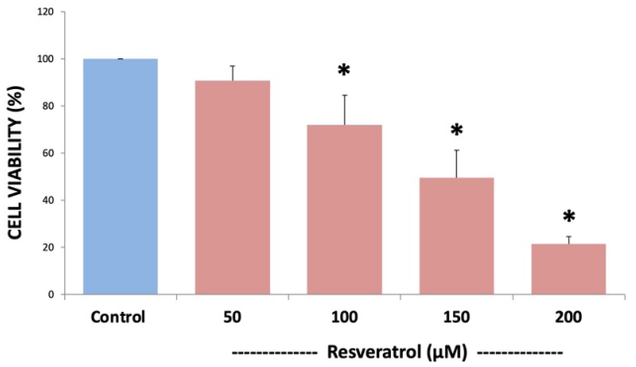


Figure 1. *Resveratrol reduced the viability of OE33 cells in a dose-dependent manner. OE33 cells were exposed to different concentrations of resveratrol for 24 hours. Subsequently, cell viability was determined by the WST-1 assay. * $p < .05$. N = 4 independent experiments.*

3.2. Resveratrol Induced Apoptosis in OE33 Cells.

To investigate whether resveratrol's viability-reducing effect occurs through the induction of apoptosis, the apoptosis rate in the cells was measured after treatment. As shown in Figure 2, exposure to resveratrol increased the apoptosis ratio in the cells from 15±2% to 52±12%, as well as decreased the percentage of normal cells from 84±2% to 34±3% significantly ($p < .05$). This data showed that resveratrol exerts its cytotoxic effect by inducing apoptosis.

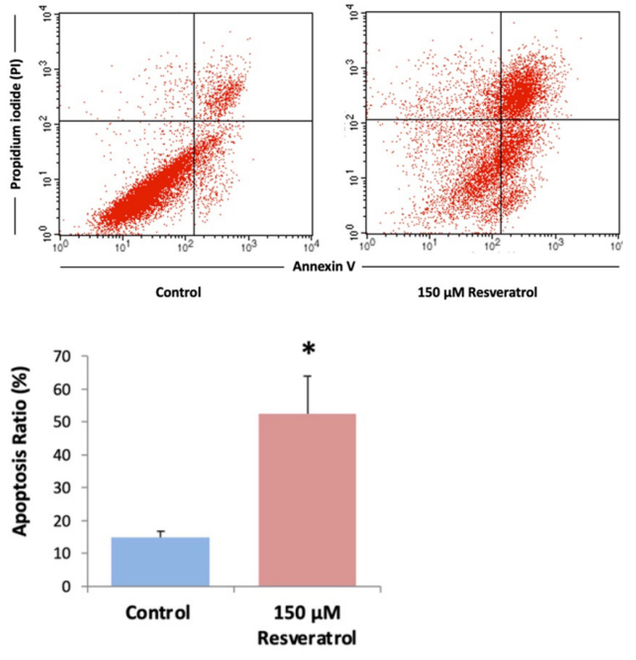


Figure 2. *Resveratrol induced apoptosis in OE33 cells. Following a 24-hour treatment with resveratrol, the cells were stained with Annexin V-FITC and PI and analyzed by flow cytometry. (A) Representative dot plots of OE33 cells after the treatment. Viable (healthy) cells were Annexin V^-/PI^-, early apoptotic cells were Annexin V^+/PI^-, late apoptotic cells were Annexin V^+/PI^+, and necrotic cells were Annexin V^-/PI^+. (B) The apoptosis ratio was calculated by summing the percentages of early and late apoptotic cells. * $p < .05$. N = 3 independent experiments.*

3.3. Resveratrol Increased Intracellular ROS Levels in OE33 Cells.

To understand the mechanism by which resveratrol reduces the viability of OE33 cells, intracellular ROS levels were measured with H2DCFDA using flow cytometry after 24 hours resveratrol treatment. It was observed that 150 µM resveratrol increased ROS levels in the cells by 236% ($p < .001$) (Figure 3). This demonstrates that resveratrol reduces viability of OE33 cells by increasing ROS levels, which leads to the activation of apoptosis.

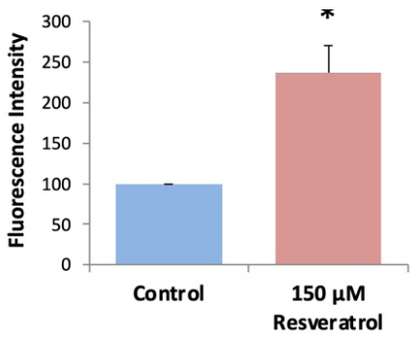
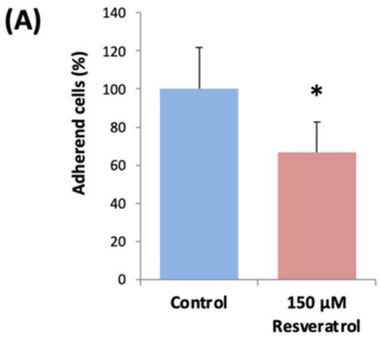
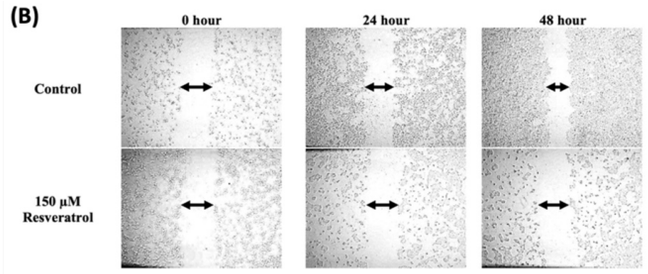


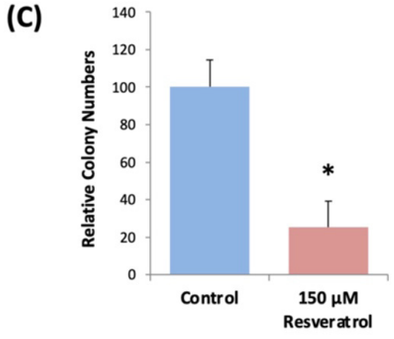
Figure 3. *Resveratrol increased intracellular ROS levels in OE33 cells. Following a 24-hour treatment with resveratrol, intracellular ROS levels were quantified using 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) staining and analyzed by flow cytometry. The fluorescence intensity reflects the intracellular ROS levels. * $p < .05$. N = 3 independent experiments.*

3.4. Resveratrol Inhibited the Metastatic Potential of OE33 Cells.

The processes of metastasis involve three key actions: migration, adhesion, and colony formation. 150 µM resveratrol treatment reduced the adhesive ability of OE33 cells, which is depicted by the lower percentage of cells (~67%) that can adhere to the surface coated with fibronectin compared to control (Figure 4A). Furthermore, wound healing assay revealed that resveratrol prevented the cells to cover the wound area (Figure 4B). This might be due to significantly decreased migration and higher mortality of the treated cells compared with untreated cells. Additionally, colony formation assay showed that resveratrol reduced colony formation by ~75% in OE33 cells compared to untreated cells (Figure 4C, 4D). These results suggested that resveratrol inhibits the metastatic potential of OE33 cells.







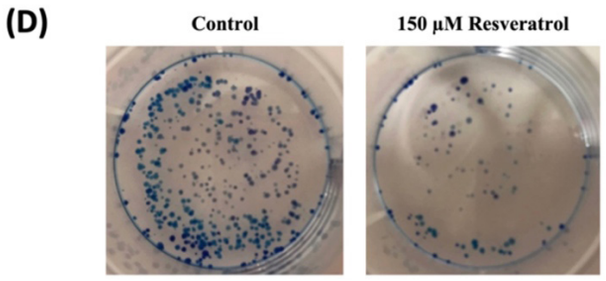


Figure 4. *Resveratrol inhibited the metastatic potential of OE33 cells. Following a 24-hour treatment with resveratrol, (A) cell adhesion ability was assessed, (B) cell migration was evaluated by microscopy, and (C, D) cell colonies were quantified. * $p < .05$. N = 3 independent experiments.*

4. DISCUSSION

The global incidence of EAC has been steadily increasing, particularly in Western countries. Despite its rising prevalence, effective therapeutic options remain limited, highlighting the urgent need for novel treatment strategies (10). Natural polyphenols have gained attention as potential anti-cancer agents due to their minimal side effects (11).

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a well-known polyphenolic compound which was initially extracted in 1940 from the roots of white hellebore (Veratrum grandiflorum) (12). Subsequently it was found in a variety of dietary sources, including grapes, nuts, apples, red fruits, black olives, capers, brown or red rice (13). A wide range of health-promoting effects of resveratrol was exhibited including hepatoprotective, neuroprotective anti-inflammatory, antioxidant and anti-carcinogenesis activities (14). Resveratrol was first identified as an inhibitor of carcinogenesis in a mouse skin cancer model, which subsequently sparked increased research interest. It has been shown that resveratrol reduces the incidence and

progression of various malignancies, including lungs, breast, prostate, cervical, ovarian liver pancreatic, colorectal, gastric, oral, and also esophageal cancers (15,16).

In a study conducted on male Sprague-Dawley rats, Woodall and colleagues examined the impact of resveratrol on the progression from reflux esophagitis to Barrett's metaplasia, dysplasia, and EAC. Their findings demonstrated that resveratrol significantly mitigated the severity of esophagitis while also reducing the incidence of carcinoma and intestinal metaplasia. These results suggest that resveratrol offers substantial protective effects against esophageal cancer development (17). A population-based case–control study observed that a diet rich in resveratrol was associated with a reduced risk of esophageal cancer in the Swedish population (8). However, the underlying mechanisms of this protective effect of resveratrol remain to be fully elucidated. Because of that, in the present study, we investigated the effects of resveratrol on EAC cells and found that resveratrol decreased cell viability in a dose dependent manner. The inhibitory effects of resveratrol on the growth of various cell types, including ESCC and EAC cells, have been previously demonstrated. Consistent with our findings, these studies also reported that the cytotoxic effects of resveratrol are both dose – and time-dependent (9,13,18,19). It is well known that resveratrol exerts dual effects. At high concentrations (≥25 µM), it induces cytotoxicity (19), whereas at lower concentrations, it promotes the expression of cell survival proteins (20). Consistently, we also observed the cytotoxic effects of resveratrol at higher doses (≥100 µM).

Previous studies have shown that the underlying mechanism of resveratrol's cytotoxic effect involves the induction of apoptosis and cell cycle arrest (14,21). Resveratrol has been shown to induce apoptosis in human esophageal carcinoma cells in a dose – and time-dependent manner, primarily through decreased expression of the anti-apoptotic protein Bcl-2 and increased expression of the pro-apoptotic protein Bax (9). Apoptosis aside, previous research has also linked resveratrol-induced growth inhibition with cell cycle arrest (14). Resveratrol is known to activate p53 and p21, leading to cell cycle arrest and senescence. In EAC cells, resveratrol treatment increased p27Kip1 levels, leading to cell cycle arrest and suppression of cell proliferation (22). Furthermore, Tang et al. demonstrated that resveratrol inhibits the growth of ESCC cells by inducing cell cycle arrest at the sub-G1 phase in a dose-dependent manner (18). Dhir et al. (23) observed that resveratrol stimulates apoptosis through ROS production. They suggested that resveratrol influences key cellular pathways, including DNA repair, transcription, and proliferation, via this mechanism. In line with their findings, we also demonstrated that resveratrol enhances apoptosis by increasing ROS levels. We extended these findings by demonstrating that resveratrol's pro-apoptotic effect via ROS also contributes to its antimetastatic activity in EAC cells.

Metastasis, the final stage of carcinogenesis, is the leading cause of death in cancer patients, including those with esophageal cancer (24). Previous studies on pancreatic, liver, renal, ovarian, and kidney cancer cell lines have shown that resveratrol significantly reduces the metastasis of cancer cells (24,25,26). In the current study, we observed that all three steps of metastasis, i.e. migration, adhesion, and colony formation were inhibited by resveratrol treatment. Based on our knowledge, this is the first study to highlight the anti-metastatic effects of resveratrol on EAC cells. Metastasis of cancer cells requires disruption of the cell-extracellular matrix (ECM) interaction and degradation of the ECM. A disintegrin and metalloproteinase (ADAM) proteins, which are proteolytic enzymes responsible for destroying the ECM, are overexpressed in many types of cancers, including esophageal cancers, and correlate with cancer progression and metastasis. Lin et al. (24) observed that resveratrol reduced the migration of ESCC cells by decreasing ADAM9 protein levels, which supports our findings.

On the other hand, it is also known that resveratrol has biphasic, dose-dependent effects, altering its role in oxidative stress. These biphasic concentration-dependent effects of resveratrol, acting as an antioxidant at low doses and a prooxidant at high doses, have been observed both in vitro and in vivo. Both low and high concentrations offer beneficial effects: low doses contribute to cancer prevention, while high doses are cytotoxic and aid in cancer treatment (19). It has been suggested that at lower doses, the antioxidant potential of resveratrol is mediated through both free-radical scavenging and the upregulation of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, which protect cells and DNA from ROS-induced damage through various mechanisms (13). At higher doses, increased ROS generation may lead to organelle and DNA damage, resulting in apoptosis, autophagy, necrosis, or senescence in cancer cells (16). Based on our data, we also hypothesize that the inhibitory effect of resveratrol on cell viability at higher concentrations is mediated by apoptosis, which is triggered by increased ROS production in EAC cells.

Despite accumulating evidence supporting resveratrol's anticancer properties in vitro and in preclinical studies, its clinical translation remains limited, which results in conflicting reports on its efficacy. A key limitation is the compound's poor bioavailability and low aqueous solubility. Resveratrol is rapidly metabolized and eliminated, making it difficult to achieve and sustain therapeutically effective plasma concentrations (27). Although generally considered safe, adverse effects such as diarrhea, nausea, and anemia have been reported, particularly at high doses. In rare cases, complications such as nephrotoxicity, impaired lipid metabolism, and thrombocytopenia have also been observed. These findings underscore the importance of dose optimization and improved delivery strategies to enhance the clinical applicability of resveratrol (20).

This study has several limitations. First, the use of in vitro cell line models may not fully replicate the biological diversity and complexity of tumors in vivo. Therefore, the effects of resveratrol observed in our study may differ in a clinical setting. Second, our study primarily focused on short-term

effects of resveratrol, and long-term treatment studies are needed to evaluate potential prolonged effects and outcomes. Additionally, although significant findings were made regarding resveratrol's impact on cell proliferation and apoptosis, the exact molecular mechanisms underlying these effects remain incompletely understood, and further research is needed to elucidate these pathways.

5. CONCLUSION

In conclusion, our findings demonstrate that resveratrol reduces the viability of EAC cells in a dose-dependent manner by inducing apoptosis through increased intracellular ROS production. Additionally, resveratrol suppresses the metastatic potential of these cells, highlighting its ability to modulate key cellular processes involved in esophageal cancer progression. These results suggest that resveratrol holds potential as a complementary agent alongside conventional chemotherapy for the treatment of EAC. However, further in vivo studies and clinical investigations are required to fully elucidate its therapeutic potential and mechanisms of action.

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Clinical and Experimental Health Sciences

Turkish Validity and Reliability Study of the Negative Menstrual Attitudes Assessment Scale: A methodological study

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ABSTRACT

Objective: The objective of this study is to determine the validity and reliability of the Negative Menstrual Attitudes Assessment Scale (NMAS) in Türkiye. The NMAS is a scale that evaluates women's negative attitudes towards menstruation..

Methods: This study employed a methodological approach and concentrated on female students registered in the Faculty of Health Sciences at a public institution from June to August 2023. The scale underwent a three-phase Turkish adaption procedure, comprising linguistic validity, content validity, and a pilot implementation. The pilot research had a cohort of 30 individuals. Following this, construct validity was assessed using Kaiser-Meyer-Olkin, Exploratory Factor Analysis (EFA), and Confirmatory Factor Analysis (CFA). Furthermore, assessments were performed to evaluate reliability, encompassing Cronbach's α internal consistency coefficient, item-total correlation, test-retest analysis, and ROC analysis for determining cut-off scores.

Results: According to the EFA analysis, which confirmed the validity of the 15-item scale, it was determined that no items had factor loadings below 0.30. The subsequent CFA analysis validated the three-factor structure of the NMAS, exhibiting good fit indices. The resultant 15-item scale consists of three sub-dimensions: Debilitating Event, Bothersome Event, and Natural Event. The internal consistency coefficient of NMAS, as determined by Cronbach's alpha, was 0.861, 0.855, 0.784, and 0.772 for the overall measure and its sub-dimensions, respectively. Moreover, the item-total correlation and test-retest analysis of the scale demonstrate a robust association.

Conclusion: A rigorous and accurate instrument has been created to evaluate unfavorable menstruation attitudes among women in Turkish culture. The scale demonstrated robust psychometric properties, confirming its validity and reliability for assessing negative menstrual attitudes in Turkish culture.

Keywords: Negative menstrual attitudes, NMAS, Menstrual attitude, Reproductive age, Scale

1. INTRODUCTION

Menstruation and the menstrual cycle occur every month during a woman's reproductive years, impacting a significant portion of her life (30-35 years) (1). Although menstrual bleeding is a natural physiological process, it is considered "unclean" in certain cultures, leading to restrictions in daily activities, both religious and socially (2,3). Studies have shown that negative attitudes and misinformation related to menstruation can have detrimental effects on reproductive health (4). These negative attitudes include viewing menstruation as an obstacle or embarrassment, perceiving it as a physical and psychological burden, discomfort, feeling obligated to follow certain restrictions during menstruation and an erroneous belief that certain activities are forbidden (5).

Factors that contribute to negative attitudes towards menstruation include a lack of knowledge, age, personal experiences, myths, traditions, social and cultural beliefs, and pain and difficulties associated with menstruation. These factors have been identified in studies (1,4,6) on the topic. Research conducted across various cultures has indicated that the expression of the connection between menstruation and culture may differ. For instance, previous research has suggested that menstruation is often viewed

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as a symbol of femininity, as well as a biological process that involves the removal of contaminated blood or toxins and can cause discomfort, including disruption to sports activities (6,7). Studies conducted in Türkiye have found that individuals with high levels of health self-efficacy tend to have more positive attitudes towards menstruation (7), while negative menstrual attitudes can have a detrimental impact on genital hygiene behaviour (8). Additionally, the majority of participants in a study reported negative initial reactions to their first menstrual bleeding (6).

Negative menstrual attitudes encompass beliefs that menstruation is shameful, disruptive or distressing. These attitudes are shaped by sociocultural norms, intergenerational messages and individual psychological experiences. Contemporary studies emphasize the role of menstrual stigma, where menstruation is framed as a private and polluting phenomenon, reinforcing silence and concealment (9). Cultural taboos, restrictive gender norms and early negative experiences—such as pain or embarrassment—contribute to internalized negativity (10). These attitudes influence not only emotional responses but also health behaviors, highlighting the need for culturally adapted assessment tools.

Recent qualitative and quantitative studies have illuminated the challenges confronted by women and adolescent girls during menstruation, identifying their detrimental effects on health, education, employment and well-being (11–13). Negative attitudes towards menstrual health have been associated with poorer health outcomes, while positive attitudes have been linked to psychological well-being and better overall health (4,12). Negative menstrual attitudes are known to exacerbate menstrual discomfort (13). It is crucial for health practitioners to address negative attitudes toward menstruation. Therefore, in light of the influence of social and cultural factors on menstrual attitudes, it is imperative to devise culturally-specific assessment methods. In Türkiye, a menstrual attitude assessment scale was previously developed by Brooks and Gunn and was subsequently validated and shown to be reliable by Fırat et al. (14,15). The scale comprises of 33 items and 5 sub-dimensions evaluating positive and negative attitudes towards menstruation. In order to determine negative attitudes, Kawata et al. developed a 15-item, 3-subdimensional scale evaluating negative menstrual attitudes based on the menstrual attitude scale.

In this study, the Turkish validity and reliability of the scale developed by Kawata et al. Since it only measures negative attitudes, it is thought to make an important contribution to the literature. In addition, the fact that the Turkish version of the scale has a cut-off score will allow individualization of the service provided to women.

Although various instruments have been developed to assess attitudes toward menstruation, most encompass both positive and negative dimensions and therefore lack conceptual precision in capturing the distinct features of menstrual stigma and discomfort (14,15). The Negative Menstrual Attitudes Scale (NMAS), by contrast, was specifically constructed to assess exclusively negative perceptions grounded in sociocultural norms, misconceptions and emotional responses related to menstruation (3). This theoretical specificity enhances its utility in both research and practice, particularly in cultural contexts where menstruation remains a stigmatized and underdiscussed phenomenon (16,17). Within the Turkish literature, although general menstrual attitude measures exist, no validated tool currently isolates negative attitudes as a distinct construct (18). The adaptation of the NMAS into Turkish thus responds to a significant methodological and conceptual gap, offering researchers and healthcare professionals a culturally relevant, psychometrically sound instrument that can facilitate targeted interventions and contribute to the broader advancement of menstrual health equity.

2. METHODS

This methodological study examined female students enrolled in the Faculty of Health Sciences at a state university between June and August 2023. Various methods can be used to determine the sample size for adapting measurement tools to different cultures. For this study, the calculation was based on at least 10 times the number of items on the scale (15 items) (19). The minimum sample size was set at 300 and 328 participants took part in the study. Additionally, a pilot study was conducted with 30 participants and a test-retest analysis was performed with 33 participants. None of these participants were included in the exploratory and confirmatory factor analyses. Participants were randomly selected from the relevant population based on inclusion criteria. The study included participants aged 18–25 who were single, had no communication barriers and voluntarily agreed to participate in the research. Participants with chronic reproductive health or psychiatric issues were excluded. To ensure test-retest reliability, participants were contacted and appointments were scheduled. This criterion was developed due to the prevalence of single university students in Türkiye and the cultural perception that sexual behaviour is specific to married women. For test-retest reliability assessment, a subsample of 33 participants was selected using the same inclusion criteria. These participants were randomly chosen from individuals not involved in the initial exploratory or confirmatory factor analyses. They were contacted two weeks after the first administration to complete the scale again under similar conditions.

2.1. Data Collection Tools

2.1.1. Personal Information Form

It is a survey composed of ten queries prepared by the researchers to determine the socio-demographic and menstrual characteristics of the participants who took part in the study.

2.1.2. Negative Menstrual Attitudes Assessment Scale (NMAS)

The NMAS was created by Kawata and associates in 2022, drawing upon the Brooks-Gunn and Ruble "menstrual attitude questionnaire" to measure women's unfavorable perceptions of menstruation (3,14). The measure employs a five-point Likert scale (1 = never, 2 = rarely, 3 = sometimes, 4 = often and 5 = always) and has three sub-dimensions: "Debilitating Event," "Bothersome Event," and "Natural Event." Technical terminology is elucidated at initial usage. Cronbach's alpha coefficients were computed, resulting in values of 0.76 and 0.69 for the "Debilitating Event" and "Bothersome Event" sub-dimensions and 0.82 for the "Natural Event" sub-dimension, respectively. The comprehensive scale demonstrated an internal consistency value of 0.861. The "Debilitating Event" sub-dimension revealed an internal consistency coefficient of 0.855, the "Bothersome Event" sub-dimension indicated a coefficient of 0.784 and the "Natural Event" sub-dimension showed a coefficient of 0.772.

2.1.3. Procedure

The scale was translated into Turkish using a three-stage procedure consisting of language validity, content validity and pilot testing. In the language validity stage, the research team translated the 'Negative Menstrual Attitudes Assessment Scale' from English into Turkish. The translation was then evaluated by qualified linguists from Türkiye and compared with the original scale. The back-translated version was compared with the original by the research team and minor revisions were made to ensure semantic and conceptual equivalence. Language equivalence was confirmed by experts with high item-level agreement. Experts (4 women's health and nursing nurses, 5 midwives, 1 statistician) developed the English and Turkish versions of the scale, which were then evaluated by experts for content validity. Ten faculty members, in collaboration with the experts, evaluated each item on a scale of 1 to 3 (1: item should be removed, 2: item should be revised, 3: item is appropriate) and assessed the appropriateness and clarity of the items within the scale. The degree of agreement among expert opinions was assessed using Kendall's W analysis (Kendall W=0.092; $\chi 2$=14.740, p=0.544>0.05). Expert evaluations indicated a high degree of agreement regarding the scale components (20,21). The data obtained from this study were excluded from the original sample. Following expert advice, a pilot study was conducted with 30 participants to apply the scale. The evaluation from the pilot study showed that the questions were clear and that participants were able to respond quickly and easily. Additionally, official written permission was obtained from Kazutomo Ohashi, the developer of the original scale, for the Turkish adaptation.

2.2. Psychometric Testing of The NMAS

2.2.1. Validity

To evaluate the construct validity of the scale, we employed the Kaiser-Meyer-Olkin (KMO) test to determine sample adequacy and size prior to doing factor analysis. We evaluated the appropriateness of the scale for factor analysis by Bartlett's Test of Sphericity. KMO values were employed to assess the appropriateness of the data for factor analysis. KMO values are assessed on a scale: "excellent" for 0.90-1.00, "very good" for 0.80-0.89, "good" for 0.70-0.79, "moderate" for 0.60-0.69 and "poor" for 0.50-0.59. A KMO score of 0.60 or above is necessary for factor analysis. An elevated value in Bartlett's sphericity test immediately enhances the appropriateness of data for component analysis (21).

Validity analysis refers to the accuracy of a measurement tool in measuring a specific feature without any confusion with other features (22). In this study, the preferred method of Principal Component Analysis was used to examine the factor structure of the scale. The results were evaluated based on the prerequisite that the questions' factor loadings obtained from the analysis should be at least 0.30, according to Büyüköztürk (23). Confirmatory factor analysis (CFA) was conducted to validate the accuracy of the sub-dimensions of the scale obtained through exploratory factor analysis (EFA) (17). We accepted a $\chi 2$/sd ratio of ≤5, RMSEA value of ≤0.08 and GFI, CFI, IFI values higher than 0.90 as the minimum data fit index of the model (24).

2.2.2. Reliability

The scales' reliability was assessed using Cronbach's alpha (α) coefficient. The Cronbach α coefficients range from 0 to 1, with greater values signifying enhanced reliability of the scale's internal consistency. According to the established grading system, scores below 0.50 are classified as undesirable, scores from 0.50 to 0.60 are categorized as bad and scores between 0.60 and 0.70 are regarded as dubious. values ranging from 0.70 to 0.80 are deemed acceptable and values of 0.80 and more are regarded as good to exceptional (25). The internal consistency coefficients of the scales are affected by the quantity of items included in the scale. Consequently, augmenting the quantity of items in a scale results in a heightened internal consistency coefficient (26). Nonetheless, a scale including a limited number of items requires a reliability coefficient of 0.50 to be deemed sufficient. In instances of limited item quantities, internal consistency can be assessed using Cronbach's α, composite reliability coefficients and correlation coefficients among the scale items. Correlation values over 0.20 among the items are adequate to guarantee the reliability of the test scale (27). The link between individual test item scores and the overall score of the Negative Menstrual Attitudes Evaluation Scale was assessed by the analysis of item-total correlation coefficients. To do a test-retest examination of the scale, 33 people who fulfilled the necessary research requirements

were recruited for the study. The scale's temporal invariance was assessed by test-retest correlation (28).

2.3. Data Analysis

SPSS 26.0 software was used in the study to calculate Cronbach's α for internal consistency and reliability analyses, as well as explanatory factor analysis (EFA) (29). AMOS 24 was the preferred package for conducting confirmatory factor analysis to ensure scale control with factor structure. Statistical significance level (p) was set at .05 throughout the analysis. Descriptive statistics such as mean, standard deviation, number and percentage values were reported (30).

2.3.1. Multivariate Normal Distribution Analysis

As EFA and CFA constitute multivariate analysis techniques, the associated database underwent testing for multivariate normal distributio (30). A total of 334 data forms were collected, with subsequent preparation of the study data involving rigorous scrutiny for missing observations and outliers. Six questionnaire forms were excluded from the study because they did not meet the criteria set out and the Mahalanobis distance (31). The research employed 328 questionnaire forms and a Mahalanobis Distance value of 5.393 was computed using the AMOS software. The assumption was confirmed since the computed coefficient for the multivariate normal distribution was below 8 (32,33).

2.4. Ethical Issues

At the commencement of adapting the Negative Menstrual Attitudes Assessment Scale to Turkish culture, Kazutomo Ohashi was contacted via email and received permission for the adaption. Subsequently, written consent was obtained from the institution conducting the research and the Health Sciences Non-Interventional Clinical Research Ethics Committee of a public university gave clearance (Decision No: 2023/4594). Participants were notified that they would receive no reward for their participation in the study and that their data would be used solely for research reasons. An informed consent form was distributed and eligible subjects who voluntarily satisfied the acceptance requirements were incorporated into the study.

3. RESULTS

Participant demographics are available in Table 1. The average age of participants was 21.22 ± 2.35 years, the average age at the onset of menstruation was 13.19 ± 3.16 years, the average BMI was 22.72 ± 8.1, the average number of days with menstrual bleeding was 5.05 ± 1.59, the average menstrual cycle duration was 28.46 ± 4.76 and the average severity of menstrual pain was 5.84 ± 2.889. According to Table 1, 49.1% of the participants had an equivalent income and expenses, 83.5% were unemployed and 61.9% resided in the city centre.

Table 1. *Demographic and Menstrual Cycle Characteristics of Participants (N=328)*

Variable	Groups	N	%
Income Status	Low	118	36.0
	Medium	161	49.1
	High	49	14.9
Employment Status	Yes	54	16.5
	No	274	83.5
Family Structure	Nuclear family	254	77.4
	Extended family	59	18.0
	Divorced family	15	4.6
Place of Residence	Province	203	61.9
	District	84	25.6
	Village-Town	41	12.5
Total		328	100.0
Variable		Mean ± sd	
Age		21.22 ± 2.35	
Menarche age		13.19 ± 3.16	
BMI		22.72 ± 8.1	
Menstrual cycle duration		28.46 ± 4.76	
Duration of menstrual bleeding		5.05 ± 1.59	
Pain intensity during the menstrual period		5.84 ± 2.889	

sd*: standart deviation*

3.1. Validity

The KMO study revealed a KMO coefficient of 0.85 for the participants, while Bartlett's Test of Sphericity yielded an X2 value of 1951.262. The test findings were determined to be statistically significant (p<.05). The KMO value of 0.853 indicates that the sample size is adequate and suitable for factor analysis (Table 2).

Table 2. *KMO ve Barlett's Test of Sphericity Test*

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.853
Bartlett's Test of Sphericity	Approx. Chi-Square	1951.262
	df	105
	p	.001*

df*: Degree of freedom, *p<.05; The test is test is statistically significant*

An exploratory factor analysis (EFA) was performed to evaluate the validity of the 15-item Negative Menstrual Attitudes Assessment Scale employed in the study. The factor load values indicated that the 'debilitating incident' sub-dimension includes items 1, 4, 5, 6 and 9, with a variance between 0.573 and 0.757. The 'Bothersome event' subscale includes items 2, 3, 8, 10 and 11, with a range of 0.598 to 0.842. The 'Natural event' subscale includes items 13, 14, 15, 16 and 17, with a range from 0.325 to 0.886. The overall variance was accounted for by 58.554%. The 'debilitating event' sub-dimension accounted for 33.763% of the variation, the 'bothersome event' sub-dimension accounted for 16.977% and the 'natural event' sub-dimension accounted for 7.814% (Table 3). Figure 1 illustrates the scree plot of the scale, which was analyzed by evaluating the factor loadings of items with a minimum threshold of 0.30. No items were discarded owing

to insufficient factor loadings (18). The "Negative Menstrual Attitudes Assessment Scale," comprising 15 items and three sub-dimensions, was obtained.

Table 3. *Factor Loadings and Variance Explained of NMAS*

Questions	Sub-Groups		
	Debilitating event	Bothersome event	Natural event
Q1	0.705		
Q4	0.757		
Q5	0.753		
Q6	0.573		
Q9	0.605		
Q2		0.701	
Q3		0.723	
Q8		0.598	
Q10		0.791	
Q11		0.842	
Q13			0.725
Q14			0.886
Q15			0.832
Q16			0.325
Q17			0.724
% Variance Explained	**33.763**	**16.977**	**7.814**
Total		**58.554**	

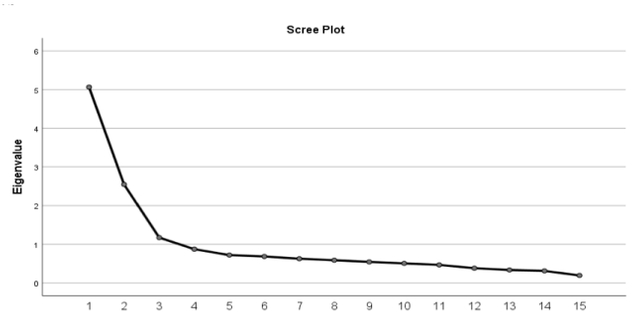

Figure 1. *Scree plot graph of the scale*

CFA was conducted to validate a scale model comprising of three components and fifteen questions subsequent to EFA as per references (32,33). CFA is a commonly used method to create latent variables through observed variables in modelling (34). Confirmatory factor analysis was conducted to verify the precision of the dimensions in the acquired scale. Confirmatory factor analysis was conducted on the acquired scale to verify the precision of the dimensions. CFA was applied to verify the scale model consisting of 3 sub-dimensions and 15 questions after EFA (32,33). The model's goodness of fit indices were calculated, yielding χ2 (CMIN, Chi-Square Goodness of Fit) 380.450, χ2/df 4.373, IFI (Incremental Fit Index) 0.846, CFI (Comparative Fit Index) 0.844, GFI (Goodness of Fit Index) 0.864 and RMSEA (Root Mean Square Error of Approximation) 0.102. Model change was executed due to measurement inaccuracies and concealed structures, since the computed values failed to achieve the requisite standard (35).

Covariances for the binary residual terms were computed and the model was then reconstructed. To maintain consistency, the plots were restricted to identical dimensions, as covariances cannot be computed for residual components across different dimensions (36). The covariances detected among these binary error terms reveal a consistent pattern, illustrating an abstract idea. This explanation relates to the collective structure formed by the two variables rather than their separate effects. The amalgamation of these two error categories reveals a statistically significant phenomena.

In the modification coefficient control, covariances among residual terms within the same sub-dimension were plotted, resulting in the formulation of a new model. The error term pairings with modifications identified were e10-e11, e2-e3, e5-e6, e8-e10, e1-e6 and e5-e6. The goodness of fit index values derived from the new NMAS model were: χ2 268.691, χ2/df 3.277, CFI 0.901, GFI 0.905, IFI 0.902 and RMSEA 0.080 (Table 4). The reduction in χ2/df and RMSEA values, together the increase in CFI, GFI and IFI values exceeding 0.90, confirmed the NMAS model as statistically adequate. An optimal model fit was attained with a reduction in χ2/df (χ2/sd ≤5) and an elevation in RMSEA (RMSEA ≤0.08), GFI, CFI and IFI (>0.90) (29,37). Confirmatory Factor study (CFA) is a subset of Structural Equation Modeling (SEM) study. In SEM analysis, many indicators are provided and analyzed to assess model fit, rather than relying on a single index (38,39).

Table 4. *CFA Fit Indexes*

	Model 1	Model 2	Good Fit	Acceptable Compliance
CMIN	380.450	268.691	The model with the smallest value is more compatible.	
P	0.001	0.001	p< .005	
X^2/df	4.373	3.277	≤ 3	3-5
CFI	0.844*	0.901	≥ .90	0.90 – 0.95
GFI	0.864*	0.905	≥ .90	0.90– 0.95
IFI	0.846*	0.902	≥ .90	0.90 – 0.95
RMSEA	0.102*	0.080	≤ .008	0.05 – 0.08

X^2:CMIN, Chi-Square Goodness of Fit; ***CFI***: Comparative Fit Index; ***GFI***: Goodness of Fit Index, ***IFI***: Incremental Fit Index ***RMSEA***: Root Mean Square Error of Approximation * Values are not in the desired range.

Figure 2 shows the diagram of the Negative Menstrual Attitudes Assessment Scale according to the second CFA model.

The NMAS validation analyses showed that the model, comprising 3 sub-dimensions and 15 questions, was statistically valid.

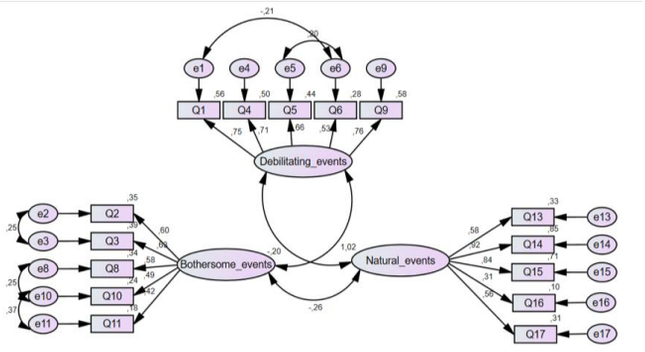

Figure 2. *NMAS modified CFA Mode*

3.2. Reliability

Cronbach's alpha (α) coefficient of internal consistency was used to analyse the reliability of the scale. Following an examination of the NMAS' internal consistency through Cronbach's α reliability analysis, the total scale exhibited an internal consistency coefficient of 0.861. Furthermore, the 'debilitating event' sub-dimension and 'bothersome event' sub-dimension revealed internal consistency coefficients of 0.855 and 0.784, respectively. Lastly, the 'natural event' sub-dimension displayed an internal consistency coefficient of 0.772 (Table 5). The correlation coefficients of the scale items ranged from 0.259 to 0.588 and no item was omitted due to a value lower than 0.20 (27).

Table 5. *Item Total Correlation and Cronbach's α of Negative Menstrual Attitudes Assessment Scale*

Questions	Sub-Groups		
	Debilitating event	Bothersome event	Natural event
Q1	0.517		
Q4	0.588		
Q5	0.511		
Q6	0.327		
Q9	0.585		
Q2		0.402	
Q3		0.423	
Q8		0.534	
Q10		0.481	
Q11		0.405	
Q13			0.299
Q14			0.330
Q15			0.483
Q16			0.259
Q17			0.334
Cronbach's α	**0.811**	**0.738**	**0.765**
Total		**0.717**	

3.3. Test-retest Reliabilty

A test-retest analysis was conducted to assess the scale's temporal invariance, using 33 individuals in the research (28). The correlation coefficients between the mean scores of the NMAS used in the study varied from 0.210 to 0.565 in the initial application and the subsequent application after a 15-day gap (Table 6). A highly statistically significant positive correlation was identified for the total score and all subscales of the NMAS (p<.05). Nonetheless, for the sub-dimensions of Debilitating event, Bothersome event and Natural event, as well as the overall scale, no statistically significant change was seen in either measurement (p>.05) and the notion of invariance over time was upheld. The results indicate a strong statistically significant connection between the responses gathered at two separate locations, hence demonstrating the scale's consistency.

Table 6. *Test-retest Reliability of NMAS*

Groups		Mean ± sd	Median (Min-Max)	t value	p^a value	r value	p^b value
Debilitating event	Test	16.27 ± 4.87	17(7-25)	1.327	.194	0.268	.015*
	Re-test	14.91 ± 4.89	15(7-25)				
Bothersome event	Test	15.18 ± 4.78	17(5-22)	0.251	.803	0.210	.004*
	Re-test	14.91 ± 5.13	15(5-25)				
Natural event	Test	12.18 ± 4.22	11(6-22)	-0.061	.952	0.257	.003*
	Re-test	12.24 ± 4.11	12(5-20)				
SCALE	Test	43.64 ± 9.45	45(21-56)	0.735	.468	0.565	.001
	Re-test	42.06 ± 9.61	44(20-56)				

sd*: standart deviation,* ***t****; paired t test value,* ***r****; pearson correlation coefficent, p^a>.05; here is no difference between pretest and posttest.* ***p^b****<.05; There is a very strong significant relationship between the two values.*

In the assessment of the measure, a 5-point Likert-type scale was employed (1; never, 2; rarely, 3; sometimes, 4; often, 5; always). Questions 13, 14, 15, 16 and 17 were subjected to reverse coding. For the 328 participants analysed for the NMAS, the lowest score from the total scale was 15 and the highest score was 65, the mean score was 45.05 ± 7.95, the lowest score from the "Debilitating event" sub-dimension was 5 and the highest score was 25, the mean score was 16. 78 ± 4.39, the lowest score from the "Bothersome event" sub-dimension was 5, the highest score was 25 and the mean score was 16.93 ± 4.46, the lowest score from the "Natural event" sub-dimension was 5, the highest score was 25, and the mean score was 11.33 ± 3.91 (Table 6). When assessing the total NMAS score, a score of 14-25 is deemed low, a score of 26-43 is considered medium and a score of 44-65 is considered high level negative menstrual attitude. Based on this study, the participants scored an average of 45.05 ± 7.95 on the NMAS and had a high level of negative menstrual attitude (Table 7).

Table 7. *Average scale scores and minimum-maximum values can be obtained and utilized*

SCALES	Mean ± sd	Min – Max	Min-Max Scores to Receive From the Scale
Debilitating event	16.78 ± 4.39	5 – 25	5-25
Bothersome event	16.93 ± 4.46	5 – 25	5-25
Natural event	11.33 ± 3.91	5 – 25	5-25
SCALE	45.05 ± 7.95	15 – 64	15-75

sd*: standart deviation*

3.4. Scale Estimation Point Calculation

ROC analysis was used to ascertain the cut-off point. The ROC curve provides the optimal cut-off point for the instrument, together with the sensitivity and specificity ratios of the judgments based on this cut-off point. Sensitivity is defined as the proportion of individuals who are truly ill and are identified as such by the test's cut-off point, while specificity is defined as the proportion of individuals who are truly healthy and are accurately identified as such by the test. The superior the test, the greater the upward shift of the curve (indicating high sensitivity) and the leftward shift (indicating a low false positive rate). An AUC of 0.5 indicates a lack of discrimination; an AUC between 0.5 and 0.7 signifies statistically negligible discriminating power of the test. A discrimination score between 0.7 and 0.8 is regarded as adequate, between 0.8 and 0.9 as very good and above 0.9 as exceptional. The findings of the analysis are presented in Table 8.

The cut-off scores determined through ROC analysis were established by examining the distributional characteristics of the total NMAS scores in conjunction with the corresponding sensitivity and specificity values. The selected thresholds—14–25 (low), 26–43 (moderate) and 44–65 (high)—were derived from the inflection points observed in the ROC curve, reflecting optimal discrimination between varying levels of negative menstrual attitudes. This categorization was informed by previous psychometric applications of the NMAS, where similar score intervals were used to delineate severity levels, albeit without formally defined cut-off points (3). Moreover, comparable score-based interpretive frameworks have been utilized in other menstrual attitude studies to stratify respondents for targeted analysis (12). The adoption of these thresholds thus enhances the interpretability and clinical relevance of the scale, while maintaining consistency with existing research on attitudinal assessments related to menstruation.

Table 8. *NMAS ROC Analysis Results*

Scale Total Score	Cut Off	Sensitivity	Specificity	p	AUC
Low	14-25	0.95	0.75	.001*	0.719
Medium	26-43	0.87	0.68	.001*	0.845
High	44-65	0.96	0.73	.001*	0.985

p<.05;* ***AUC**: Area under the curve*

Table 8 depicts the outcomes of the NMAS ROC analysis. Please refer to Figure 3 for the ROC curve presentation. Participants who had a score between 14-25 points demonstrated a low level of negative menstrual attitude. A moderate level of negative menstrual attitude was observed for those who scored between 26-43 points and a high level of negative menstrual attitude was noted in those who scored between 44-65 points.

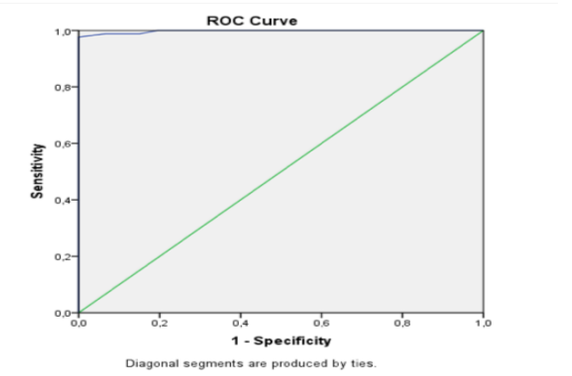

Figure 3. *ROC Analysis Curve indicating the high level*

4. DISCUSSION

This study aimed to establish the Turkish validity and reliability of a measurement tool assessing negative menstrual behavior in women. The scale's validity and reliability are vital aspects requiring careful consideration. Through psychometric analysis, the NMAS was found to be valid and reliable. The study results were discussed in accordance with existing literature.

To assess the construct validity of the NMAS, EFA was conducted followed by CFA to confirm the validity of the obtained structure. The analysis revealed no item with a factor loading below 0.30 and the scale items were categorized into three factors, accounting for 58.554% of total variance. In studies involving scales, if the explained variance ratio remains above ≥30, it suggests that the scale has construct validity. In studies involving scales, it is expected that the items will possess high loadings on the factor they belong to. If a group of items demonstrates a significant correlation with a particular factor, it implies that these items are capable of measuring a concept-structure-factor simultaneously (30). In the 1980 Menstrual Attitude Scale developed by Books et al., the factor loadings for the 'natural event' subscale varied from 0.59 to 0.72, the 'debilitating event' subscale ranged from 0.32 to 0.73 and the 'bothersome event' subscale ranged from 0.32 to 0.72 (14). Kawata et al. observed that the EFA values of the "Negative Menstrual Attitude Questionnaire" were 0.76 for the 'debilitating event' subscale, 0.69 for the 'bothersome event' subscale and 0.82 for the 'natural event' subscale (3,12). Furthermore, two questions were eliminated from the original scale owing to insufficient factor loadings: "It is easier for me to feel unwell than usual before menstruation" and "The only benefit of menstruation is to indicate that I am not pregnant." The Turkish version has a resemblance to the original scale regarding factor loadings.

The three-factor structure (Depleting Event, Distressing Event and Natural Event) has been preserved based on theoretical consistency with both empirical evidence and the original NMAS developed by Kawata et al. (3). These dimensions address different aspects of negative menstrual attitudes: perceived physical and emotional burden, social discomfort

and distorted perceptions regarding the naturalness of menstrual bleeding. When compared to the broader Menstrual Attitude Questionnaire (MAQ) developed by Brooks-Gunn and Ruble (1980), which includes five subscales covering both positive and negative dimensions, the NMAS offers a more focused framework specifically designed to capture stigmatising beliefs (40). While the 'Diminishing Event' and 'Natural Event' dimensions conceptually align with those in the MAQ, the 'Distressing Event' subscale reflects a more contemporary recognition of menstrual-related social distress. In this context, the retention of these factors supports both cultural specificity and psychometric consistency in the assessment of negative menstrual attitudes.

When evaluated in relation to the original version developed by Kawata and colleagues in 2022, the Turkish adaptation of the NMAS preserved the original three-factor structure, yet demonstrated slight variations in factor loadings and internal consistency coefficients (3). Unlike the original study, which excluded two items due to insufficient psychometric performance, all 15 items were retained in the Turkish version, indicating strong structural coherence within this sample. The slightly higher reliability coefficients observed in the Turkish version may reflect the internal consistency of menstrual perceptions shaped by prevailing social norms. Bobel et.al. explain that in societies where menstruation is socially stigmatized and framed as a source of bodily shame, attitudinal homogeneity is more pronounced due to the internalization of silence and taboo (16). Similarly, Sommer and colleagues highlight how social norms and health discourses significantly influence how menstruation is perceived, discussed and experienced (17). In the Turkish context, Özdal argues that menstrual stigma is reinforced through culturally embedded expectations of modesty, bodily control and limited public discourse (18). Taken together, these insights suggest that while the NMAS demonstrates structural stability across cultures, its psychometric performance may be influenced by the sociocultural environment in which it is applied.

The CFA was performed on a three-dimensional scale consisting of 15 items derived from the EFA. The precision of the dimensions was assessed and the goodness of fit indices were considered to ascertain the appropriateness of the model produced by CFA for the data. The precision of the dimensions was assessed and the fit indices were considered to ascertain the appropriateness of the model produced by CFA for the data. Nevertheless, achieving an adequate fit proved unfeasible. The fit index values for the first model of the scale were: $\chi 2$ 380.450, $\chi 2/df$ 4.377, RMSEA 0.102, GFI 0.864, CFI 0.844 and IFI 0.846. Upon analyzing the modification indices of the model, it was determined that the residual terms e10-e11, e2-e3, e5-e6, e8-e10, e1-e6 and e5-e6 exhibited the largest values. The covariances among these binary residual terms were delineated, leading to the reconstruction of the model and subsequent computations. A secondary CFA model was implemented by correlating the error covariances of the relevant elements. The CFA fit index values for the newly formed model, incorporating error covariances among the binary residual components, were $\chi 2$ 268.691, $\chi 2/df$ 3.277, RMSEA 0.080, GFI 0.905, CFI 0.901 and IFI 0.902. A strong concordance was observed with the $\chi 2/df$ and RMSEA values (Table 4). The statistical model demonstrated an adequate match. The confirmatory factor analysis corroborated the three-component scale structure established by exploratory factor analysis (EFA). The results from the original scale yield fit indices of CMIN/df=174.529/87 ($p<.001$), AIC=240.529, GFI=0.867, AGF=0.817, CFI=0.526 and RMSEA=0.076 (95% CI=0.59-0.92). These results validate the three-factor structure of the scale and demonstrate parallels between the Turkish version and the original version regarding confirmatory factor analysis. Moreover, it is important to acknowledge that the NMAS has not been modified for application in cultures distinct from the original form. Consequently, comparisons were conducted solely with the original form of the scale.

The NMAS's reliability was evaluated by Cronbach's α internal consistency coefficient, item-total correlation and test-retest analysis. The Cronbach α reliability analysis conducted on the NMAS revealed that the 'debilitating event' subscale had an internal consistency coefficient of 0.811, the 'bothersome event' subscale recorded a coefficient of 0.738 and the 'natural event' subscale demonstrated a coefficient of 0.765. The overall dependability of the analysis was determined to be 0.717 (Table 5). Given that the Cronbach's α internal consistency coefficient, deemed adequate for a measurement instrument, should approach 1, the internal consistency coefficients obtained for the total and all sub-dimensions of the NMAS were regarded as acceptable and indicative of good reliability ($p<.05$). The results indicated that the scale is homogeneous and appropriate for Turkish culture. In the Greek adaptation of the Menstrual Attitude Scale created by Brooks-Gunn and Ruble, the internal consistency coefficient for the 'debilitating event' subscale was 0.754, for the 'bothersome event' subscale it was 0.739 and for the 'natural event' subscale it was 0.754 (14). An examination of Cronbach's alpha was performed on the original scale to assess its reliability, revealing satisfactory internal consistency coefficients. Consequently, the Turkish adaptation of the scale resembles its original counterpart in this regard.

The research indicated that all item-total correlation coefficients surpassed the permissible threshold for item selection (i.e., ≥0.20), ranging from 0.259 to 0.588. Since no items possessed coefficients below 0.20, none were eliminated (27). The findings demonstrate that the scale items are effective and sufficient for assessing the targeted behavior (20).

The test-retest analysis of the study indicated that the correlation values from the second application, conducted 15 days after the original assessment with 33 individuals, varied from 0.210 to 0.565 and were deemed statistically significant (Table 6). This study validated that the NMAS is a dependable scale, exhibiting temporal invariance since the

level of agreement remained consistent during this period. A statistically significant link was identified between the replies obtained at two distinct time points.

Analysis of the NMAS mean scores revealed that the average total score was 45.05 ± 7.95. The 'debilitating event' subscale averaged 16.78 ± 4.39, the 'bothersome event' subscale averaged 16.93 ± 4.46 and the 'natural event' subscale averaged 11.33 ± 3.91. According to the ROC analysis, scores ranging from 14 to 25 were categorized as low level, those from 26 to 43 as medium level and those from 44 to 65 as high level. The subjects demonstrated a pronounced unfavorable attitude towards menstruation. Lawal et al. obtained mean scores of 13.85 ± 3.67 for the 'natural event' subscale, 36.96 ± 7.13 for the 'debilitating event' subscale and 18.92 ± 4.36 for the 'bothersome event' subscale (12). The original 15-item scale had a total mean score of 42±14.9, with the "Debilitating event" subscale averaging 13±5.9, the "Bothersome event" subscale averaging 19±5.5 and the "Natural event" subscale averaging 10±3.5. The overall mean score is comparable to the original. Women's attitudes towards menstruation are associated with menstrual discomfort, cycle duration, cultural variances and knowledge about menstruation (1,3,41) and unfavorable attitudes towards menstruation exacerbate anxiety and concern (4,12). Accordingly, it is advised that health practitioners organize training programs to cultivate good menstruation attitudes among women of reproductive age and ascertain coping skills. Achieving the specified outcomes can enhance the living conditions of women in the reproductive age demographic.

A 15-item, 3-dimensional scale was obtained by exploratory factor analysis (EFA). Exploratory Factor Analysis (EFA) indicated no items with factor loadings beneath 0.30. The precision of the acquired three-dimensional structure was assessed using CFA. The NMAS is a reliable and valid tool within Turkish culture, demonstrated by strong correlations in Cronbach's α internal consistency coefficient, item-total correlation and test-retest analysis.

The findings of this study offer a meaningful contribution to the menstrual health literature by providing evidence for the validity and reliability of a culturally adapted instrument that exclusively measures negative menstrual attitudes. Unlike comprehensive scales that address both positive and negative dimensions, the NMAS facilitates a focused assessment of stigmatizing perceptions, misconceptions and discomfort associated with menstruation. Such attitudes are closely linked to behavioral outcomes including limited menstrual hygiene practices, reduced health service utilization and emotional distress. The availability of a validated Turkish version of the NMAS enables researchers and clinicians to identify attitudinal patterns specific to young women in a university context, thereby supporting the development of culturally and demographically tailored interventions. Furthermore, the use of this tool in clinical and educational settings may inform preventive strategies aimed at reducing internalized stigma and enhancing menstrual well-being across the reproductive lifespan (9,10,16,17).

This study is subject to certain limitations. The sample is limited to university students aged 18 to 25, which may affect the generalisability of the findings to other populations, such as older women, adolescents or women with different educational and socioeconomic backgrounds. Menstrual attitudes are known to vary according to life stages and cultural contexts. Future studies should aim to validate the validity of NMAS in more diverse samples to ensure its broader application. Including women from rural areas, various age groups and those with different reproductive health experiences will provide deeper insights into the cultural nuances of negative menstrual attitudes.

5. CONCLUSIONS

A 3-dimensional 15-item scale was obtained using AFA. The AFA results demonstrated that no items exhibited factor loadings below 0.30. The DFA was utilised to assess the validity of the obtained 3-dimensional structure. The Cronbach's α internal consistency coefficient, item-total correlation and test-retest analysis of the scale demonstrated high correlations. The findings of this study demonstrate that the NMAS, developed to ensure validity and reliability, is a valid and reliable tool in the context of Turkish culture and the scale form is provided as an appendix.

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Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: ZÖ, SGS, YAD
Design of the study: ZÖ, SGS
Acquisition of data for the study: ZÖ, SGS
Analysis of data for the study: ZÖ, YAD
Interpretation of data for the study: ZÖ, YAD
Drafting the manuscript: ZÖ, SGS
Revising it critically for important intellectual content: YAD
Final approval of the version to be published: ZÖ, SGS, YAD

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Clinical and Experimental Health Sciences

Validity and Reliability Study of Postpartum Specific Anxiety Scale Short Form Into Turkish

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ABSTRACT

Objective: In this study, it was aimed to adapt the Short Form of Postpartum Specific Anxiety Scale (PSAS-SF) into Turkish.

Methods: The sample of the methodological study consisted of 300 pregnant women. The data of the study were collected between July and August 2022 by using the Personal Information Form, PSAS-SF, and State-Trait Anxiety Inventory. There are 12 items in the PSAS-SF. The scale has 4 sub-dimensions. These are; Maternal Competence and Attachment, Infant Safety and Welfare, Practical Infant Care, and Psychosocial Adjustment to Motherhood. The data were evaluated in the SPSS 26 program and the LISREL 9.1 program. Validity analysis of the data was performed by content validity index, exploratory factor analysis, and confirmatory factor analysis, and reliability analysis was performed by Cronbach's Alpha reliability coefficient. Ethics committee permission was obtained.

Results: The content validity index of the scale was found to be 0.80. In Kendall's W Agreement analysis, it was found that the opinions of the experts were compatible (p>.05). As a result of Exploratory Factor Analysis; KMO: 0.745, x2: 1184, 964, Df= 66 were found. As a result of Confirmatory Factor Analysis, x2: 221,164 (degrees of freedom: 48), x2/df: 3.608, and RMSEA: 0.079 were found, and the model indicated a good/excellent fit. Item factor loadings of the scale. 51-.84 and the effect of the scale items on the sub-dimension was found statistically significant (p<.05). The total Cronbach's alpha coefficient of the scale was found to be 0.79.

Conclusion: This study showed that the PSAS-SF is a valid and reliable measurement tool.

Keywords: Postpartum period, anxiety, nursing, reliability, and validity

1. INTRODUCTION

The postnatal period is a time when the mother returns to her pre-pregnancy state and undergoes intense physical, social, and psychological changes (1). During this time, family members and the mother may experience a range of emotions, from joy and excitement to stress and challenges as they adapt to the new situation (2).

Women may experience mental health issues when they struggle to adapt to the changes that occur during this period (3). Postnatal depression and anxiety are common mental health issues during this time. Postnatal anxiety is characterized by constant and excessive worry, increased fear and tension, and difficulty concentrating (4-5). The frequency of postpartum anxiety ranges from 2% to 45% (6).

Etiological factors identified for postpartum anxiety are socio-demographic characteristics, previous history of anxiety, unwanted pregnancy, inadequate prenatal care, complications during pregnancy or childbirth, insufficient social support, low self-esteem, and breastfeeding problems (7-10). Anxiety during pregnancy and postpartum is a problem that has multiple negative effects on the whole family, especially the newborn (11-12). Although DSM-5 includes standardized diagnostic criteria specific for postpartum depression, there are no diagnostic criteria for postpartum anxiety disorders. In recent years, there have been many studies about postpartum depression, while studies on postpartum anxiety have remained limited (13-14).

It is known that the limited number of postnatal anxiety studies, instruments assessing general anxiety were used, not instruments assessing anxiety specific to the postnatal period. [11] Since the psychological well-being of women during the postpartum period, which is one of the

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most vulnerable periods in terms of psychiatric diseases throughout women's lives, is not yet routinely evaluated in our country, many women with postpartum anxiety have to take care of themselves (13). This situation affects the whole family, especially the newborn has a multifaceted negative impact. High levels of anxiety, mother and infant attachment, as well as adversely affecting the baby's behavioral, social, and emotional development negatively (13).

Therefore, it is important to assess mothers' anxiety levels in the postnatal period. Due to the high number of items in the instruments used to measure the level of anxiety in the postnatal period in Turkey, the aim was to examine the validity and reliability of the Postpartum Special Anxiety Scale Short Form and to adapt it to Turkish.

2. METHODS

2.1. Type of Study

This study is a methodological study in which the validity and reliability of the Postpartum Special Anxiety Scale Short Form was examined.

2.2. Study Population and Sample

According to TÜİK (Turkish Statistical Institute) data for 2021, the total fertility rate in Turkey is 1.71, and the total fertility rate in Konya is 1.81. The reason for choosing the city where the sample was taken is that the fertility rate is above the country's average. The population of the study consisted of mothers who were within 0-6 months after birth and living in Konya. It is stated that 50 is very poor, 100 is poor, 200 is average, 300 is good, 500 is very good, and 1000 is excellent for the number of samples for exploratory factor analysis (15). To have a moderate sample size, 300 participants were included in the study. The study was completed with 300 participants.

2.3. Inclusion Criteria

- Volunteering to participate in the study
- Being within 0-6 months after birth
- No communication problems
- No psychiatric diagnosis (self-report)

2.4. Exclusion Criteria

- -Illiterate in Turkish
- -History of psychiatric illness (self-report)
- -Did not complete the questionnaire

2.5. Data Collection

Personal Information Form, Postpartum Special Anxiety Scale Short Form, and STAI (State-Trait Anxiety Inventory) were used to collect the data. Data collection for the study was online from women who were members of social media groups (Facebook, WhatsApp, Instagram) between July and August 2022 and met the inclusion criteria.

2.5.1. Personal Information Form; consists of 21 questions, including socio-demographic characteristics of women in the postnatal period (13, 16).

2.5.2. ***Postpartum Special Anxiety Scale Short Form (PSAS-SF);*** It is a 4-point Likert-type scale (Never, Not very often, often, almost always) developed by Sergio A. Silverio et al.2021 PSAS-SF measures the prevalence of postnatal anxiety in mothers with infants aged 0-6 months. Items 6,8,10, and 12 are reversed. The scale has 4 subdimensions. These are; Maternal Competence and Attachment (1,2,3), Infant Safety and Welfare (4,5,6), Practical Infant Care (7,8,9), and Psychosocial Adjustment to Motherhood (10,11,12). Since the scale reflects mothers' experiences in the last 7 (seven) days, it can be used to monitor changes in postnatal reactions and their frequency. Participants should take into account how they have felt in the last 7 (seven) days while answering the scale questions. The score obtained from the scale is between 12 and 48, and high scores indicate that mothers have high anxiety levels (16). In this study, Cronbach's Alpha reliability coefficient was found to be 0.798.

2.5.3. STAI (State-Trait Anxiety Inventory); The State-Trait Anxiety Inventory (STAI) is a self-report scale consisting of 40 items, developed by Spielberger et al. in 1970 and adapted to Turkish by Öner and Le Compte in 1983. Each subscale contains 20 items and is rated on a 4-point Likert scale. The scale is divided into two subscales: The STAI-1 measures current anxiety, while the STAI-2 measures general anxiety. All items are followed by a 4-point scale rating the frequency of feelings from 'not at all' to 'very much' and scoring from 1 to 4. The score ranges from 20 to 80 points, with higher scores indicating a higher level of anxiety. A score greater than 45 on the state-trait anxiety inventory (STAI), developed by Spielberger et al. in 1970, is classified as severe anxiety, while scores smaller than or equal to 45 are classified as mild anxiety (17-18). In this study, the mean STAI-I dimension score was 41.94±6.50 points. Cronbach's Alpha reliability coefficient was found to be 0.740. The mean STAI-II dimension score was 46.97±7.53 points. Cronbach's Alpha reliability coefficient was found to be 0.784.

2.6. Data Analysis

The statistical package program used for data analysis was IBM SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, New York, USA). Descriptive statistics were presented as n (number of units), % (percentage), Mean (mean), SD (standard deviation), M (median), min (minimum), and max (maximum) values. The normality of numerical variables was assessed using the Shapiro-Wilk normality test. The Independent Samples t-test was used to compare two groups with normally distributed variables. For variables with more than two categories, we used analysis of variance for comparisons. If the result of the Analysis of Variance was

significant, we conducted multiple comparisons using the Bonferroni test. We evaluated the relationships between numerical variables using the Pearson correlation coefficient. We considered p<.05 to be statistically significant. During the validity and reliability study phase of the PSAS-SF, we conducted analyses for language validity, content validity, construct validity, internal consistency, and item analysis.

2.7. Ethics

For the adaptation of the scale into Turkish, permission was obtained from Sergio A. Silverio via e-mail, and the original scale was requested. Ethics committee permission was obtained from KTO Karatay University Drug and Non-Medical Device Research Ethics Committee dated 17.06.2022 and decision number 2022/006. Women who met the criteria for participation in the study were informed about the study and consent was obtained. The research was conducted by the principles of the Helsinki Declaration.

2.8. Research Questions/Hypothesis

- Is the Postpartum Special Anxiety Scale Short Form (PSAS-SF) a valid measurement tool for the Turkish population?
- Is the Postpartum Special Anxiety Scale Short Form (PSAS-SF) a reliable measurement tool for the Turkish population?

3. RESULTS

When the sociodemographic characteristics of the participants were analyzed, all 300 participants were women who had given birth. The median age of the participants was 27 years. 98 (32.7%) of the participants were high school graduates, and 97 (32.3%) had a university education or higher. 65 (21.7%) were employed, and 66 (22%) were smokers. The place of residence was distributed as a provincial center for 267 (89%) and a district center for 33 (11%) participants.

The average age of the spouses of the participants was 31 years. Among the spouses of the participants, 110 (36.7%) were high school graduates, and 107 (35.7%) were university graduates or above. The spouses of 158 (52.7%) of the participants were employed as laborers. There were 237 (79%) people living as a nuclear family. There were 46 (15.3%) people with low income, 225 (75%) people with moderate income, and 29 (9.7%) high-income individuals. The median duration of marriage was 1 year. The families had 2 children. 212 (70.7%) women had planned pregnancies. There were 56 (18.7%) people who had health problems in their last pregnancy. There were 54 (18%) people who had problems during the last birth. Of the children born, 150 (50%) were girls and 150 (50%) were boys. 136 (45.3%) of the mothers had a normal delivery. There were 252 (84%) people who received information from health personnel during pregnancy, 252 (84%) people who received information from health personnel in the postnatal period, and 171 (57%) people who helped family members to care for the baby.

3.1. Validity Results

Language, content, and construct validity methods were used to ensure the validity of the scale. After the scale was translated into Turkish by the translation-back translation method, it was reviewed by the researchers, and the content validity was evaluated. Five (5) experts were consulted to determine the content validity index. According to the evaluations, the content validity index value was found to be 0.80. The KMO value used to evaluate the adequacy of the sample size was 0.745, and the result of the Barlett Sphericity Test used to evaluate the suitability of the scale for factor analysis was X2=1184.964 (p<.01). Exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were used to evaluate construct Validity (19). Firstly, EFA was conducted for factor analysis. As a result of the exploratory factor analysis performed on the final form of the scale, a structure with 4 subdimensions explaining 67.17% of the total variance was obtained in parallel with the original form.

The first sub-dimension explains 16.20% of the scale, the second subdimension explains 18.41% of the scale, the third subdimension explains 16.35% of the scale, and the fourth subdimension explains 16.21% of the scale. The factor loadings of the scale ranged between 0.51 and 0.84. As a result of the EFA conducted on the scale, the factor structure was confirmed by the CFA results. Accordingly, it can be said that the items have high factor loadings.

Figure 1 shows the path diagram of the CFA. Each of the path coefficients of the dimensions for 12 questions is statistically significant (p<.05). Accordingly, the maternal competence and attachment dimension consists of questions 1, 2, and 3, the infant safety and well-being dimension consists of questions 4, 5, and 6, the practical infant care dimension consists of questions 7, 8, and 9, and psychosocial adjustment to motherhood dimension consists of questions 10, 11 and 12. The effect of all questions on the subdimension was statistically significant (p<0.05).

Within the scope of CFA, χ2 /df, RMSEA, SRMR, IFI, TLI, CFI, and GFI were used to evaluate the factor validity of the models (Table 1). As a result of CFA, χ2/sd: 1184, 964; RMSEA: 0.079; SRMR: 0.077; IFI: 0.95; GFI: 0.89; TLI: 0.917; CFI: 0.948 were found. RMSEA is an index that is least affected by sample size. However, RMSEA fit index criteria are inconsistent in different literature. In this field, a cut-off value close to 0.06 or 0.08 is generally considered acceptable (15). IFI, TLI, CFI, and GFI fit indices exceeding 0.90 are considered evidence of adequate model fit. In this study, RMSEA≤0.05, IFI, TLI, CFI ≥0.90, and GFI≥0.85 were determined as acceptable (Table 1). The fit indices obtained as a result of CFA meet the acceptable fit index criteria (20-21).

Table 1. *Statistical values for the fit of the PSAS-SF model*

Measurement	Good Fit	Acceptable Fit	Model Fit Index Values
(χ^2/sd)	≤ 3	≤ 4-5	3.608**
RMSEA	≤ 0.05	0.06-0.08	0.079*
SRMR	≤ 0.05	0.06-0.08	0.077*
IFI	≥ 0.95	0.94-0.90	0.950*
CFI	≥ 0.95	0.94-0.90	0.948*
GFI	≥ 0.90	0.89-0.85	0.891*
TLI	≥ 0.95	0.94-0.90	0.917*

** Acceptable fit; ** good fit*

χ2/df, root mean square error of estimate (RMSEA), the goodness of fit index (GFI), standardized root mean square error (SRMR), comparative fit index (CFI), excess fit index (IFI), and Turker-Lewis index (TLI)

Correlation analyses were conducted to investigate the relationships between the PSAS and anxiety measures that are theoretically related (STAI-I-II). When Table 2 is analyzed, there is a statistically significant positive correlation between STAI-I and PSAS-SF infant safety and welfare dimension (r=0.240, p<.05). There is a statistically significant positive relationship between STAI-II and PSAS-SF and its subdimension (r=0.253, p<.05, Table 2).

Table 2. *Correlations between PSAS-SF and STAI-I and STAI-II.*

PSAS-SF	STAI-I	STAI-II
Maternal Competence and Attachment	*r*=-0.051 *p*=.376	***r*=0.140 *p*=.016**
Infant Safety and Welfare	***r*=0.240 *p*<.001**	***r*=0.146 *p*=.011**
Practical Infant Care	*r*=0.014 *p*=.813	***r*=0.208 *p*<.001**
Psychosocial Adjustment to Motherhood	*r*=-0.05 *p*=.393	***r*=0.237 *p*<.001**
PSAS-SF	*r*=0.079 *p*=.171	***r*=0.253 *p*<.001**

r: Pearson Correlation Coefficient; Bolded sections are statistically significant (p<.05).

0.00: no relationship; 0.01 – 0.29: low-level relationship; 0.30 – 0.70: moderate relationship; 0.71 – 0.99: high-level relationship; 1.00: perfect relationship.

3.2. Reliability Results

Reliability is the basic feature that every measurement tool should have. This feature determines that the data of the measurement tool is collected correctly and is repeatable (22). "Cronbach Alpha Correlation Analysis" and "Item Total Score Correlation" can be used to determine the reliability, independent inter-observer agreement, and internal consistency of measurement. Consistency is defined as the harmony between the results of observations and measurements repeated on the same people, under the same conditions, by the same observer. Cronbach's Alpha method, which is recommended for testing the reliability of Likert-type scales, is a measure of the internal consistency of the items in the measurement tool (23). Cronbach's alpha values of the scale and its sub-dimensions are given in Table 3. According to the table, the factor loadings of the questions in the maternal competence and attachment dimension vary between 0.611 and 0.841, the factor loadings of the questions in the infant safety and well-being dimension vary between 0.729 and 0.845, the factor loads of the questions in the practical infant care dimension vary between 0.720 and 0.771, and the factor loads of the questions in the psychosocial adjustment to motherhood dimension vary between 0.517 and 0.780. Since Cronbach's Alpha (α) was above 0.70, its reliability was considered sufficient.

Therefore, it can be said that the four dimensions of the postnatal anxiety scale measure the sub-features. According to these results, the questionnaire is a reliable measurement tool. An overall correlation above 0.20 indicates that the item is important for the question. According to the results obtained, the overall correlation values are between 0,287 and 0,631. The scale was retested 15 days later, with 30 individuals enrolled. As a result of the test-retest analysis, r = 0.835 was found, and the correlation between repeated measurements was determined to be highly significant (p<.001). According to these results, the adapted scale is a valid measurement tool (Table 3).

Table 3. *PSAS-SF validity and reliability results*

Factor	Item No	Factor Loadings				Total Correlation	Explained Variance %	Cronbach Alpha
		1	2	3	4			
Maternal Competence and Attachment	1				0.725	0.579	16.20	0.766
	2				0.841	0.298		
	3				0.611	0.631		
Infant Safety and Welfare	4	0.729				0.597	18.41	0.772
	5	0.818				0.428		
	6	0.845				0.462		
Practical Infant Care	7		0.753			0.382	16.35	0.691
	8		0.771			0.448		
	9		0.720			0.581		
Psychosocial Adjustment to Motherhood	10			0.779		0.287	16.21	0.617
	11			0.517		0.288		
	12			0.780		0.347		
Scale							67.17	0.798
KMO=0.745 *Df=66* χ^2=1184.964 *p*<.001								

KMO: Kaiser–Meyer–Olkin test; Df: Degree of Freedom

Table 4. *PSAS-SF descriptive statistics*

PSAS-SF and its Dimensions	*Mean±SD*	*M (min-max)*	Subdimension 1	Subdimension 2	Subdimension 3	Subdimension 4
Maternal Competence and Attachment	4.16±1.65	3 (3-12)	**1**			
Infant Safety and Welfare	6.39±2.38	6 (3-12)	***r*=0.382 *p*<.001**	**1**		
Practical Infant Care	5.31±2.04	5 (3-12)	***r*=0.424 *p*<.001**	***r*=0.375 *p*<.001**	**1**	
Psychosocial Adjustment to Motherhood	4.82±1.64	5 (3-12)	***r*=0.340 *p*<.001**	***r*=0.202 *p*<.001**	***r*=0.296 *p*<.001**	**1**
PSAS-SF	20.68±5.48	20 (12-48)	***r*=0.725 *p*<.001**	***r*=0.748 *p*<.001**	***r*=0.750 *p*<.001**	***r*=0.599 *p*<.001**

r: Pearson Correlation Coefficient; Summary statistics are given as mean ± standard deviation and Median (minimum, maximum) value. Bolded sections are statistically significant (p<.05).

All subdimensions had a highly statistically significant impact on the questions. Each of the path coefficients of maternal competence and attachment, infant safety and well-being, practical infant care, and psychosocial adjustment to motherhood on the postnatal anxiety scale is statistically significant (p<.05). The dimension with the highest effect was found to be the maternal competence and attachment dimension, while the lowest effect was found in the infant safety and well-being dimension. The mean score of the maternal competence and attachment dimension of the scale was calculated as 4.16±1.65 points. The mean total score of the scale was found to be 20.68±5.48 points. The total score of the scale was obtained by summing the number of questions and items 6, 8, 10, and 12 are reversed items. The lowest score on the scale was 12 points, and the highest score was 48 points. There was a statistically significant positive correlation between the PSAS-SF and its dimensions (p<.05).

4. DISCUSSION

The main purpose of this research is to introduce the PSAS to the Turkish community. The lack of a brief and easy-to-administer scale specifically tailored to the postpartum period, despite the availability of general anxiety measurement tools, played a role in determining the research topic. Therefore, this study focused on the validity and reliability of a short-form anxiety scale suitable for use during the postpartum period. Construct validity is the scientific expression of the accuracy of the information about the construct to be measured by the researchers (24). Factor analysis is one of the analyses used to evaluate construct validity. Two approaches, EFA and CFA, are generally used in the scale development or adaptation process (25).

Before factor analysis, it is necessary to evaluate whether the sample size is sufficient. In this study, the sample size was calculated at 300 individuals. This value shows that the sample size is sufficient (25-26). In addition, the adequacy of the determined sample for factor analysis is evaluated by KMO analysis. The KMO value varies between 0 and 1. However, for the application of factor analysis, there should be a relationship between “n” and the selected variables. The existence of this relationship is determined by Barlett’s Test of Sphericity. If p<.05 in Barlett’s Test of Sphericity, there is a significant relationship between the variables for factor analysis (27). In this study, the KMO value was 0,745> 0.70, and the Barlett sphericity test was significant (p<.01). As a result, it shows that the sample is sufficient and suitable for factor analysis.

As a result of the factor analysis of the scale, a structure with four factors explaining 67.17% of the total variance was obtained. In factor analysis, the factor load of the items should be higher than 0.30. In this study, it was determined that the factor load of each item on the scale was. It has been determined that the scale consists of four subdimensions. In the research, Chi-square/degree of freedom (χ2/sd), root mean square errors (RMSEA), goodness of fit index (GFI), CFI, adjusted index of fit (AGFI), Approximate, and standardized root mean errors (SRMR) were studied. As a result of DFA, it was determined as RMSEA: 0.079, χ2 /sd:3.608, GFI: 0.891, CFI: 0.948, and SRMR: 0.077. The fit indices obtained as a result of CFA were found to be sufficient.

The reliability of the scale was evaluated with Cronbach’s α coefficient, item-total score correlation, and test-retest methods. Cronbach’s α coefficient ranges from 0 to 1 (28). In this study, Cronbach’s coefficients of the scale and its subdimensions were calculated. The Cronbach α number of the scale was 0.798, and the Cronbach α number of the subdimensions was 0.76, 0.77, 0.69, and 0.61 respectively. With these results, it can be said that the scale has high reliability.

Item-total score correlation expresses the relationship between the scores of the scale items and the total score of the scale. Items with an item-total score correlation of 0.30 and higher distinguish well; items with 0.20–0.30 should be corrected, or the item can be included in the scale if deemed necessary; and items lower than 0.20 should be removed from the Scale (29-30).

The relationship between the total scale score and the items on the scale ranged from 0.35 to 0.85. It was determined that there was a strong and significant relationship between scale total score and scale items. Therefore, it was determined that the items should be included in the scale. The test-retest method expresses the stability of the scale. In this method,

the reliability of the same measurement tool is evaluated by applying it to the same individuals twice. If the results of the two applications are the same, it is assumed that the scale is reliable. The accepted criterion for the reliability coefficient is that it is above 0.80 (24). In this study, r=0.835 was found as a result of the test-retest (p<.001). When the scale was re-applied to individuals after 15 days, it gave similar results. In this direction, the invariance of the scale concerning time has been proven.

The results of this study show that the PSAS-SF is a valid and reliable measurement tool in the Turkish population. The four-factor structure obtained in the study is largely compatible with the original scale. Confirmatory factor analysis (CFA) results showed good fit indices and were calculated as $\chi 2/df$= 3.608, RMSEA= 0.079, SRMR= 0.077, CFI= 0.948, GFI= 0.891, TLI= 0.917 and IFI= 0.95. Although the total variance explained was found to be 67.17%, which is slightly lower than the 75% value in the original study, it is seen that the scale preserved its general factor structure. This suggests that some items may be perceived differently in the Turkish cultural context. The test-retest reliability was found to be high (r= 0.835), similar to the original study, supporting the scale as a stable measurement tool. In addition, the scale was found to show significant correlations with the STAI scale and its convergent validity was found to be strong. However, the fact that additional scales such as EPDS and Parenting Sense of Competence Scale (PSoC) used in the original study were not included in this study can be considered as a limitation. Sampling differences should also be taken into consideration; the present study included mothers who were 0-6 months postpartum, whereas the original study evaluated mothers who were 0-12 weeks postpartum. This situation requires taking into account the change in postpartum anxiety over time. The long version of the PSAS scale has been previously adapted into Turkish.

However, in this study, the short form was adapted considering the ease of use. In conclusion, the Turkish version of the PSAS-SF seems to be an appropriate tool for measuring postpartum anxiety, but it is recommended that the validity of the scale be tested with larger and more diverse samples.

5. CONCLUSION

The results of the language, content, and construct validity and reliability analyses of the PSAS-SF were proven to have acceptable measurement value results. The scale is a valid and reliable measurement tool to determine the anxiety levels of women in the postnatal period. It is thought that this study may contribute to future research. Since no practical and validated measurement tool can be used specifically for the postpartum period in our country, it will contribute significantly to the literature.

A limitation of this study is that the data were obtained based on the participants' self-reports, and observation was not possible. The possibility that the participants may have given the answers expected within the framework of social norms for various reasons, such as the situation they were in while answering the questions, time, etc., may have caused bias. Despite these limitations, according to the results obtained from the PSAS-SF, it can be used as a measurement tool to evaluate the anxiety levels of women in the postpartum period due to the high validity and reliability of the measurement values.

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Clinical and Experimental Health Sciences

Comparative Molecular Docking Analysis of Carbapenem Antibiotics With NDM-1 and OXA-23 B-Lactamases: Insights Into Binding Mechanisms

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ABSTRACT

Objective: This study aimed to investigate the molecular interactions between carbapenem antibiotics (imipenem and meropenem) and two β-lactamase enzymes, NDM-1 (class B) and OXA-23 (class D), to understand the structural basis of carbapenem resistance.

Methods: The study employed molecular docking methods using the crystal structures and homology models of the NDM-1 and OXA-23 enzymes to analyze binding affinities and interaction patterns.

Results: The results revealed distinct binding characteristics for each enzyme. NDM-1 exhibited a polar, hydrophobic active site, with imipenem forming hydrogen bonds with ASP124, ASN220, and LYS211, while meropenem relied on weaker interactions like Van der Waals forces. In contrast, OXA-23 featured a hydrophobic cavity with charged residues, where imipenem bonded with ARG259 and meropenem interacted with SER79, TRP219, and ARG259. A key finding was the significantly lower binding energy for the OXA-23-imipenem complex in the crystal structure (-8.06 kcal/mol) compared to its homology model (-5.34 kcal/mol), highlighting the importance of accurate structural representation. The study demonstrated strong agreement between the computational and experimental data.

Conclusion: These findings provide critical insights into the structural basis of carbapenem resistance, offering potential avenues for designing inhibitors to combat β-lactamase-mediated antibiotic resistance. The integrated approach validates the utility of computational methods in elucidating molecular interactions and guiding therapeutic strategies.

Keywords: NDM-1, OXA-23, imipenem, meropenem, molecular docking

1. INTRODUCION

Antibiotics are among the most essential therapeutic agents used in the treatment of infectious diseases, playing a pivotal role in modern medicine (1). Among these, carbapenems stand out as powerful β-lactam antibiotics, effectively combating a broad spectrum of bacterial pathogens. They function by inhibiting bacterial cell wall synthesis through the irreversible binding to penicillin-binding proteins (PBPs). This binding disrupts the critical process of peptidoglycan cross-linking, which is vital for maintaining the structural integrity of bacterial cell walls (2).

However, the widespread and often inappropriate use of antibiotics has precipitated an alarming rise in antibiotic resistance, significantly undermining the efficacy of these vital medications. Antibiotic resistance has escalated into a global health crisis, characterized by the emergence of bacteria that have developed sophisticated mechanisms to circumvent the effects of antibiotics. One prominent defense mechanism involves the production of β-lactamases—enzymes that specifically inactivate β-lactam antibiotics, thereby diminishing their therapeutic effectiveness (1).

β-lactamases are categorized into two major groups based on their enzymatic mechanisms: serine β-lactamases (which encompass Classes A, C, and D) and metallo-β-lactamases (Class B). Serine β-lactamases utilize a pivotal catalytic serine residue located in their active site to facilitate the hydrolysis of β-lactam antibiotics. In contrast, metallo-β-lactamases rely on zinc ions for their hydrolytic activity, which creates a different enzymatic environment for the inactivation of these antibiotics (3).

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The role of zinc ions extends beyond merely assisting in the enzymatic process; they are also crucial for the recognition and binding of various β-lactam antibiotics. Additionally, specific amino acid residues within the active site of β-lactamases contribute significantly to substrate binding and catalysis, influencing the overall activity and specificity of these enzymes (4). Understanding the intricate mechanisms by which β-lactamases operate is essential for developing strategies to combat antibiotic resistance and enhance the effectiveness of existing antibiotics (5).

Among clinical isolates, New Delhi metallo-β-lactamase (NDM) has emerged as the most prevalent and concerning variant of metallo-β-lactamase enzymes. NDM was first identified in 2009 in a male patient who had recently traveled to New Delhi, India, highlighting the global connectivity that can facilitate the spread of antibiotic resistance (6). The identification of the NDM-1 variant, along with its numerous genetic variants, has sparked significant alarm within the medical community due to its remarkable ability to inactivate nearly all β-lactam antibiotics, with the notable exception of aztreonam. This extensive resistance renders infections caused by NDM-producing bacteria particularly challenging to treat and often leads to severe, life-threatening complications, necessitating the urgent need for alternative therapeutic strategies (7).

In the realm of β-lactamases, Class D enzymes are particularly noteworthy, with over 250 distinct variants documented to date. These enzymes exhibit considerable structural diversity, which is closely linked to their ability to confer broad-spectrum antibiotic resistance across various bacterial species. Among these, some Class D β-lactamases are capable of hydrolyzing carbapenems—powerful antibiotics often used as a last resort—earning them the designation of carbapenem-hydrolyzing class D β-lactamases (CHDLs). One of the prominent members of this group is OXA-23, characterized by a distinct structural framework that comprises two α-helices and six β-sheets. Initially observed in Scotland, OXA-23 has since shown a widespread geographical distribution, raising concerns about its presence in various healthcare settings worldwide (8-9).

In particular, during an outbreak investigation at Kocaeli University Hospital in Turkey, a significant number of blaOXA-23-positive Acinetobacter baumannii isolates were identified. This finding underscores the clinical relevance of OXA-23 in the context of nosocomial infections and the urgent need for ongoing surveillance and research to understand the mechanisms of resistance and to develop effective countermeasures against such formidable pathogens (10).

Molecular docking studies have emerged as a crucial methodology in modern scientific research, particularly in the fields of biochemistry and pharmacology. This computational technique allows researchers to simulate and analyze the interactions between chemical compounds and biologically relevant macromolecules, such as proteins or nucleic acids, within three-dimensional structures that have been resolved using techniques like X-ray crystallography or NMR spectroscopy (11). By accurately positioning potential drug candidates in these target sites, molecular docking facilitates the prediction of various binding modes, interaction energies, and the resulting molecular conformations of both the ligand and its target (11,12).

The significance of docking methods is particularly pronounced in drug discovery, where they serve as powerful tools for identifying novel ligands that can modulate specific biological targets effectively. This predictive capability not only accelerates the early-stage screening of compounds but also enhances the likelihood of discovering therapeutic agents that might not be readily apparent through traditional experimental methods (11). Furthermore, docking studies provide substantial advantages in terms of time efficiency and resource conservation; they allow for preliminary assessments of compound efficacy and specificity without the need for extensive laboratory work, thus streamlining the drug development process and reducing costs associated with experimental screening (12).

In this study, molecular interactions between β-lactam antibiotics, specifically imipenem and meropenem, and both the crystallographic structure and the homology model of two important β-lactamases, New Delhi Metallo-β-lactamase-1 (NDM-1) and Oxacillinase-23 (OXA-23), were explored in this study. Advanced molecular docking techniques were utilized to analyze the binding affinities of these antibiotics to the enzyme targets, with a focus on the various interaction patterns that emerge during the binding process. By systematically comparing the docking results, insights were provided into the structural mechanisms underlying antibiotic resistance facilitated by these enzymes. This investigation not only enhances our understanding of the biochemical basis for resistance but also aids in the development of more effective therapeutic strategies against β-lactam-resistant bacterial infections.

2. METHODS

The crystal structures of NDM-1 (PDB ID: 5YPL) and OXA-23 (PDB ID: 4JF5) were retrieved from the Protein Data Bank database. Using these template structures, homology models of OXA-23 and NDM-1 were generated using Swiss-Model (13-17). Model validation was performed using ERRAT (18), VERIFY3D (19,20), PROCHECK (21,22), and Swiss-Model's built-in quality assessment tools (13-17).

The ligand preparation of meropenem and imipenem in pdbqt format was conducted using MGLTools (23). Molecular docking were performed with AutoDock4.2 (24), targeting the known binding sites of both the homology models and crystal structures. The hydrolyzed forms of meropenem and imipenem were used in this study. The binding site coordinates were defined as (-7.925, 39.154, 11.045) for OXA-23 and (2.250, 7.779, 24.903) for NDM-1.

The docking protocol utilized an advanced Genetic Search Algorithm, which was specifically designed to assess the conformational relationships between potential binding partners. This was complemented by the Lamarckian Genetic Algorithm, which played a crucial role in determining the optimal binding pose for each complex. As a result of this

Table 1. *Validation Metrics of NDM-1 and OXA-23 Homology Models*

	QMEANDisCo Global		MolProbity Score		Clash Score		Ramachandrana Favoured (%)		ERRAT		VERIFY (%)	
	Crys. Strc.	Hom. Strc.	Crys. Strc.	Hom. Strc.	Crys. Strc.	Hom. Strc.	Crys. Strc.	Hom. Strc.	Crys. Strc.	Hom. Strc.	Crys. Strc.	Hom. Strc.
NDM-1	0.94	0.93	1.06	0.63	2.68	0.28	99.12	97.91	97.1963	91.5929	91.70	97.10
OXA-23	0.87	0.89	1.11	0.96	0.96	0.00	98.33	96.27	90.411	94.3723	86.36	93.00

NDM-1; New Delhi metallo-β-lactamase-1, OXA-23; Oxacillinase-23

Table 2. *Comparative binding analysis of carbapenems with NDM-1 and OXA-23 β-lactamases*

System	Binding Energy (kcal/mol)		Key Interacting Residues	
	Homology	Experimental	Homology Model	Experimental Structure
NDM-1/Imipenem	-4.26	-3.82	ASN220, ASP124, LYS211	ASN220, GLN123, LYS211
NDM-1/Meropenem	-6.19	-5.74	LYS211, ASN220, ASP124	LYS211, ASN220, ASP124
OXA-23/Imipenem	-5.34	-8.06	ARG259	THR217, ARG259, VAL128
OXA-23/Meropenem	-6.57	-7.59	ARG259, TRP219, SER79	LYS82, LYS216, ARG259,

NDM-1; New Delhi metallo-β-lactamase-1, OXA-23; Oxacillinase-23

comprehensive approach, a total of 10 distinct binding models were generated for each complex analyzed.

To visualize and analyze these generated models, PyMol and Schrödinger Maestro were employed, both of which are powerful tools in molecular visualization (25-26). The analysis process involved creating two-dimensional interaction diagrams in Schrödinger Maestro. These diagrams were constructed based on carefully defined parameters, which included a maximum hydrogen bond distance of 2.8 Å to ensure relevant interactions were captured, a minimum donor angle of 120.0° to optimize the spatial orientation of hydrogen bond donors, and a minimum acceptor angle of 90.0° to maintain effective interaction geometry. This structured approach facilitated a thorough evaluation of the binding interactions within the complexes, providing insight into their potential biological implications.

3. RESULTS

The quality of the generated homology models for NDM-1 and OXA-23 was rigorously assessed using a combination of validation tools, and the results are summarized in Table 1.

In this study, an in-depth examination of the molecular interactions between imipenem and meropenem with two distinct classes of β-lactamases: the class D OXA-23 and the class B NDM-1 were conducted. Utilizing both crystallographic structures and homology models of these enzymes, molecular docking to elucidate the binding characteristics of these antibiotics was performed . A comparative analysis was performed between the docking poses generated in this work and the native ligand conformations observed in the relevant crystal structures retrieved from the Protein Data Bank (PDB) (Table 2).

4.DISCUSSION

The models demonstrated a high degree of reliability, as evidenced by their scores across all metrics. The ERRAT scores for NDM-1 and OXA-23, respectively, indicate a high-quality model with a significant percentage of the protein structure falling within the 91% rejection limit. Similarly, the VERIFY3D analysis showed that over 80% of the residues for both models had an averaged 3D-1D score of >0.2, confirming that the models' 3D structures are consistent with their amino acid sequences (Table 1).

Further validation by PROCHECK confirmed the stereochemical quality of the models. The Ramachandran plots revealed that residues for the NDM-1 model and for the OXA-23 model were located in the most favored regions, with no residues in the disallowed regions. This favorable residue distribution highlights the stereochemical accuracy of the generated models. The built-in quality assessment tools from Swiss-Model also supported these findings, with high Global Model Quality Estimation (GMQE) scores of and for NDM-1 and OXA-23, respectively. Collectively, these validation results confirm that the generated homology models are of high quality and reliable for subsequent analyses.

The extensive binding energy calculations to quantify the strength of the interactions were carried out. Our comparative analysis revealed that while the binding energies of imipenem and meropenem showed only minimal differences across the various systems studied, a significant anomaly in the binding profile of the OXA-23-imipenem complex was identified. Notably, the binding energy derived from the crystal structure of OXA-23 was markedly lower at – 8.06 kcal/mol compared to the binding energy of the homology model, which registered at – 5.34 kcal/mol (Table 2). This discrepancy implies that the binding affinity of imipenem to the OXA-23 enzyme is notably enhanced in the experimentally determined crystal structure, highlighting the importance of accurate structural representation in predicting molecular interactions.

To enable comparison, the interactions of imipenem and meropenem with both a homology model and the crystal structure of NDM-1 were analyzed using 2D diagrams,

alongside the interaction diagram of the native complex from the Protein Data Bank (PDB).

Two-dimensional structure of the NDM-1 (homology model) – Imipenem complex, highlighting the intricate molecular interactions that imipenem forms within the active site of the NDM-1 enzyme. In this analysis, it was found that imipenem establishes critical hydrogen bonds with the residues located at positions ASP124, ASN220, and LYS211 in the NDM-1 sequence, which play a pivotal role in stabilizing the drug-enzyme interaction (Figure 1a). Similar interaction poses were also observed in the molecular docking study performed using the experimental structure of NDM-1. Furthermore, analysis of the crystal structure from the Protein Data Bank (PDB) revealed that this binding mode is largely conserved (Figure 1c). Beyond these primary interactions, additional amino acids surrounding the active site are predicted to engage in weaker interactions, further contributing to the overall binding stability. Notably, the interacting residues collectively create a negatively charged polar hydrophobic binding pocket, which is essential for accommodating the imipenem molecule. In the context of NDM-1 (New Delhi metallo-beta-lactamase-1), Lysine 211 (Lys211) is a crucial amino acid residue that plays a significant role in the enzyme's catalytic activity and broad substrate specificity. The NDM-1 enzyme relies on zinc ions in its active site to hydrolyze and inactivate beta-lactam antibiotics (27).

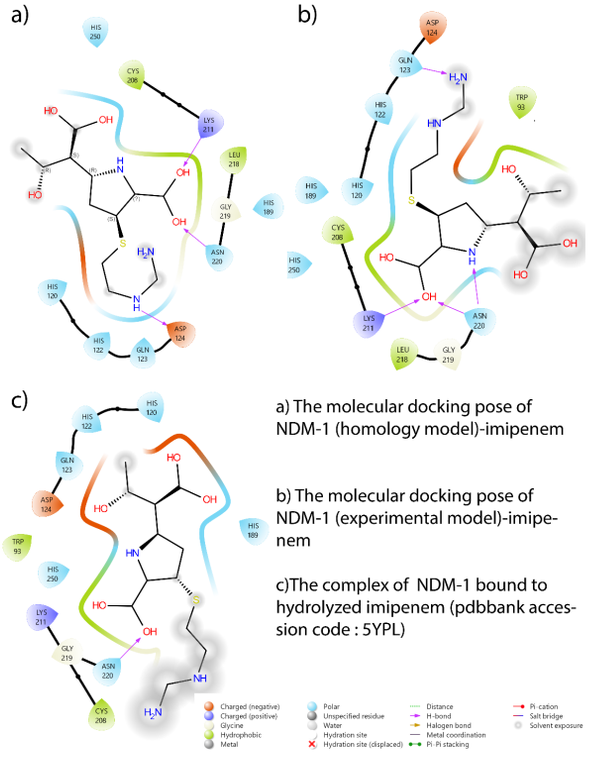

Figure 1. *Two-dimensional schematic representation of protein-ligand binding modes a) NDM1 (homology model)-Imipenem, b) NDM1 (experimental model)-Imipenem, c) The complex of NDM1-Imipenem (pdb code: 5YPL)*

The structural representation of the NDM-1 (homology model) – Meropenem complex, along with an analysis of the interaction profile of meropenem. Unlike imipenem, meropenem does not form direct hydrogen bonds with any of the NDM-1 residues; however, the presence of weak molecular interactions—including Van der Waals forces and hydrophobic contacts—significantly contributes to the stabilization of the complex. Furthermore, analysis of the post-docking pose in the experimental model revealed a hydrogen bond with Lys211 (Figure 2b). Upon examining the structure of the NDM-1-meropenem complex from the Protein Data Bank (PDB), it was observed that while the overall binding cavity was consistent with previous findings, meropenem formed hydrogen bonds with residues Asp124, Asn220, and Lys211, based on established hydrogen bonding criteria (Figure 2c). The binding site for meropenem mirrors that of the imipenem complex, characterized by a negatively charged polar hydrophobic pocket that is critical for binding efficacy.

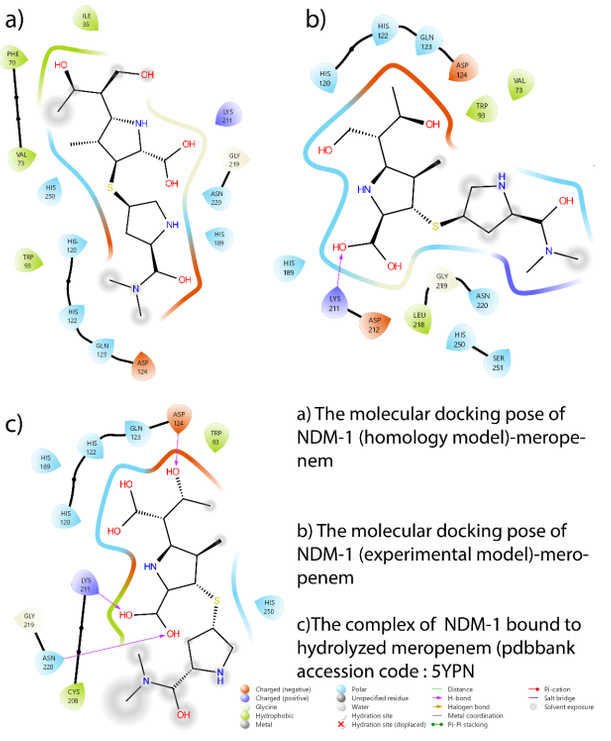

Figure 2. *Two-dimensional schematic representation of protein-ligand binding modes a) NDM1 (homology model)-Meropenem, b) NDM1 (experimental model)-Meropenem, c) The complex of NDM1-Meropenem (pdb code: 5YPN)*

The two-dimensional interaction map of the OXA-23 (homology model) – Imipenem complex was shown. Here, imipenem establishes a direct hydrogen bond with the residue located at ARG259 in the OXA-23 enzyme, which is crucial for effective binding (Figure 3a). In addition to this strong interaction, other amino acids within the proximity of the binding site support the interaction through weaker forces. Based on the 2D analysis of the post-docking results from the experimental model, imipenem formed hydrogen bonds with Val128, Thr217, and Arg259. For the control

group structure, meropenem was observed to form hydrogen bonds with Ser79 and Arg259 (Figure 3b-c). The interaction site of OXA-23 is formed as a polar hydrophobic binding cavity, optimizing the fit of the imipenem molecule.

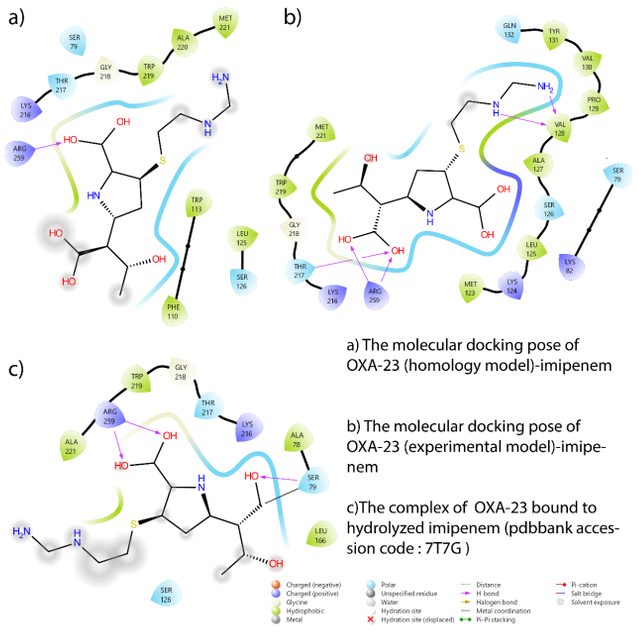

Figure 3. *Two-dimensional schematic representation of protein-ligand binding modes a) OXA-23 (homology model) – Imipenem, b) OXA-23 (experimental model) – Imipenem, c) The complex of OXA-23 – Imipenem (pdb code: 7T7G)*

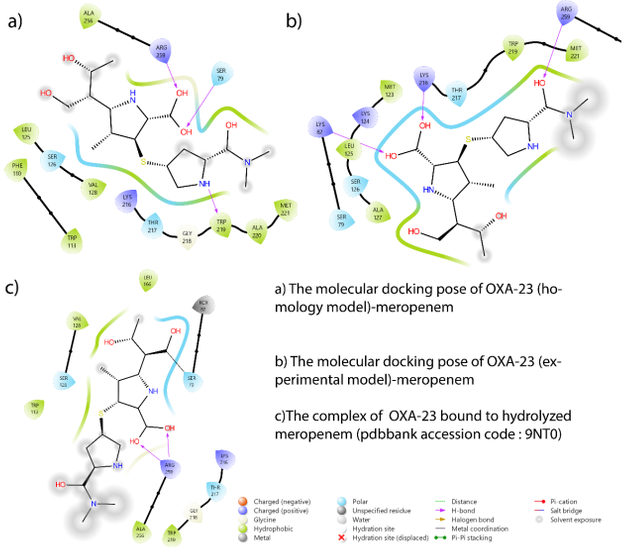

Figure 4. *Two-dimensional schematic representation of protein-ligand binding modes a) OXA-23 (homology model) – Meropenem, b) OXA-23 (experimental model) – Meropenem, c) The complex of OXA-23 – Meropenem (pdb code: 7T7G)*

Finally, Figure 4 provides a detailed structural analysis of the OXA-23/Meropenem complex. In this complex (homology model), meropenem forms significant hydrogen bonds with the residues SER79, TRP219, and ARG259 in the OXA-23 enzyme, which not only stabilizes the binding but also enhances the overall affinity of the complex (Figure 4a). Weak interactions from surrounding amino acids further augment this binding process. Docking analysis of meropenem to the experimental model revealed hydrogen bond interactions with Lys82, Lys216, and Arg259. Conversely, the control group complex exhibited a single hydrogen bond interaction, exclusively with Arg259 (Figure 4b-c). The binding site of OXA-23 is also organized as a polar hydrophobic pocket, showcasing structural similarities to the NDM-1 complex and illustrating the strategic nature of these interactions in antibiotic binding and efficacy. Arg259 is a fundamental part of the OXA-23 active site, playing a direct role in substrate binding and orientation. Its interactions are essential for the enzyme's carbapenemase activity, making it a critical residue to consider in the design of new inhibitors aimed at overcoming antibiotic resistance (28).

5. CONCLUSION

This study conducted a comprehensive comparative analysis of computational and experimental models to examine the binding interactions between carbapenem antibiotics (imipenem and meropenem) and β-lactamase enzymes (NDM-1 and OXA-23). Molecular docking studies revealed distinct binding characteristics for each enzyme. In the case of NDM-1, the binding pocket was found to consist of polar, hydrophobic, and partially charged residues, forming a well-defined active site. Imipenem demonstrated specific hydrogen bonding interactions with ASP124, ASN220 and LYS211 residues, while additional weaker interactions contributed to complex stabilization. Notably, the computational models showed strong agreement with experimental data, confirming a consistent binding mode. For OXA-23, structural analysis identified a predominantly hydrophobic binding cavity containing charged residues. While imipenem formed a hydrogen bond with ARG259, meropenem exhibited more extensive interactions with SER79, TRP219 and ARG259. Both computational and experimental approaches confirmed similar binding geometries for the antibiotics in OXA-23, suggesting a conserved recognition mechanism. The comparative analysis highlighted key differences between the enzymes, with NDM-1 displaying a more polar character in its binding site compared to the more hydrophobic nature of OXA-23's active site. Importantly, both enzymes maintained consistent binding architectures across different analytical methods, with minor variations reflecting their distinct catalytic requirements. These findings provide valuable structural insights that could inform the development of new β-lactamase inhibitors and strategies to overcome carbapenem resistance. The strong correlation between computational predictions and experimental observations validates the integrated approach used in this study for investigating antibiotic-enzyme interactions at the molecular level. Despite these valuable insights, this study is limited by its reliance on a purely computational model, which may not fully capture the dynamic complexity of biological systems.

Therefore, future work will focus on validating these findings through *in vitro* and *in vivo* experiments to further our understanding of the binding mechanisms and to inform the rational design of new inhibitors.

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Clinical and Experimental Health Sciences

Assessment of Dietary Behaviors and Influencing Factors Among Patients with Heart Failure: A Cross-Sectional Study

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ABSTRACT

Objective: This study was conducted to determine the nutritional behaviours and related factors in individuals with heart failure (HF).

Methods: The study was descriptive in nature and was conducted with a sample of 178 individuals over 18 years of age and diagnosed with HF. Data were collected using the Patient Information Form and the Scale for Dietary Behaviors in Heart Failure (SDBHF)

Results: It was found that SDBHF total scores of the participants differed according to variables such as comorbidity, hypertension, smoking duration, special diet application status, reason for skipping lunch and snack consumption status ($p<.05$). In addition, it was determined that the scores of the Healthy Habits Subscale of the SDBHF differed according to the snack consumption status of the participants ($p<.05$). It was found that the Salt Restriction Subscale scores differed according to variables such as hypertension and special diet application status ($p<.05$). It was determined that the Sugar Restriction subscale scores differed according to variables such as gender, employment status, presence of diabetes, smoking duration, alcohol consumption status and special diet application status ($p<.05$). Prevention of Fluid Retention subscale scores were found to differ according to variables such as gender, marital status, educational status, employment status, income level, smoking duration, chewing difficulty after memory, and presence of constipation after diagnosis ($p<.05$).

Conclusion: The results showed that the nutritional status of HF patients is in accordance with the current guidelines.

Keywords: Diet, heart failure, nutrition

1. INTRODUCTION

Heart failure (HF) is a condition in which the heart fails to pump enough blood to meet the needs of the tissues as a result of a structural and functional disorder of the heart. This syndrome, which has high mortality and hospitalisation rates worldwide, causes a great increase in healthcare expenditures (1). While 1-2% of the adult population in developed countries have a diagnosis of HF, it is known that the number of patients diagnosed with HF worldwide is approximately 64.3 million (2). The prevalence of heart failure is gradually increasing with the aging of societies (3).

Adequate and balanced nutrition plays an important role in preventing or alleviating the severity of symptoms of heart failure. In addition, nutritional behaviours are also highly determinant in preventing comorbidity or reducing the severity of diseases (3). Therefore, determination of patients' nutritional behaviours and related factors is very important in terms of developing nutritional treatment strategies (4).

Regulation of dietary habits is very important in HF management. The main aim of nutritional recommendations for patients with HF is to prevent further strain by reducing the workload of the heart (5). Noncompliance with diet worsens HF symptoms and increases rehospitalisation (6). Therefore, monitoring the nutritional behaviour of HF patients is very important in reducing the health and economic burden (7).

Studies investigating the relationship between heart failure and nutrition focus on the effect of sodium-restricted diets (8) and nutrient deficiencies observed in patients with HF (9). However, these studies largely emphasize physiological aspects such as electrolyte balance and do not sufficiently address the broader concept of patients' overall nutritional behaviours, including food choices, meal patterns, and lifestyle-related influences. Studies examining the factors affecting the nutritional behaviours of patients and determining the frequently encountered nutritional problems are very limited

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(3,10). As a result, there is a clear gap in the literature regarding a holistic understanding of dietary behaviour in individuals with HF, particularly from a behavioural and psychosocial perspective. In this study, it was aimed to determine the nutritional behaviours and related factors of individuals diagnosed with heart failure. By focusing on both behavioural patterns and associated socio-demographic variables, this study intends to contribute a broader perspective to the literature and inform future dietary interventions tailored to HF patients.

2. METHODS

2.1. Ethical Principles

The informed consent form was read to the volunteers by the researcher, their written consent was obtained and they were included in the study. The informed consent form included explanations that the participants could leave the study at any time, that their answers to the questions would be kept confidential and would not be shared with anyone, that participation in the study was completely voluntary and that there was no obligation to participate. Written permission for use was obtained from the author who developed the scale used in the study. Ethical approval was obtained from the Non-Interventional Scientific Research Ethics Committee of Ordu University and institutional approval was obtained from Giresun Provincial Health Directorate.

2.2. Participants

The population of the study consisted of all patients who were hospitalised in the cardiology clinic of Giresun Training and Research Hospital between January 2024 and October 2024 and diagnosed with HF. In the descriptive and correlational study, the data collection form was applied face-to-face. Based on power analysis, a minimum sample size of 138 was considered sufficient and 178 people participated in the study. Individuals who were older than 18 years of age, diagnosed with HF at least six months ago, and had no communication problems were included in the study, while individuals who requested to withdraw from the study for any reason after participating in the study were excluded from the study.

Patients were recruited during their inpatient stay in the cardiology clinic, based on their willingness to participate and eligibility according to inclusion criteria. No randomisation was performed, as the study did not involve any intervention. Therefore, all patients who met the criteria and agreed to participate were included consecutively. Although refusal rates were not systematically recorded, there were no reports of active withdrawal once participation had commenced. This non-randomised, single-center recruitment approach may pose a risk of selection bias and limit the representativeness of the sample to the broader heart failure population.

2.3. Collection of Data

Data were collected in face-to-face interviews using the Patient Information Form and Scale for Dietary Behaviors in Heart Failure. To minimize interviewer bias and ensure consistency, all face-to-face interviews were conducted by two nurses with experience in patient communication, both of whom were employed at the hospital where the study was conducted. A standardized interview protocol was followed throughout the data collection process to promote uniformity in the administration of questionnaires. Interviewers were instructed to avoid leading questions and to maintain a neutral tone and body language during all interactions. Although face-to-face interviews allowed for clarification of items and enhanced participant understanding, the potential for social desirability bias cannot be entirely ruled out and is acknowledged as a limitation of the study.

2.3.1. Patient Introduction Form: In the first part of the patient introduction form, which was created by the researchers in line with the literature (5,6,9), there are 9 questions (age, gender, marital status, educational status, occupation, income status, presence of comorbidity, smoking status, alcohol use status) to define demographic and clinical characteristics. In the second part of the form, there are questions evaluating nutrition and dietary habits. The questionnaire was developed by one of the researchers, who holds expertise in nutrition and dietetics, based on a comprehensive review of relevant literature in the fields of cardiology and nutrition. Although no formal expert panel was convened, the content was structured in alignment with evidence-based sources.

2.3.2. Scale for Dietary Behaviors in Heart Failure (SDBHF): The scale developed by Boy and Enç consists of 4 sub-dimensions (healthy habits, salt restriction, sugar restriction, and prevention of fluid retention) and 19 items (11). The "'Healthy Habits"' sub-dimension of the scale consists of items 4, 5, 11, 12, 16; "'Salt Restriction"' sub-dimension: items 1, 3, 7, 9, 10, 15; "'Sugar Restriction"' sub-dimension: items 6, 17, 19; "'Prevention of Fluid Retention"' sub-dimension: items 2, 8, 13, 14 and 18. Items 3, 6, 7, 9, 10, 15, 19 should be reverse coded. The lowest score of 19 points and the highest score of 76 points can be obtained from the scale. A score of 46 and above on the scale indicates that HF patients exhibit nutritional behaviours in accordance with the guidelines, whereas a score of 45 and below indicates that HF patients do not eat in accordance with the nutritional recommendations of the guidelines. In the Turkish adaptation of the scale, Cronbach's alpha value was found to be 0.72 (11). In this study, it was determined as 0.58.

2.4. Statistical Evaluation

IBM SPSS Statistics 22 (IBM SPSS, Turkey) programme was used for statistical analysis of the findings obtained in the study. The normality of the data was assessed using the Kolmogorov-Smirnov test, and all variables were found to be normally distributed ($p>.05$). Therefore, parametric tests were applied. In addition to descriptive statistical methods (mean, standard deviation, frequency), Independent Sample T Test was used for comparisons between two groups and One-Way Anova test was used for comparisons between more than two groups. Pearson correlation analysis was conducted to examine the relationships

between the SDBHF total and subscale scores and selected independent variables. For statistically significant results (p<.05), effect sizes were calculated using Cohen's *d* for t-tests and eta squared (η^2) for ANOVA, in order to determine the magnitude of the differences. Significance was accepted as p<.05.

3. RESULTS

The mean total SDBHF scale score of individuals with chronic heart failure was 45.92±6.18. The subscale scores of the scale were determined as healthy habits subscale 12.20±2.38, salt restriction 16.76±3.97, sugar restriction 8.70±2.03, and prevention of fluid retention 8.24±1.77, respectively. Given that the cut-off point of the SDBHF is 46, this average score suggests that more than half of the participants did not adhere to dietary recommendations for heart failure management. The individuals included in the study were predominantly over 75 years of age (38.76%), male (63.5%) and married (72.5%). It was determined that 50.6% of the participants were primary school graduates, 47.8% were retired, and 71.3% of them had an income equivalent to their expenses. Significant differences in dietary behaviours were observed according to gender, marital status, educational level, income status, smoking and alcohol use, and presence of comorbidities (p<.05). It was determined that the sugar restriction subscale score of the participants differed according to gender and employment status and these differences were statistically significant (p<.05). In addition, it was determined that the prevention of fluid retention subscale scores showed statistically significant differences according to gender, marital status, educational status and income level (p<.05) (Table 1).

Table 1. *Sociodemographic characteristics and SDBHF scale (n=178)*

Sociodemographic Characteristics		SDBHF Total Score	Healthy Habits Subscale Score	Salt Restriction Subscale Score	Sugar Restriction Subscale Score	Prevent Retention Subscale Score
	(n,%)	X±SD	X±SD	X±SD	X±SD	X±SD
All participants		45.92±6.18	12.20±2.38	16.76±3.97	8.70±2.03	8.24±1.77
Age						
18-39	1 (0.56)	48.00	13.00	14.00	10.00	11.00
40-64	51 (28.65)	45.11±6.20	11.90±2.51	16.13±3.66	8.49±2.12	8.58±1.60
65-74	57 (32.02)	45.94±6.57	12.08±2.03	17.10±4.75	8.50±2.20	8.24±1.94
≥75	69 (38.76)	46.46±5.90	12.52±2.55	16.98±3.48	9.01±1.81	7.94±1.68
		p=.685[b]	p=.517[b]	p=.500[b]	p=.380[b]	p=.094[b]
Gender						
Female	65 (36.5)	45.96±5.92	12.23±2.69	16.83±3.16	9.18±1.86	7.72±1.65
Male	113 (63.5)	45.89±6.35	12.19±2.19	16.72±4.39	8.43±2.08	8.53±1.77
		p=.938[a]	p=.923[a]	p=.866[a]	**p=.017[a*] d=.027**	**p=.003[a**] d=.050**
Marital Status						
Married	129 (72.5)	46.24±6.10	12.21±2.40	16.76±4.08	8.79±2.05	8.46±1.76
Single	49 (27.5)	45.06±6.37	12.18±2.36	16.75±3.71	8.46±2.00	7.65±1.65
		p=.254[a]	p=.934[a]	p=.985[a]	p=.337[a]	**p=.006[a**] d=0.042**
Education level						
Literate	39 (21.9)	45.15±4.86	11.79±2.24	17.05±3.48	8.82±1.99	7.48±1.80
Primary school	90 (50.6)	45.72±6.86	12.35±2.46	16.42±4.33	8.77±2.13	8.16±1.62
Secondary school	28 (15.7)	46.28±5.53	12.10±2.60	16.57±3.09	8.78±2.00	8.82±1.67
High school	18 (10.1)	48.61±6.18	12.38±2.09	18.83±3.85	8.00±1.78	9.38±1.91
University	3 (1.7)	42.33±1.15	13.00±1.73	12.66±2.08	8.66±1.52	8.00±1.00
		p=.268[b]	p=.740[b]	p=.059[b]	p=.660[b]	**p=.001[b**] η^2=0.101**
Employment Status						
Unemployed	60 (33.7)	46.66±5.62	12.36±2.74	17.31±3.26	9.28±1.77	7.70±1.73
Employed	33 (18.5)	44.81±5.88	11.96±2.31	15.51±3.73	8.21±1.88	9.12±1.76
Retired	85 (47.8)	45.82±6.66	12.18±2.14	16.85±4.43	8.49±2.19	8.28±1.67
		p=.381[b]	p=.742[b]	p=.107[b]	**p=.021[b*] η^2=0.062**	**p=.001[b**] η^2=0.078**
Income						
Income < Expenses	40 (22.5)	45.45±6.89	12.10±2.72	17.05±3.57	8.70±2.25	7.60±2.04
Income = Expenses	127 (71.3)	45.97±6.09	12.26±2.33	16.55±4.09	8.78±1.97	8.37±1.56
Income > Expenses	11 (6.2)	47.00±4.71	11.90±1.57	18.18±3.94	7.81±1.88	9.09±2.34
		p=.752[b]	p=.847[b]	p=.376[b]	p=.320[b]	**p=.014[b*] η^2=0.048**

*a: Independent – Samples T Test, b: One-Way ANOVA, *p<.05; **p<.01; ***p<.001, SDBHF: The Scale for Dietary Behaviors in Heart Failure*
Effect sizes were calculated using Cohen's d for comparisons between two groups, and eta squared (η^2) for comparisons involving more than two groups

It was determined that the majority of the individuals in the study had comorbidities (84.8%), the most common comorbidity was hypertension (73.1%), and they did not smoke (71.9%) or drink alcohol (88.8%). It was found that SDBHF total score showed a significant difference according to the presence of comorbidity, hypertension and smoking duration (p<.05). Salt restriction subscale scores were found to differ according to the presence of hypertension (p<.05). It was determined that sugar restriction subscale scores differed statistically significantly according to the presence of diabetes, smoking duration and alcohol use (p<.05). In addition, statistically significant differences were found between prevention of fluid retention subscale scores according to smoking duration (p<.05) (Table 2).

Table 2. *Clinical characteristics and SDBHF scale (n=178)*

Clinical Characteristics		SDBHF Total Score	Healthy Habits Subscale Score	Salt Restriction Subscale Score	Sugar Restriction Subscale Score	Prevent Retention Subscale Score
Comorbidity	**(n,%)**	**X±SD**	**X±SD**	**X±SD**	**X±SD**	**X±SD**
Yes	151 (84.8)	46.41±6.11	12.29±2.41	17.00±4.04	8.82±1.95	8.29±1.79
No	27 (15.2)	43.18±5.95	11.70±2.18	15.44±3.32	8.07±2.38	7.96±1.62
		p=.012[a*] d=0.001	p=.234[a]	p=.061[a]	p=.079[a]	p=.376[a]
Hypertension						
Yes	130 (73.1)	46.74±6.20	12.36±2.45	17.18±4.00	8.85±1.91	8.33±1.76
No	48 (26.9)	43.68±5.60	11.77±2.13	15.62±3.69	8.31±2.30	7.97±1.76
		p=.003[a] d=0.003**	p=.138[a]	**p=.020[a*] d=0.015**	p=.151[a]	p=.230[a]
Diabetes Mellitus						
Yes	75 (42.1)	46.46±5.41	12.25±2.32	16.33±3.50	9.54±1.73	8.33±1.66
No	103	45.52±6.69	12.17±2.43	17.07±4.27	8.09±2.03	8.17±1.84
		p=.369[a]	p=.829[a]	p=.218[a]	**p<.001[a***] d=0.002**	p=.557[a]
Stroke						
Yes	6 (3.4)	44.16±2.40	11.16±1.94	15.50±2.50	9.16±2.13	8.33±1.36
No	172 (96.6)	45.98±6.27	12.24±2.39	16.80±4.01	8.69±2.03	8.23±1.78
		p=.477[a]	p=.278[a]	p=.430[a]	p=.576[a]	p=.898[a]
COPD						
Yes	22 (12.4)	45.22±5.99	12.09±1.97	17.00±3.36	8.40±1.89	7.72±1.63
No	156 (87.6)	46.01±6.22	12.22±2.44	16.73±4.06	8.75±2.05	8.31±1.78
		p=.553[a]	p=.807[a]	p=.767[a]	p=.464[a]	p=.146[a]
Chronic Renal Failure						
Yes	7 (3.9)	46.57±3.73	11.85±0.89	18.71±3.49	8.57±1.90	7.42±2.29
No	171 (96.1)	45.89±6.27	12.22±2.42	16.68±3.98	8.71±2.04	8.27±1.74
		p=.800[a]	p=.368[a]	p=.186[a]	p=.857[a]	p=.216[a]
Smoking						
Yes	50 (28.1)	44.96±5.91	11.88±2.53	16.02±3.44	8.48±1.96	8.58±1.60
No	128 (71.9)	46.29±6.27	12.33±2.31	17.05±4.14	8.79±2.06	8.10±1.81
		p=.196[a]	p=.252[a]	p=.119[a]	p=.353[a]	p=.111[a]
Years of smoking						
1-10 years	8 (4.5)	45.42±4.50	12.28±3.03	17.71±2.28	9.71±1.38	9.57±1.39
11-30 years	21 (11.8)	45.76±5.84	12.00±2.44	15.38±3.69	8.66±2.37	8.19±2.11
31-70 years	21 (11.8)	43.28±5.78	11.95±2.29	16.04±4.28	7.47±2.20	7.61±1.46
		p=.011[b*] η^2=0.045	p=.952[b]	p=.382[b]	**p=.049[b*] η^2=0.051**	**p=.049[b*] η^2=0.005**
Alcohol consumption						
Yes	20 (11.2)	44.65±6.91	11.45±2.21	17.20±6.73	7.65±1.95	8.35±2.13
No	158 (88.8)	46.08±6.09	12.30±2.39	16.70±3.50	8.84±2.01	8.22±1.72
		p=.331[a]	p=.132[a]	p=.752[a]	**p=.013[a*] d=0.002**	p=.772[a]

*a: Independent – Samples T Test, b: One-Way ANOVA, *p<.05; **p<.01; ***p<.001, SDBHF: The Scale for Dietary Behaviors in Heart Failure, COPD: Chronic Obstructive Pulmonary Disease*

Effect sizes were calculated using Cohen's d for comparisons between two groups, and eta squared (η^2) for comparisons involving more than two groups

Table 3. *Nutrition, dietary habits and SDBHF scale (n=178)*

Clinical Characteristics		SDBHF Total Score	Healthy Habits Subscale Score	Salt Restriction Subscale Score	Sugar Restriction Subscale Score	Prevent Retention Subscale Score
	(n,%)	X±SD	X±SD	X±SD	X±SD	X±SD
Special diet application situation						
Yes	44 (24.7)	49.27±6.58	12.60±2.79	19.05±4.96	9.17±1.95	8.45±2.35
No	134 (75.3)	44.97±5.76	12.09±2.36	16.10±3.39	8.57±2.03	8.20±1.56
		p<.001[a***] **d=0.005**	p=.243[a]	**p<.001**[a***] **d=0.014**	p=.100[a]	p=.445[a]
Commonly applied diet types (n=44)						
Diabetic diet	8 (18.2)	46.14±5.72	11.71±2.81	15.00±2.94	11.42±0.53	8.00±2.38
Salt-free diet	19 (43.2)	50.50±7.78	13.27±3.00	20.77±6.19	8.50±1.79	7.94±2.26
Salt-free diabetic diet	13 (29.6)	49.16±5.14	12.50±2.61	18.33±2.22	9.16±1.89	9.16±1.99
Low fat salt free diet	2 (4.5)	49.00±8.48	10.50±2.12	22.50±0.70	7.00±0.00	9.00±5.65
Weight loss diet	2 (4.5)	51.00	12.00	18.00	10.00	11.00
		p=.704[b]	p=.585[b]	p=.078[b]	**p=.003**[b**] **η^2=0.017**	p=.498[b]
Daily meal frequency?						
1 meal	1 (0.6)	43.00	14.00	14.00	7.00	8.00
2 meals	59 (33.1)	44.61±5.86	12.00±2.48	16.00±3.21	9.00±2.01	7.91±1.76
3 meals	118 (66.3)	46.60±6.27	12.29±2.33	17.16±4.27	8.72±2.05	8.40±1.76
		p=.116[b]	p=.558[b]	p=.143[b]	p=.701[b]	p=.218[b]
Skipped meal (n=60)						
Breakfast	18 (30.0)	44.16±5.31	12.00±2.40	15.55±2.95	8.44±2.14	8.16±1.20
Lunch	38 (63.3)	45.13±6.12	12.07±2.58	16.42±3.11	8.89±1.91	7.73±1.94
Dinner	4 (6.7)	41.25±4.99	11.75±2.36	13.50±4.50	7.50±2.38	8.50±2.08
		p=.426[b]	p=.967[b]	p=.180[b]	p=.365[b]	p=.553[b]
Breakfast skipping reason (n=18)						
Loss of appetite	6 (33.4)	45.00±7.48	13.16±3.43	15.50±4.03	8.33±1.63	8.00±1.54
Not used to it	8 (44.5)	43.25±4.65	11.25±1.83	16.25±2.43	8.00±2.39	7.75±0.88
Not ready	1 (5.5)	49.00	13.00	17.00	10.00	9.00
Time constraints	2 (11.1)	42.00±1.41	11.00±0.00	13.50±2.12	8.00±2.82	9.50±0.70
Other	1 (5.5)	46.00	12.00	13.00	12.00	9.00
		p=.838[b]	p=.657[b]	p=.713[b]	p=.488[b]	p=.367[b]
Lunch skipping reason (n=38)						
Loss of appetite	9 (23.6)	49.00±7.82	13.33±3.16	17.77±3.23	9.44±1.42	8.44±2.18
Not used to it	22 (57.9)	43.77±4.40	11.45±2.08	16.13±3.16	8.63±1.98	7.54±1.76
Not ready	2 (5.3)	37.00±5.65	11.00±2.82	14.50±0.70	6.00±1.41	5.50±0.70
Time constraints	3 (7.9)	45.00±2.00	12.33±1.15	14.33±2.51	10.33±1.15	8.00±2.64
Other	2 (5.3)	57.00	18.00	20.00	9.00	10.00
		p=.012[b*] **η^2=0.040**	p=.052[b]	p=.275[b]	p=.106[b]	p=.257[b]
Dinner skipping reason (n=4)						
Loss of appetite	1 (25.0)	46.00	12.00	17.00	9.00	8.00
Not used to it	2 (50.0)	37.00±1.41	12.50±3.53	10.50±4.94	5.50±0.70	8.50±3.53
Other	1 (25.0)	45.0	10.00	16.00	10.00	9.00
		p=.164[b]	p=.864[b]	p=.634[b]	p=.171[b]	p=.981[b]
Snack consumption						
Yes	144 (80.9)	46.56±6.36	12.54±2.30	16.95±4.19	8.81±2.03	8.25±1.79
No	34 (19.1)	43.17±4.48	10.79±2.21	15.94±2.77	8.26±2.03	8.17±1.69
		p=.001[a**] **d=0.018**	**p<.001**[a***] **d=0.084**	p=.180[a]	p=.159a	p=.812[a]
Snack time (n=144)						
Mid-morning	1 (0.7)	46.00	15.00	15.00	8.00	8.00
Afternoon	104 (72.2)	46.35±6.06	12.53±2.34	16.65±3.64	8.91±1.99	8.25±1.73
Night	39 (27.1)	47.15±7.23	12.48±2.22	17.82±5.39	8.56±2.14	8.28±1.97
		p=.799[b]	p=.563[b]	p=.301[b]	p=.610[b]	p=.985[b]

Table 3. *(Continued)*

Clinical Characteristics		SDBHF Total Score	Healthy Habits Subscale Score	Salt Restriction Subscale Score	Sugar Restriction Subscale Score	Prevent Retention Subscale Score
	(n,%)	X±SD	X±SD	X±SD	X±SD	X±SD
Appetite change after HF diagnosis?						
No	107 (60.1)	45.84±6.08	12.06±2.15	16.83±4.20	8.55±2.19	8.39±1.81
Increase	1 (0.6)	40.00	14.00	9.00	10.00	7.00
Decrease	70 (39.3)	46.12±6.38	12.40±2.69	16.77±3.53	8.92±1.77	8.02±1.70
		p=.605[b]	p=.498[b]	p=.146[b]	p=.398[b]	p=.321[b]
Body weight change after HF diagnosis?						
Yes	27 (15.2)	46.07±5.51	12.74±2.33	16.33±3.28	9.11±1.92	7.88±1.50
No	112 (62.9)	45.25±6.42	11.81±2.27	16.67±4.40	8.46±2.07	8.30±1.92
Uncertain	39 (21.9)	47.71±5.68	12.97±2.51	17.30±3.01	9.12±1.94	8.30±1.47
		p=.100[b]	**p=.014[b*] η^2=0.048**	p=.580[b]	p=.115[b]	p=.534[b]
Frequent chewing difficulty after HF diagnosis?						
Yes	7 (3.9)	44.28±4.02	12.85±1.77	15.85±2.79	8.14±1.46	7.42±0.53
No	171 (96.1)	45.98±6.25	12.18±2.40	16.80±4.01	8.73±2.05	8.27±1.79
		p=.477[a]	p=.464[a]	p=.540[a]	p=.456[a]	**p=.004[a**] d=0.009**
Frequent swallowing difficulty after HF diagnosis?						
Yes	4 (2.2)	41.50±2.64	12.50±2.65	13.50±30.00	8.00±0.81	7.50±1.00
No	174 (97.8)	46.02±6.21	12.20±2.38	16.83±3.97	8.72±2.05	8.25±1.78
		p=.149[a]	p=.805[a]	p=.097[a]	p=.174[a]	p=.398[a]
Frequent indigestion/bloating after HF diagnosis?						
Yes	71 (39.9)	45.77±6.79	12.29±2.40	16.83±5.14	8.52±1.99	8.12±1.98
No	107 (60.1)	46.01±5.78	12.14±2.37	16.71±2.98	8.83±2.06	8.31±1.61
		p=.797[a]	p=.690[a]	p=.855[a]	p=.321[a]	p=.482[a]
Frequent diarrhea after HF diagnosis?						
Yes	15 (8.4)	47.06±6.04	13.40±2.41	16.80±4.12	8.93±2.05	7.93±1.83
No	163 (91.6)	45.81±6.20	12.09±2.35	16.76±3.97	8.68±2.04	8.26±1.76
		p=.455[a]	**p=.043[a*] d=0.023**	p=.971[a]	p=.656[a]	p=.483[a]
Frequent constipation after HF diagnosis?						
Yes	69 (38.8)	45.18±5.75	11.88±2.34	16.69±3.52	8.76±2.11	7.84±1.67
No	109 (61.2)	46.38±6.42	12.41±2.39	16.80±4.25	8.66±1.99	8.49±1.78
		p=.210[a]	p=.150[a]	p=.856[a]	p=.755[a]	**p=.016[a*] d=0.033**

*a: Independent – Samples T Test, b: One-Way ANOVA, *p<.05; **p<.01; ***p<.001, SDBHF: The Scale for Dietary Behaviors in Heart Failure, HF: Heart Failure Effect sizes were calculated using Cohen's d for comparisons between two groups, and eta squared (η^2) for comparisons involving more than two groups*

It was determined that the majority of the participants did not follow a special diet (75.3%), and those who followed a diet mostly followed a salt-free diet (43.2%). It was determined that 66.3% of the participants consumed 3 main meals a day, 63.3% of those who skipped main meals skipped lunch, and the most common reason for skipping meals for all main meals was that the food was not ready (44.5%). It was determined that 80.9% of the participants consumed snacks and the most snacks were consumed in the afternoon (72.2%). It was determined that 60.1% of the participants diagnosed with heart failure had no change in appetite, 62.9% experienced a change in body weight, and most of the participants did not experience chewing (96.1%) and swallowing (97.8%), digestion (60.1%), diarrhoea (91.6%), and constipation (61.2%) problems (Table 3). It was determined that the total SDBHF scores of the participants differed according to variables such as the status of applying a special diet, the reason for skipping lunch, and the status of consuming snacks, and these differences were statistically significant (p<.05). A significant difference was found between the healthy habits subscale scores of individuals diagnosed with HF according to variables such as the status of consuming snacks, the change in body weight after being diagnosed with HF, and diarrhoea problems frequently experienced after being diagnosed with HF (p<.05). It was determined that the salt restriction subscale scores of the participants showed a significant difference according to the status of applying a special diet (p<.05). It was found that the

sugar restriction subscale scores of the participants differed significantly according to the types of diet they commonly followed (p<.05). It was found that there was a statistically significant difference in the prevention of fluid retention subscale scores according to the status of experiencing chewing problems and constipation frequently after being diagnosed with HF (p<.05) (Table 3).

Only statistically significant correlations between the SDBHF total and subscale scores and selected independent variables are presented in Table 4. No significant associations were observed with the remaining variables (p>.05). A significant correlation was found between the SDBHF total score and gender, education level, income status, and the presence of frequent constipation after heart failure diagnosis. For the Healthy Habits Subscale, significant correlations emerged with snack consumption and the presence of frequent diarrhea after diagnosis. Regarding the Sugar Restriction Subscale, statistically significant associations were identified with gender, education level, presence of comorbidity, hypertension, adherence to a special diet, and frequent constipation. Additionally, the Prevent Retention Subscale scores showed significant correlations with gender, marital status, education level, and income status (p<.05). These findings indicate that demographic and clinical characteristics may influence specific dimensions of dietary behaviors in patients with heart failure (Table 4).

Although statistically significant differences were observed between dietary behaviours and various demographic and clinical variables, an evaluation of the effect sizes presented in the tables indicates that most of these differences are of small or limited clinical significance. The majority of effect sizes were in the small range (η^2<.06; Cohen's d<0.5), with only a few approaching a moderate level. This suggests that while the findings are statistically significant, their practical or clinical impact may be limited.

Table 4. *Correlation between SDBHF total and subscale scores and selected independent variables (n=178)*

Variables		SDBHF Total Score	Healthy Habits Subscale Score	Salt Restriction Subscale Score	Sugar Restriction Subscale Score	Prevent Retention Subscale Score
Gender	r	0.148	-0.007	0.030	0.165	0.223
	p	**.049***	.923	.691	**.028***	**.003****
Marital Status	r	-0.141	-0.006	-0.036	-0.132	-0.205
	p	.061	.934	.629	.079	**.006****
Education level	r	0.206	0.067	0.040	0.154	0.285
	p	**.006***	.372	.595	**.040***	**<.001*****
Income	r	0.163	0.005	0.071	0.124	0.219
	p	**.030***	.950	.347	.099	**.003****
Comorbidity	r	0.037	-0.090	0.066	0.179	-0.067
	p	.621	.234	.385	**.017***	.376
Hypertension	r	0.054	-0.092	0.122	0.175	-0.112
	p	.484	.238	.115	**.023***	.150
Special diet application situation	r	0.073	-0.089	0.117	0.182	-0.058
	p	.333	.243	.121	**.016***	.445
Snack consumption	r	-0.135	-0.289	0.037	-0.052	-0.018
	p	.072	**<.001*****	.626	.488	.812
Frequent diarrhea after HF diagnosis?	r	-0.012	-0.152	0.026	0.070	0.053
	p	.874	**.043***	.730	.352	.483
Frequent constipation after HF diagnosis?	r	0.180	0.108	0.022	0.157	0.181
	p	**.016***	.150	.768	**.036***	.016

*Pearson correlation analysis; *p<.05; **p<.01; ***p<.001; SDBHF: The Scale for Dietary Behaviors in Heart Failure, HF: Heart Failure*

4. DISCUSSION

Although nutrition is a determining factor in the development of heart failure, it also has a significant effect on the course of the disease. It has been reported that HF patients who are fed in accordance with the guidelines have reduced disease symptoms and improved quality of life (12,13,14). In this study, the SDBHF score of the participants was found to be 45.92. This finding shows that the nutritional behaviours of the participants were not compatible with the recommendations of guidelines and expert academicians. Educational level, cultural factors and economic conditions are among the reasons for this situation. This situation related to nutrition may make it difficult to control the symptoms of the disease and negatively affect the quality of life. What makes this finding particularly noteworthy is that, to our knowledge, this is one of the few studies in Türkiye to quantitatively assess dietary behaviour among hospitalized

HF patients using a validated scale. By numerically identifying this gap in dietary behaviours, our findings highlight a specific target for clinical intervention—namely, improving adherence to evidence-based dietary recommendations through structured education. These results suggest that nutrition education may have positive effects on disease symptoms and quality of life in patients with HF. Individuals who are fed in accordance with the guidelines may manage the disease better (15). Therefore, future studies examining the effects of dietary behaviours in accordance with the guidelines on the disease in more detail should be planned.

HF patients constitute an important risk group due to high mortality risk, high healthcare costs and comorbidities associated with unfavourable clinical outcomes. The presence of concomitant chronic diseases constitutes an important problem in HF management. It has been reported that a significant proportion of HF patients have at least two chronic diseases (16). The most common complications accompanying heart failure are obesity, diabetes and hypertension, followed by COPD and renal dysfunction (17). In this study, in accordance with the literature, it was concluded that the participants had similar comorbidities, especially hypertension and diabetes mellitus frequently accompany patients with HF. Accordingly, it is possible to say that hypertension and diabetes should be considered as priority targets in the management of HF. Controlling hypertension and diabetes with appropriate nutritional therapy may slow down or prevent the progression of HF (18). As reported in the literature, the prevalence of these two comorbidities suggests that nutrition programmes specific for these diseases may play an important role in the treatment of HF (18,19,20). In addition, COPD and renal dysfunction were found to be highly prevalent in our study. This finding suggests that individualised diet plans prepared by considering pulmonary and renal dysfunctions in HF patients may positively affect the course of the disease. This study also provides important contributions to the limited literature on how comorbidities such as diabetes and hypertension influence dietary behaviours in patients with heart failure. Contrary to the common assumption that comorbidities may negatively impact self-care behaviours, patients diagnosed with hypertension and diabetes were found to have significantly higher SDBHF total and subscale scores. In particular, those with hypertension had higher total scores and greater adherence to salt restriction, while those with diabetes had higher scores on the sugar restriction subscale. These findings suggest that the presence of certain comorbid conditions—such as hypertension and diabetes—may actually motivate individuals to adopt more conscious and guideline-consistent dietary behaviours. To the best of our knowledge, these associations have not been previously demonstrated using the SDBHF scale. In this respect, the study offers a novel perspective to the literature by emphasizing the need for individualized dietary interventions not only for patients with heart failure alone but also for those with coexisting hypertension and diabetes.

Smoking, which is considered to be an important risk factor in the occurrence of HF and worsening of the disease prognosis, not only damages the cardiovascular system but also indirectly causes progression of HF by negatively affecting nutritional habits and food metabolism (21). The fact that smoking decreases taste and smell senses and appetite increases the risk of malnutrition in HF patients. In addition, smoking may adversely affect the absorption of nutrients and cause micronutrient deficiencies, especially antioxidant vitamins (vitamin C and E), folate and magnesium. Deficiencies in these nutrients lead to increased inflammation and oxidative stress in HF patients, resulting in a poor prognosis. In addition, the fact that smoking promotes a tendency to consume processed and salty foods may lead to sodium and fluid imbalance in HF patients (22,23). The lower SDBHF scores of smokers compared with nonsmokers in this study support this finding. In addition, the fact that the scale scores of the longest-time smokers were the lowest is similar to the literature. Although the difference in dietary behaviour scale scores between smokers and non-smokers was not statistically significant, smokers generally had lower scores. However, when smoking duration was taken into account, the negative impact on dietary behaviours became more pronounced. As the duration of smoking increased, a significant decrease was observed in the total SDBHF scores (η^2=0.045), as well as in the Sugar Restriction (η^2=0.051) and Prevent Retention (η^2=0.005) subscale scores. These findings suggest that prolonged exposure to smoking may adversely affect adherence to dietary behaviours, particularly regarding sugar intake and fluid/sodium management. Notably, a moderate effect size was observed for sugar restriction behaviours (η^2=0.051), which may have clinical relevance. Although the effect sizes for the other subscales were small, the differences were still statistically significant. To the best of our knowledge, this is the first study to demonstrate these associations using the SDBHF scale, offering a novel perspective on how smoking duration may weaken dietary self-management in patients with HF.

The relationship between nutrition and alcohol consumption in patients with heart failure is of great importance for the management of the disease. A balanced diet supports general health by providing the vitamins and minerals required by the body. However, excessive alcohol consumption may disrupt this balance (24). Alcohol may prevent the absorption of nutrients in the body and lead to deficiency of some vitamins (especially B vitamins) (25). Furthermore, alcohol consumption may worsen heart failure by damaging the heart muscle and increase blood pressure (26). Therefore, it is recommended that individuals with heart failure limit or completely avoid alcohol consumption. Healthy eating habits and control of alcohol consumption may improve the quality of life of these patients and slow the progression of the disease. In this study, the low alcohol consumption in HF patients may reflect the awareness of patients or healthcare professionals that alcohol can worsen heart failure. Since the adverse effects of alcohol are known, these individuals may be avoiding alcohol consumption.

Healthy eating habits and low alcohol consumption may improve the quality of life of HF patients and slow disease progression. This study is among the few that quantitatively examine the relationship between alcohol consumption and specific dietary behaviours in patients with heart failure. Our findings showed that participants who consumed alcohol had lower total scores on the dietary behaviour scale, as well as lower scores on the Healthy Habits subscale compared to non-drinkers, although these differences were not statistically significant. Nevertheless, the overall trend in the data suggests that alcohol consumption may negatively influence dietary behaviour. In contrast, a statistically significant difference was observed in the Sugar Restriction subscale, where alcohol consumers scored significantly lower ($p<.05$; $\eta^2=0.002$). Although the effect size was small, this finding may still be clinically relevant from a behavioural standpoint. These results support the notion that alcohol consumption may impact not only physiological outcomes but also self-care behaviours such as adherence to dietary recommendations. Moreover, the fact that the analysis was based on the presence or absence of alcohol consumption, rather than its duration or quantity, is methodologically noteworthy. In this respect, the study contributes new insights to the literature by highlighting the need to consider alcohol-related behavioural patterns in HF patients and underscores the value of incorporating this factor into future intervention-based research.

In this study, it was determined that the frequency of a special diet was low in patients with HF. In the literature, it has been reported that dietary therapy plays a very important role in improving the disease prognosis and reducing complications in individuals with heart disease (1,3,10,12,14). Especially salt-free diet has been reported to control symptoms by decreasing fluid retention in patients with heart failure (1,10). Similarly, positive effects of diabetic diet on blood glucose regulation and the role of low-fat diet in the management of dyslipidaemia have been reported (27). However, in this study, low rates of implementation of these diets were observed. This suggests that dietary recommendations are not sufficiently adopted by patients or not sufficiently emphasised by healthcare professionals. It is thought that this may be due to individual, cultural or socioeconomic factors affecting diet compliance of patients. In addition, lack of dietary education and difficulties in implementing the diet in daily life may also be contributing factors. This lack of dietary adherence may be attributed to multiple factors, including insufficient awareness of dietary recommendations, inadequate nutritional counselling, low health literacy, and limited access to dietitian services within the healthcare system. Socioeconomic barriers such as financial constraints and limited educational background may also hinder patients' ability to implement structured dietary practices. Clinically, poor adherence to recommended dietary guidelines in heart failure patients can contribute to fluid retention, uncontrolled symptoms, increased hospitalization rates, and diminished quality of life. Therefore, routine nutritional screening and individualized dietary counselling should be integrated into heart failure management programs. Building on these findings, our study adds novel evidence regarding the behavioural impact of following a special diet among HF patients. Participants who reported adhering to a special diet had significantly higher total SDBHF scores, as well as higher scores in the Salt Restriction subscale ($p<.05$). Although the differences were not statistically significant in other subscales—namely Healthy Habits, Sugar Restriction, and Prevent Retention—their scores were also consistently higher, indicating a positive behavioural trend. These results suggest that adherence to a special diet may be associated not only with salt reduction, which is frequently emphasized in HF management, but also with broader improvements in dietary behaviour. While the effect sizes were small ($d=0.005$ for total score; $d=0.014$ for salt restriction), they nonetheless reinforce the potential value of structured dietary planning in supporting nutritional self-care. To our knowledge, this is one of the first studies in Türkiye to demonstrate these associations using a validated dietary behaviour scale. These findings highlight a critical gap between clinical recommendations and patient behaviour and emphasize the importance of encouraging special diet adherence through tailored counselling in HF care.

Regular meal consumption in patients with HF is critical for disease management. In our study, it was observed that most of the patients had the habit of consuming regular meals, but some participants skipped meals for reasons such as 'lack of habit' and 'lack of appetite'. Skipping meals may lead to disturbances in blood glucose regulation, energy deficiency and negative eating behaviours such as overeating at the next meal (28). This may adversely affect the course of the disease by making it difficult to control fluid and sodium intake in heart failure. Therefore, it is extremely important for individuals with heart failure to maintain regular meal consumption, develop individual solutions to minimise the reasons for skipping meals, and receive support from healthcare professionals in this regard in terms of disease management and improving quality of life. In support of this, our findings also indicated a positive trend between meal frequency and dietary behaviour scores. As the number of daily meals increased, both the total SDBHF score and the Salt Restriction subscale score tended to be higher, although the differences did not reach statistical significance. This trend suggests that regular meal consumption may be associated with greater adherence to dietary recommendations, particularly in areas such as sodium control, which is critical in HF management. While the relationship was not statistically significant, the consistency of this behavioural pattern reinforces the potential value of meal regularity as a modifiable target in dietary counselling. To our knowledge, few studies have examined this association using a validated dietary behaviour scale in a HF population, making this an important contribution to understanding the role of meal patterns in nutritional self-care.

Appetite and weight loss are frequently reported in patients with HF (29). This has been associated with metabolic and hormonal effects of heart failure (30). In this context, Arslan

et al. (2023) reported that both underweight and obese patients with acute coronary syndromes had higher GRACE risk scores, suggesting that extremes of body composition may reflect worse prognosis. This highlights the importance of monitoring anthropometric indicators and adjusting nutritional strategies accordingly in patients with HF (31). Especially loss of appetite may decrease the quality of life by increasing the risk of nutritional deficiency in patients. In addition, chewing and swallowing difficulties are common problems in patients with heart failure, which may make food intake more difficult (32). Digestive system problems such as indigestion, bloating, diarrhoea and constipation are generally associated with side effects of the drugs used, low physical activity level and inadequate fluid and fibre intake (33,34,35). These symptoms may limit patients' activities of daily living and worsen their nutritional status. Therefore, early diagnosis of such problems in individuals with heart failure, development of personalised nutrition plans and multidisciplinary approaches for symptom management are very important. These problems may be caused by several interrelated factors, including advanced age, medication side effects (e.g., diuretics, ACE inhibitors), poor oral health, fatigue, or depressive symptoms—all of which are prevalent among patients with chronic heart failure. From a clinical perspective, these issues may result in inadequate energy and nutrient intake, weight loss, and ultimately malnutrition, which is a known predictor of poor prognosis, higher readmission rates, and increased mortality in HF patients. Addressing these barriers through multidisciplinary approaches—including dental care, appetite-stimulating strategies, and appropriate food texture modifications—may help improve dietary intake and overall health outcomes.

In our study, symptom-specific analyses provided further insights into how common physiological problems in HF may affect dietary behaviours. Participants who reported chewing difficulties after HF diagnosis had lower scores in the total SDBHF and all subscales, with a statistically significant decrease observed in the Prevent Retention subscale ($p<.05$, $d=0.009$). This finding suggests that chewing difficulties may particularly impair behaviours aimed at managing fluid and sodium retention. Conversely, patients reporting diarrhoea had higher scores in all domains, with a statistically significant increase in the Healthy Habits subscale ($p<.05$, $d=0.023$), possibly reflecting increased dietary awareness in response to digestive discomfort. Finally, individuals experiencing constipation had lower total and subscale scores, with a significant reduction again in the Prevent Retention subscale ($p<.05$, $d=0.033$). While other differences in each symptom group were not statistically significant, the consistent directional trends observed highlight the potential impact of gastrointestinal and oral symptoms on patients' ability to follow recommended dietary practices. To our knowledge, this is one of the first studies to examine these associations in detail using a validated dietary behaviour scale in HF patients. These findings reinforce the importance of early identification of such symptoms and the integration of tailored nutritional strategies—such as texture-modified diets and symptom-specific counselling—into heart failure management.

Beyond their impact on dietary behaviours, limiting factors such as chewing difficulties, constipation, and diarrhoea may also contribute directly to compromised nutritional status. For instance, chewing difficulties can reduce overall food intake and lead to avoidance of nutrient-dense, high-fibre, or protein-rich foods. Constipation may reduce appetite and delay gastric emptying, further limiting caloric and micronutrient intake. Diarrhoea, while sometimes prompting healthier eating behaviours, may also cause malabsorption and loss of fluids, electrolytes, and nutrients. Although our study focused primarily on behavioural outcomes, the observed patterns suggest a potential pathway through which these symptoms may contribute to malnutrition risk. Future research should investigate how symptom-specific dietary behaviour changes correlate with objective measures of nutritional status, such as BMI, serum albumin levels, or muscle mass.

4.1. Strengths and Limitations

Scale for Dietary Behaviours in Heart Failure used in this study constituted the strength of this study because it had not been used before, but it was quite limiting in the discussion of the findings.

Cronbach's alpha value of the SDBHF used in our study was 0.58. A higher internal consistency of the scale would have provided more robust and reliable results. This relatively low alpha value may be attributable to cultural or contextual differences in dietary behaviour expressions among patients with HF. This represents a notable methodological limitation and may affect the reliability of subgroup comparisons and the interpretation of observed differences. Therefore, further validation and possible revision of the scale is recommended in future studies to enhance its reliability and applicability in different populations. Additionally, conducting item-level analysis or exploratory factor analysis may help identify specific items that reduce internal consistency and support scale refinement efforts.

In addition, the study was conducted in a single tertiary care hospital located in one region of the country, which may limit the generalizability of the findings to broader populations with different sociodemographic or healthcare access characteristics. Future research involving multicenter and regionally diverse samples is needed to confirm the representativeness and transferability of these results to other settings.

Although data on age, sex, marital status, education and income were collected, more nuanced indicators of cultural and ethnic diversity were not explored, which may limit the interpretation of dietary behaviours in broader sociocultural contexts.

Moreover, although a standardized protocol was used during interviews, the face-to-face data collection method may have

introduced social desirability bias. In addition, the use of self-reported data to assess dietary behaviors may introduce recall bias or reporting bias, as participants might underreport or overreport certain habits. Although interviews were conducted face-to-face using a standardized protocol, the reliance on self-reports remains a methodological limitation that could affect data accuracy.

Another important limitation is the cross-sectional design of the study, which does not allow for causal inferences. Although associations between dietary behaviors and socio-demographic or clinical variables were identified, the directionality or causality of these relationships cannot be established.

5. CONCLUSION

Based on the specific findings of this study, several clinically relevant implications can be drawn to enhance dietary management in patients with heart failure. First, the observation that patients with comorbid hypertension and diabetes demonstrated higher adherence to salt and sugar restriction respectively suggests that nutrition counselling should be tailored to reflect these coexisting conditions. Incorporating comorbidity-specific dietary goals may increase patient motivation and engagement. Second, the negative association between smoking duration and dietary adherence—particularly in sugar restriction and fluid management—underscores the need to integrate nutritional education with smoking cessation strategies, particularly among long-term smokers. Third, symptoms such as chewing difficulty and constipation were associated with lower scores in the fluid retention subscale, highlighting the importance of symptom screening and the use of texture-modified or fiber-adjusted diets. Additionally, the positive association between snack consumption and healthy dietary habits supports the inclusion of structured, nutrient-rich snacks in dietary planning. The data also revealed that patients who adhered to special diets, particularly salt-restricted or diabetic diets, showed better overall dietary behaviours, reinforcing the value of individualized dietary prescriptions. Skipping meals—especially lunch—due to lack of appetite or routine was associated with lower adherence, suggesting the need for behavioural strategies to support meal regularity. Furthermore, lower education and income levels were associated with poorer dietary scores, indicating that simplified educational tools and culturally adapted materials may benefit socioeconomically disadvantaged groups. Although the effect sizes observed in many associations were small, their cumulative impact may hold clinical relevance. Therefore, a comprehensive and multidisciplinary approach—incorporating comorbidity management, behavioural support, nutrition education, and symptom-specific interventions—should be adopted to improve dietary behaviours and, consequently, health outcomes in patients with heart failure.

Based on the findings of this study, it can be concluded that there is a need for improved and individualized dietary planning in heart failure patients. Developing personalized and guideline-based nutrition interventions tailored to the specific needs and clinical conditions of HF patients is essential for effective disease management and improving quality of life. To put these recommendations into practice, structured nutrition education programs should be developed not only for HF patients but also for their families and caregivers. Including dietitians as integral members of multidisciplinary care teams could enhance the effectiveness of dietary interventions. In addition, incorporating routine dietary behavior assessments into follow-up visits can help monitor progress and tailor interventions. On a broader level, institutional policies, such as adjusting hospital food menus to align with HF-specific dietary guidelines, could support sustainable dietary changes.

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Clinical and Experimental Health Sciences

Does Physical Activity Level, Anthropometric Measurements, and Nutritional Status Affect Stool Type in Adult Women?

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ABSTRACT

Objective: The objective of this study was to determine the stool types of adult women and evaluate their relationships with physical activity status, anthropometric measurements and nutritional status.

Methods: This study was conducted with 1479 women between the ages of 18-65 years residing in Ankara, Turkey. To determine the nutritional status of the participants, a daily food consumption record was obtained using the 24-hour food recall method. The International Physical Activity Questionnaire Short Form (IPAQ) was used to determine the physical activity status of the participants, and the Bristol Stool Scale was used to determine stool types. The Bristol Stool Scale was created based on the idea that stool type indicates intestinal transport time. Body weight, height, waist, and hip circumferences of the participants were measured by the researcher. After the measurements, the waist-to-hip ratio, waist-to-height ratio and body mass index were also calculated.

Results: Among 1479 participants with a mean age of 37.72 ± 11.51 years, 81.3% had normal, 11.3% hard, and 7.4% soft stools. The rate of obesity according to hard, normal, and soft stools was 28.75%, 28.15%, and 34.85%, respectively. The group with hard stools had significantly higher total energy (kcal/day) and dietary fat (g/day) intake than the groups with normal and soft stools ($p<.05$). There were no significant differences between IPAQ classifications and stool types. Logistic regression analysis showed that waist-to-height ratio (OR: 17.1, 95% CI: 2.46-118.92) increased the likelihood of soft stools by 17.1 times and protein intake (OR: .98, 95% CI: .96-.996) reduced the likelihood of soft stools by a factor of .979 ($p<.05$).

Conclusions: There were no significant differences between IPAQ classifications and stool types. Normal stool type is common among adult women in Turkey. Physical activity levels do not affect the type of stools.

Keywords: Fiber intake, obesity, physical activity, stool type.

1. INTRODUCTION

Nutrition forms the basis of health throughout every stage of the life cycle. Adequate and balanced nutrition entails the intake of sufficient amounts of energy and nutrients necessary for the growth, renewal, and functioning of the body and their appropriate use in the body (1). There are important physiological, neurological, and hormonal differences in women's health that affect their nutritional needs throughout the life cycle. Women, the elderly, and individuals of low socioeconomic status are more prone to experiencing constipation (2). The overall prevalence of constipation among adults ranges from 7% to 10%. Constipation prevalence varies between the sexes, with women experiencing rates approximately 6% higher than men. Constipation is a common health problem that negatively affects health-related quality of life due to decreased physical, mental, and social well-being (3). Many patients are advised to consume more high-fiber foods and engage in more physical exercise to relieve constipation (4).

Diarrhea, a prevalent digestive system symptom associated with obesity, significantly impacts the quality of life and the health of patients. The precise etiology of chronic diarrhea in obese individuals remains unclear; however, multiple studies indicate a higher prevalence of bile acid malabsorption in obese individuals compared to those with normal body mass index (BMI) values (5, 6). Compared to individuals with normal BMI values, obese individuals exhibit quicker colonic transit, and obesity is linked to heightened intestinal permeability, microbial dysbiosis, and endotoxemia (i.e., increased lipopolysaccharide levels) (7).

Physical inactivity, poor nutrition, and unmanaged stress have detrimental effects on both physical and mental health,

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significantly impacting the overall quality of life. Recent findings have shown that physical activity can improve the gut microbiome, genetic components, and microbial biodiversity, which may be beneficial for healthy aging. Several factors, including low dietary fiber intake and inadequate physical activity, might contribute to the onset of both obesity and constipation (8).

The objective of this study was to determine the stool types of adult women and evaluate their relationships with physical activity status, anthropometric measurements and nutritional status.

2. METHOD

2.1. Study Design

This study was conducted with women between the ages of 18 and 65 years residing in the Mamak district of Ankara, Turkey, during June and July 2022. G*Power analysis was used to calculate a sufficient number of samples to represent the research population. Accordingly, the number of samples to be selected from the research population of 2800 people was calculated as 580 people for power of 0.95 and an acceptable type I error of 0.05, based on an effect size of 0.30, and 1479 women were included in the research. Before beginning the study, approval was received from the Lokman Hekim University Non-Interventional Clinical Research Ethics Committee with decision dated 07.07.2022 and numbered 2022/10. Additionally, participants were informed about the study and signed a consent participation form that adhered to the protocols of the Declaration of Helsinki (World Medical Association).

Individuals under 18 or over 65 years of age, males, and those using medications or having conditions (such as celiac disease) that could affect bowel habits or stool type were excluded from the study.

The researchers administered a survey form in person to individuals who willingly agreed to participate in the study. The survey form included sections for participants' personal information (age, marital status, education level, occupation, and income level) in the first part, their health status in the second part, tobacco use in the third part, and nutritional habits in the fourth part. To determine the nutritional status of the participants, a daily food consumption record was obtained using the 24-hour food recall method (9). The International Physical Activity Questionnaire (IPAQ) Short Form was used to determine the physical activity status of the individuals and the Bristol Stool Scale was used to determine stool types (10, 11).

2.2. Evaluation of Nutritional Status and Outcome Measures

In order to evaluate the nutritional status of the participants, a daily food consumption record was obtained using the 24-hour food recall method. The "Meal and Food Photo Catalog" was used to determine the amount of food or beverages consumed by the participants (12). The contents of the meals consumed by participants were calculated using a standard recipe book (13). Average daily dietary energy carbohydrates, protein, fat, soluble fiber and insoluble fiber intake were analyzed using the Nutrition Information Systems Package Program (BeBiS, EBISpro for Windows, Germany; Turkish Version/BeBiS 8) (14).

2.3. Bristol Stool Scale

The Bristol Stool Scale was created based on the idea that stool type indicates intestinal transport time. This chart aims to estimate intestinal transport time based on 7 different stool forms (11). According to the Rome IV diagnostic criteria, types 1 and 2 reflect hard stools (constipation); types 3, 4, and 5 reflect normal stools; and types 6 and 7 reflect soft stools (diarrhea) in the Bristol Stool Scale (15).

2.4. International Physical Activity Questionnaire (Short Form)

The IPAQ Short Form was used to determine participants' physical activity levels. This form evaluates vigorous activities, activities of moderate intensity, and walking performed for at least 10 minutes in the last 7 days. Additionally, the average sedentary time spent in a day is noted. Respondents are asked how many days a week and for how long the activities are performed. While calculating the total score, the weekly MET-min value is obtained by multiplying the metabolic equation (MET) values assigned to the activities (vigorous activity = 8 MET, moderate activity = 4 MET, walking = 3.3 MET) by the number of days the activity was performed and the duration (min). According to the scores obtained, individuals' physical activity levels are grouped as "inactive," "moderately active," and "active" (10).

2.5. Anthropometric Measurements

Anthropometric measurements were carried out by the researchers. Body weight measurements and detailed body analyses were performed with a Hometta HMY-1500B device while participants were in light clothing, without shoes, and after defecation if possible. Height measurements were performed with a stadiometer while ensuring that the feet were together and participants were in the Frankfort plane (eyes and auricles at the same level) (16). The waist-to-hip ratio, waist-to-height ratio and BMI were also calculated. Participants' BMI values were calculated by dividing their body weight in kilograms (kg) by the square of their height in meters (m). BMI values were grouped using the World Health Organization's BMI classification for adults (17).

2.6. Statistical Analysis

Statistical analysis of the data was performed using the SPSS statistical package. The compliance of the data with normal distribution was evaluated with the Shapiro-Wilk test. The t-test was used for comparisons between groups of normally distributed data and the Mann-Whitney U test

was used for comparisons of non-normally distributed data. For comparisons between more than two groups, one-way analysis of variance (ANOVA) was used for normally distributed data and the Kruskal-Wallis test was applied for non-normally distributed data. Relationships between categorical variables were evaluated with the chi-square test and relationships between numerical variables were evaluated with Spearman correlation analysis. Logistic regression analysis was performed to identify variables associated with stool evaluations. Values of p<.05 were considered statistically significant.

Table 1. *Characteristics of the participating women*

	Women (n=1479)
Age (years) (X̄±SD)	37.72±11.51
	n (%)
Level of schooling	
Illiterate	28 (1.9)
Literate	19 (1.3)
Primary school	485 (32.8)
High school	535 (36.2)
University	380 (25.7)
Master's degree/doctorate	32 (2.2)
Marital status	
Married	1134 (76.7)
Single	345 (23.3)
Occupation	
Student	162 (10.9)
Civil servant	131 (8.9)
Retiree	23 (1.6)
Self-employed	71 (4.8)
Private sector	103 (7.0)
Housewife	947 (64.0)
Unemployed	31 (2.1)
Other	11 (0.7)
Income status	
Income less than expenses	576 (38.9)
Income equal to expenses	805 (54.4)
Income exceeding expenses	98 (6.6)
Physical activity status	
Inactive (Category 1)	545 (36.8)
Minimally active (Category 2)	586 (39.6)
Very active (Category 3)	348 (23.5)
Bristol Stool Scale	
Hard (Type 1 + Type 2)	167 (11.3)
Normal (Type 3 + Type 4 + Type 5)	1203 (81.3)
Soft (Type 6 + Type 7)	109 (7.4)
	X̄±SD
Energy, macronutrients, and fiber intake	
Energy (kcal/day)	1293.2±556.2
Carbohydrates (g/day)	147.4±79.2
Carbohydrates (%)	45.9±12.1
Protein (g/day)	49.5±22.6
Protein (%)	16.1±4.6
Fat (g/day)	54.9±29.1
Fat (%)	38.0±11.1
Dietary fiber (g/day)	15.2±10.5
Soluble fiber (g/day)	5.0±4.4
Insoluble fiber (g/day)	9.7±6.4
Water (mL/day)	1696.9±990.6

SD: Standard deviation

3. RESULTS

The characteristics of the participants are shown in Table 1. Our study included a total of 1479 female volunteers. The mean age of the participants was 37.72±11.51 years. It was determined that 76.7% of the participants were married and 54.4% of them had income equal to their expenses. Upon analyzing the distribution of participants based on their physical activity status, similar numbers of individuals were found in the inactive (Category 1) and minimally active (Category 2) groups, while the lowest number was observed in the highly active group (Category 3). According to the Bristol Stool Scale classification, 11.3% (167) of the women had hard stools, 81.3% (1203) had normal stools, and 7.4% (109) had soft stools.

The distribution of the participants' anthropometric measurements according to various classifications is provided in Table 2. Upon evaluating the distribution of participants based on their BMI values, 4.2% (62) were categorized as underweight, 33.3% (492) as normal weight, 33.8% (500) as mildly overweight, and 28.7% (425) as obese. It was determined that 45.6% of women were in the very high-risk class according to waist circumference. The mean anthropometric measurement values of the participants were as follows: average body weight was 69.96±14.3 kg, mean height was 160.8±6 cm, mean BMI was 27.12±5.77 kg/m^2, mean waist circumference was 87.89±14.34 cm, mean hip circumference was 106.15±11.33 cm, mean waist-to-hip ratio was 0.82±0.08, and mean waist-to-height ratio was 0.54±0.09.

Table 2. *Evaluation of participants' anthropometric measurements*

	Women (n=1479)			
	n (%)			
BMI classification (kg/m^2)				
Underweight (<18.5 kg/m^2)	62 (4.2)			
Normal (18.5-24.9 kg/m^2)	492 (33.3)			
Overweight (25-29.9 kg/m^2)	500 (33.8)			
Obese (≥30 kg/m^2)	425 (28.7)			
Waist circumference (cm)				
Low risk (M: <94 cm; W: <80 cm)	507 (34.3)			
High risk (M: 94-102 cm; W: 80-88 cm)	297 (20.1)			
Very high risk (M: ≥102 cm; W: ≥88 cm)	675 (45.6)			
Waist/height ratio				
<0.4	108 (7.3)			
0.4-0.5	382 (25.8)			
0.5-0.6	631 (42.7)			
>0.6	358 (24.2)			
Waist/hip ratio				
≤0.8	680 (46.0)			
>0.8	799 (54.0)			
	X̄±SD	**M**	**Min.**	**Max.**
Anthropometric measurements				
Weight (kg)	70.0±14.3	68.6	35.2	129
Height (cm)	160.8±6	161	140	184
BMI (kg/m^2)	27.1±5.8	26.7	14.5	58.4
Waist circumference (cm)	87.9±14.3	87	52	145
Hip circumference (cm)	106.1±11.3	105	55	154
Waist/hip ratio	0.8±0.1	0.81	0.5	1.4
Waist/height ratio	0.5±0.1	0.53	0.3	0.9

SD: Standard deviation; Min.: minimum; Max.: maximum; M: median.

The distributions of anthropometric measurements, mean energy and nutrient intake and standard deviations, and the distribution of physical activity statuses according to Bristol Stool Scale classifications are shown in Table 3. In classifications based on the stool scale, the differences in the means of body weight, height, BMI, hip circumference, waist circumference, and waist-to-hip ratio were not found to be statistically significant (p>.05).

Table 3. *Evaluation of some variables according to the Bristol Stool Scale*

	Bristol Stool Chart			
	Hard (n=478) (Type 1 + Type 2 + Type 3)	Normal (n=705) (Type 4)	Soft (n=296) (Type 5 + Type 6 + Type 7)	p
	X̄±SD	X̄±SD	X̄±SD	
Anthropometric measurements				
Weight (kg)	69.9±13.4	69.8±14.4	72.1±15.0	.253
Height (cm)	160±6	161±6	160±6	.181
BMI (kg/m^2)	27.3±5.5	27.0±5.7	28.4±6.7	.48
Hip circumference (cm)	106.3±10.6	106.0±11.3	108.00±12.6	.197
Waist circumference (cm)	88.8±13.6	87.4±14.2	91.5±15.9	.14
Waist/height ratio	0.5±0.1	0.5±0.1†	0.6±0.1†	**.008***
Waist/hip ratio	0.8±0.1	0.8±0.1	0.8±0.1	.132
Energy, macronutrient, and fiber intake				
Energy (kcal/day)	1380.9±549.4†	1290.9±547.2	1185.2±478.6†	**.016***
Carbohydrate (g/day)	156.8±85.8	147.1±78.3	137.2±77.5	.122
Carbohydrate (%)	45.3±11.5	45.9±12.1	46.4±12.7	.765
Protein (g/day)	53.1±25.1‡	49.6±22.6‡	43.2±17.4§	**.02***
Protein (%)	16.2±4.6	16.1±4.6	15.6±4.7	.545
Fat (g/day)	59.6±33.7†	54.6±28.8	50.5±23.8†	**.029***
Fat (%)	38.3±11.0	37.9±11.2	37.9±10.9	.928
Fiber (g/day)	15.8±8.9	15.3±11.0	13.8±6.5	.292
Soluble fiber (g/day)	5.2±3.7	5.0±4.6	4.7±3.3	.653
Insoluble fiber (g/day)	9.9±6.0	9.7±6.5	8.8±4.5	.316
Water (mL/day)	1731.0±1089.0	1699.0±978.0	1620.0±972.0	.653
IPAQ-MET-Score	2109.9±3252.5	1742.4±2472.6	2013.4±2885.4	.168
	n (%)	**n (%)**	**n (%)**	
IPAQ classification				
Inactive (Category 1)	66 (39.5)	436 (36.3)	43 (39.4)	χ^2=1.53
Minimally active (Category 2)	62 (37.1)	485 (40.3)	39 (35.8)	p=.82
Very active (Category 3)	39 (23.4)	282 (23.4)	27 (24.8)	
BMI classification (kg/m^2)				
Underweight (<18.5 kg/m^2)	7 (4.2)	50 (4.1)	5 (4.6)	
Normal (18.5-24.9 kg/m^2)	45 (26.9)	421 (35.0)	26 (23.8)	χ^2=10.28
Overweight (25-29.9 kg/m^2)	67 (40.1)	393 (32.7)	40 (36.7)	p=.11
Obese (≥30 kg/m^2)	48 (28.7)	339 (28.1)	38 (34.8)	
Waist circumference (cm)				
Low risk (M: <94 cm; W: <80 cm)	47 (28.1)	381 (31.6)	24 (22.0)	
High risk (M: 94-102 cm; W: 80-88 cm)	33 (19.8)	240 (20.0)	25 (22.9)	χ^2=5.06 p=.281
Very high risk (M: ≥102 cm; W: ≥88 cm)	87 (52.1)	582 (48.4)	60 (55.1)	
Waist/height ratio				
<0.4	2 (1.2)	47 (3.9)	5 (4.6)	
0.4-0.5	47 (28.1)	365 (30.3)	24 (22)	χ^2=9.58
0.5-0.6	73 (43.7)	504 (41.9)	44 (40.4)	p=.143
>0.6	45 (26.9)	287 (23.9)	36 (33)	
Waist/hip ratio				
≤0.8	69 (41.3)	567 (47.1)	44 (40.4)	χ^2=3.48
>0.8	98 (58.7)	636 (52.9)	65 (59.6)	p=.175

**: One-way ANOVA (post hoc: Tukey's test); †: there is a significant difference between groups; ‡, §: there is no significant difference between groups with the same letter; SD: standard deviation; p<.05*

The difference in mean waist-to-height ratios between the groups with normal and soft stool types was statistically significant (p<.05). In classifications based on the stool scale, the differences in mean energy intake and fat consumption between the groups with hard and soft stool types were also statistically significant (p<.05).

In classifications based on the stool scale, the differences in mean carbohydrate intake amount, carbohydrate intake percentage, protein intake percentage, fat intake percentage, dietary fiber intake amount, soluble dietary fiber intake amount, insoluble dietary fiber intake amount, and water consumption amount were not statistically significant (p>.05). The difference in mean protein consumption amount between the group with soft stools and the groups with hard and normal stools was statistically significant (p<.05). The differences in IPAQ classification groups based on the Bristol Stool Scale were not statistically significant (p>.05).

Logistic regression analysis was conducted to determine the variables associated with stool evaluations (Table 4). Waist-to-height ratio and energy, protein, and fat intake, which were found to be significant in pairwise comparisons, were included in the analysis. The normal stool evaluation level was taken as a reference. Waist-to-height ratio and energy, protein, and fat intake did not affect the occurrence of stool hardness (p>.05). The waist-to-height ratio increased the likelihood of soft stools by 17.11 times (p<.05). Protein reduced the likelihood of soft stools by a factor of 0.979 (p<.05). Energy and fat did not affect the occurrence of soft stools (p>.05). The independent variables explained 3% of the occurrence of soft stools.

Table 4. *Logistic regression (effect of independent variables on the occurrence of hard and soft stool types)*

		B	Std. error	p	OR	95% CI		R^2
						Min.	Max.	
Hard	Waist/height ratio	1.378	.834	.098	3.967	.774	20.337	**.010**
	Energy (kcal/day)	.000	.000	.883	1.000	.999	1.001	
	Protein (g/day)	.002	.005	.673	1.002	.992	1.013	
	Fat (g/day)	.004	.005	.435	1.004	.995	1.012	
	Constant	-3.106	.507	.000	.045			
Soft	Waist/height ratio	2.840	.989	**.004**	17.110	2.462	118.918	**.030**
	Energy (kcal/day)	.000	.000	.650	1.000	.999	1.001	
	Protein (g/day)	-.021	.009	**.016**	.979	.962	.996	
	Fat (g/day)	.002	.006	.801	1.002	.990	1.014	
	Constant	-3.310	.620	.000	.037			

A bar graph depicting the classification based on the Bristol Stool Scale and body mass index is provided in Figure 1. The percentages of women who were obese according to hard, normal, and soft stool types were 28.75%, 28.15%, and 34.85%, respectively.

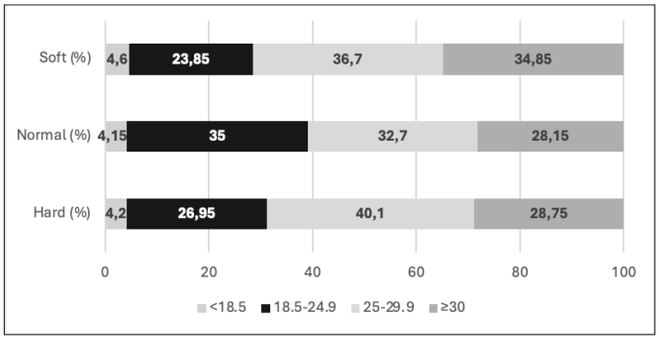

Figure 1. *Bar graph depicting the classification based on the Bristol Stool Scale and body mass index*

4. DISCUSSION

Bowel habits and stool types vary among individuals depending on their eating habits, lifestyles, and overall health statuses. Functional bowel disorders are more prevalent in women compared to men. Factors such as oral contraception, childbirth, hysterectomy, and pelvic floor dysfunction in women can be considered among the causes of intestinal disorders (18). The mechanisms behind how sex differences may affect bowel habits and stool types are not fully understood. In this study, the stool types of adult women were evaluated with the Bristol Stool Scale and their relationships with physical activity status, anthropometric measurements and nutritional status were examined.

In cases of functional bowel disorders, there are no identifiable structural or biochemical abnormalities as in other gastrointestinal disorders. Therefore, diagnosis is based almost entirely on symptoms, and the Rome diagnostic criteria constitute the most widely accepted standard for such symptom-based diagnoses. The Rome IV criteria are the most recent updated version. According to these criteria, types 1 and 2 of the Bristol Stool Scale reflect hard stools; types 3, 4, and 5 reflect normal stools; and types 6 and 7 reflect soft stools (19). Similar to other studies (20, 21), in our study, the rate of individuals with normal stools (81.3%) according to the Bristol Stool Scale was found to be higher than other types (Table 1).

Factors such as lifestyle, nutritional habits, physical activity levels, accompanying diseases, and medications used closely affect bowel habits. It is suggested that exercise affects colon motility and accelerates intestinal transit. At the same time, hormonal changes and mechanical effects during exercise can change gastrointestinal functions (22). In addition to studies that found significant relationships between physical activity and constipation (23-26), there are also studies that did not detect any such relationships (27-29). In this study, it was determined that 39.5% of individuals who reported hard stools according to the Bristol Stool Scale were inactive. The numbers of individuals who were very active were lowest for all three stool types (p>.05) (Table 3). Although physical

activity is likely to have an effect on preventing constipation or reducing its severity, different results may have emerged due to the differences in population characteristics in these studies or differences in the scales used.

Dietary fibers are known to have positive effects on bowel movements. It is suggested that water-insoluble dietary fiber increases stool volume, shortens intestinal transit time, and prevents constipation (30). There are studies in the literature reporting a significant relationship between dietary fiber intake and constipation (17, 31). Markland et al. evaluated data obtained from 10,914 adults (≥20 years old) in the 2005-2008 period of the National Health and Nutrition Examination Surveys (NHANES) and did not find a significant relationship between dietary fiber intake and constipation (32). In the present study, dietary fiber intake was found to be below the recommended levels for all three stool types determined according to the Bristol Stool Scale. Surprisingly, the daily dietary fiber intake of individuals with hard stools was found to be higher than that of participants with normal and soft stools ($p>.05$) (Table 3). The reason for this result may be the tendency of constipated individuals to increase their fiber intake. Inconsistencies between studies may be due to small sample sizes or differences in the methods used to determine daily fiber intake. The answers to many questions such as the optimal dose, type, and source of fiber affecting bowel movements are not yet clear. In order to best evaluate the relationship between dietary fiber intake and constipation and explain the underlying mechanisms, it is important that researchers determine the types of total daily fiber consumed.

It has been reported that the energy content of a meal can directly affect gastrointestinal transit and may therefore be associated with constipation. Some studies have found a significant relationship between high energy intake and a high risk of constipation (33,34). On the contrary, one study found that low energy intake in women was associated with an increased risk of constipation (35). In the present study, the energy intake of individuals with hard stools was found to be significantly higher than that of individuals with soft stools ($p<.05$) (Table 3). High energy intake may be associated with the intake of foods of high energy density, such as processed foods and ready meals. In order to best evaluate the effect of total daily energy intake on bowel movements, it is important to determine the nutritional content of total daily energy in detail, considering that each macronutrient has different effects on the gastrointestinal tract.

In this study, when nutritional intakes were compared according to stool types, significant differences were detected between the groups in terms of protein and fat intake ($p<.05$) (Table 3). Protein intake was found to reduce the likelihood of soft stools by 0.979 times (Table 4). In a different study (33), total dietary fats were associated with a lower risk of constipation, while another study (35) stated that total protein and fat intakes were associated more closely with constipation in men compared to women. Also, in the same study, total carbohydrate intake was not found to be associated with constipation. Likewise, in this study, no significant relationship was detected between carbohydrate intake and stool types. Determining the types of carbohydrates consumed will enable clearer interpretation.

It has been reported that obesity is associated with chronic gastrointestinal complaints. In a previous study, 27.7% of individuals with diarrhea were categorized in the obese class when compared to those with normal bowel habits or constipation (7). Similarly, other studies have found that there is a continuous increase in bowel movements as BMI increases (21,35). In contrast, Verkuijl et al. did not find a relationship between BMI and bowel movements (18). In our study, the number of obese individuals was found to be higher among those reporting hard stools than those reporting soft stools ($p<.05$) (Table 3). Studies to date have not been able to clearly explain the mechanisms underlying the relationship between BMI and bowel movements. Factors such as changing hormonal levels, meal frequencies, nutritional intake, and fluid consumption together with high BMI may affect these results. More studies are needed to identify the underlying mechanisms more clearly.

One of the limitations of this study is that food consumption records were based on the 24-hour retrospective recall method. This method may be affected by errors in recall. Asking participants to keep 3-day food consumption records could ensure more accurate results regarding daily nutritional intake. Another limitation of this study is that the sources of daily dietary fiber could not be determined. The strengths of the study are the large sample size and the use of the Bristol Stool Scale and the IPAQ Short Form. With disorder in bowel habits, individuals' use of gastrointestinal health services increases together with the economic burdens that these diseases bring. Therefore, determining the risk factors affecting bowel habits is important in terms of making lifestyle and nutrition recommendations and guiding individuals.

5. CONCLUSION

In this study, the rate of women reporting normal stools according to the Bristol Stool Scale was found to be high (81.3%). No significant relationship was found between physical activity, BMI classification, or fiber intake and stool type. However, significant associations between energy, protein, and fat intake and stool type were observed. In order to achieve a more comprehensive understanding of the determinants influencing bowel habits, future research should encompass diverse age groups, sexes, lifestyle behaviors and dietary patterns within well-designed, large-scale studies.

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Clinical and Experimental Health Sciences

The Effect of Text Messages and Education on Cancer Screening on Women aged 30-65

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ABSTRACT

Objective: Despite it is known that cancer screenings reduce cancer-related deaths, screening rates are still inadequate. The aim of this study is to examine the effect of text messaging and training on cancer screening in women aged 30-65.

Methods: This non-randomized controlled trial was conducted with 140 women aged 30-65 between November 2023 and April 2024 in Kırklareali, Türkiye. Participants were divided into the reminder text message group (n=46), the training group (n=48), and the control group (n=46). In the text message group, every participant received text messages about cancer and importance of screening procedures, one a day for ten days. The training group was educated for a session that lasted 45 min. Data was collected both before and 3 months after the intervention, using a questionnaire that included two parts (sociodemographic characteristics and stages of change for cancer screening practices). Data was analyzed using the Marginal Homogeneity test.

Results: In the post-test, the Attitude Scale for Cancer Screening score was statistically significantly higher in both the reminder text message group and the training group compared to the pre-test ($p<.01$), whereas no statistically significant difference was observed in the control group ($p>.05$). Participation in cancer screening behaviors increased significantly in both the reminder text message and training groups ($p<.01$). In the reminder group, the rate of Pap smear testing increased from 30.5% to 76.1%, breast self-examination from 71.8% to 97.8%, mammography from 23.9% to 41.3%, and fecal occult blood testing from 2.2% to 8.7%. In the training group, these rates increased from 41.7% to 70.9%, 66.7% to 93.8%, 25.1% to 41.7%, and 0% to 20.9%, respectively. There was no significant change in the control group ($p>.05$).

Conclusions: The results showed that both reminder text messages and training interventions effectively improved attitudes and significantly increased participation in cancer screening behaviors.

Keywords: Text messages, education, cancer

1.INTRODUCTION

Cancer is the second leading cause of death worldwide and constitutes one of the most significant global public health problems (1). Among the most prevalent and fatal cancer types are breast, colorectal, and cervical cancers. However, effective screening programs for these cancers have been shown to facilitate early diagnosis, improve treatment options, and enhance survival outcomes (2). Cancer screening plays a critical role in increasing the detection rate of malignancies and reducing cancer-related mortality (3). Notably, the regular participation of women aged 30–65 in screening programs significantly contributes to the early detection of disease and a corresponding decrease in mortality rates (4).

In Turkey, cancer screening is carried out for three types of cancer in line with World Health Organization (WHO) recommendations. For breast cancer, monthly breast self-examinations, annual clinical breast examinations, and biennial mammography for women aged 40–69 are recommended. For cervical cancer, women aged 30–65 are advised to undergo HPV-DNA testing every five years. For colorectal cancer, women aged 50–70 are screened via biennial fecal occult blood testing and decennial colonoscopy (5).

Interventions aimed at increasing cancer screening uptake are designed to support individuals in making informed screening decisions, understanding the appropriate steps to take, and identifying accessible healthcare services. These initiatives focus on enhancing knowledge, raising awareness, and fostering long-term health-promoting behaviors (6). Mobile health (mHealth) applications represent an innovative form of medical technology that improves

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healthcare accessibility, facilitates service navigation, and supports behavioral change (7). mHealth interventions targeting cancer-related behaviors include telephone-based counseling, web-based interventions, and reminder text messaging. Such interventions have been shown to strengthen patient-provider communication, encourage active patient participation in disease management, enhance motivation, and enable secure data collection. In particular, text-based reminders have been associated with improvements in patient communication and self-care behaviors among individuals diagnosed with cancer (8,9).

This study aims to evaluate the effectiveness of reminder text messages on early cancer screening behaviors among women aged 30–65, and to determine whether their impact is comparable to that of face-to-face educational interventions.

2. METHODS

2.1. Design

It is a non-randomized experimental study with a pre-test/post-test design, conducted between November 2023 and April 2024, consisting of two intervention groups—the reminder text message group (RTMG) and the training group (TG)—and one control group (CG), in which the three groups are compared

2.2. Participant

This study was conducted with 140 female participants aged 30–65 who were registered at a fami ly health center located in a district in western Türkiye (RTMG: 46, TG: 48, CG: 46).

2.2.1 Inclusion Criteria:

- Aged between 30-65 years
- Mobile phone user

2.2.2 Exclusion Criteria:

- Having a disability that prevents communication
- Having a diagnosed mental disorder
- Diagnosed with cancer
- Illiterate

2.3. Data Collection

Data were collected through self-reported measures using the Descriptive Questionnaire and the Attitude Scale for Cancer Screening. Data collection was conducted at two time points —prior to the intervention and three months after the intervention—for all groups (Figure 1).

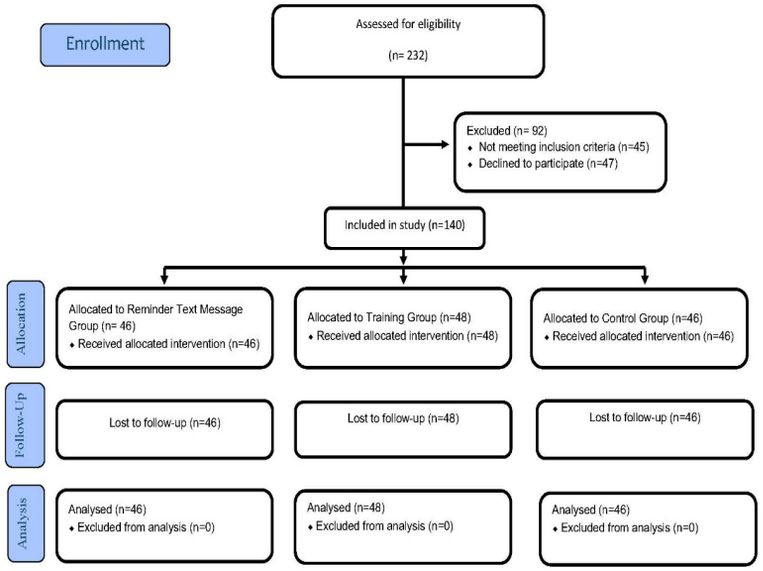

Figure 1. *Consort Diagram*

2.4. Descriptive Questionnaire

This questionnaire, developed by the researchers, consists of questions regarding age, educational level, marital status, income level, family history of cancer, and behaviors related to undergoing cancer screening tests. Behaviors related to cancer screening tests were structured based on the Stages of Change within the Transtheoretical Model, which conceptualizes behavior change as a process occurring through a series of stages. For example, the question related to mammography has been formulated as follows:

What is your current status regarding having a mammography?

1. *Precontemplation: I haven't had mammography, and I don't plan on having one in the next month.*
2. *Contemplation: I haven't had mammography done but I'm thinking of having it done in the next 6 months.*
3. *Preparation: I haven't had mammography done yet but I'm planning to have it done within the next month.*
4. *Action: I had mammography done.*
5. *Maintenance: I have had mammography done and will continue to have it done in the future.*

2.5. Attitude Scale for Cancer Screening

The scale was developed by Öztürk et al. in 2020. It comprises 24 items and is unidimensional. The scale utilizes a five-point Likert-type response format. The items are scored on a range from 1 to 5, as follows: '5: Strongly agree, 4: Agree somewhat, 3: Neither agree nor disagree, 2: Disagree somewhat, 1: Strongly disagree.' The scale is designed for use with individuals aged 30 to 70, who are at least literate, and do not have cognitive or visual impairments that would hinder their ability to respond. The minimum possible score on the scale is 24, and the maximum score is 120. No specific cutoff point has been established for the scale. As participants' scores approach 24, this indicates a negative attitude towards cancer screenings, while scores closer to 120 reflect a positive attitude towards cancer screenings. The Cronbach's alpha coefficient for the scale is 0.95 (10). In this study, the Cronbach's alpha coefficient was .80

2.6. Intervention

A face-to-face group training was conducted for the training group, which was developed by the researchers and reviewed by experts. For the reminder text message group, short messages were sent based on content developed by the researchers and reviewed by experts. No intervention was made in the control group beyond the routine practice.

The objective of the training program and the reminder messages was to foster and encourage cancer screening behaviors among the participants. The training content was designed to be consistent with the content of the reminder text messages (the importance of early screening, cervical, breast, and colorectal cancer screening tests, breast self-examination, and the promotion of screening centers). The training sessions were conducted as 30-minute group sessions, and participants were invited to the Family Health Center in groups of 15.

The reminder text messages were designed to guide participants, incorporating video content (mammography procedures, directions to the local Cancer Early Diagnosis, Screening, and Education Center-KETEM – contact point, appointment scheduling links). The reminder text messages were sent to the participants' mobile phones once a day for a period of 10 days (Table 1).

***Table 1**. Content of training and text messages*

Learning Outcomes	Training	Text-messages
Explains the importance of early cancer screening.	Presentation Ask and Question	"Early cancer screening saves lives in the fight against cancer. You can get information about the screening tests suitable for your age group at the nearest health institution, Kırklareli Vize KETEM contact point."
		"It is not cancer that kills but not having a screening. Do you want to get screened? Let's schedule your appointment immediately and invite you to the Vize KETEM contact point." (Additionally, the location information of the screening center is shared).
Participants will know and perform cervical cancer screening, and the importance of the Pap-smear test every five years for women aged 30-65.	Presentation Ask and Question	"Cervical cancer ranks 3rd among women worldwide. Join the fight against cancer and make an appointment for a Pap-smear test at the Vize KETEM contact point."
Discusses the importance of the Pap-smear test for women.		"To protect against cervical cancer, the Pap-smear test, conducted every five years for women aged 30-65, is offered free of charge at the Vize KETEM contact point. Call ..... to make an appointment now."
		"The Pap-smear test, performed to prevent cervical cancer, is a simple and painless procedure. Before coming for the screening, you should not be on your period, should not have washed the vagina or applied medication, cream, or gel at least two days prior, and should not have had sexual intercourse within the last two days. Participants coming for screening are kindly requested to be careful in this regard." (The location and phone number of the Vize KETEM contact point will be shared.)
Explains the importance of breast cancer for women.	Presentation Ask and Question Demonstration	"Studies show that the recovery rate for breast cancer detected early is 98%. Take action to prevent breast cancer and visit the Vize KETEM contact point for a mammogram." (The location information of the contact point is shared.)
Explains the importance of breast cancer screenings for women.		"Self-breast examination has been found to be 90% effective in early detection of breast cancer. If you do not know how to perform it, you can visit the Vize KETEM contact point." (A self-breast examination video is sent)
Practices self-breast examination and explain clinical breast examination.		"Women aged 40-69 must have a mammogram every two years. Schedule your appointment at the Vize KETEM contact point as soon as possible." (A video prepared with a radiology technician explaining the mammography process is sent.)
Undergoes a mammography		"The best time for a mammogram is the first week after your menstrual period when breast tissue is least sensitive. Please take this into account before coming for the screening." (A video on the mammography procedure is sent.)
		"You should perform self-breast examination every month, between the 7th and 10th day of your menstrual cycle. If you are menopausal, perform self-breast examination on the same day each month." (A self-breast examination video is sent.)
		Come to the Vize KETEM contact point for a clinical breast examination. After the age of 40, visit KETEM centers every year for a breast examination. (A self-breast examination video is sent.)
Participants define colorectal cancer.	Presentation Ask and Question demonstration	"Be aware of colorectal cancer, protect your health, and undergo screening tests. For more information, visit the Vize KETEM contact point." (The location information of the Vize KETEM contact point is shared.)
Applies the fecal occult blood test		"The fecal occult blood test is a simple and highly effective method in the fight against colorectal cancer. You can request it from your family health unit or the Vize KETEM contact point." (A video explaining how the fecal occult blood test is performed and what to consider is sent.)
Believes in the necessity of early cancer screening tests.	Presentation Ask and Question Discussion	"Cancer is a preventable disease. We must undergo screening tests in the fight against cancer. For more information and to schedule an appointment, visit the Vize KETEM contact point."
visit to a screening center for early cancer screening tests.		"Cancer screening tests enable early diagnosis, prevention, and treatment. Therefore, you can visit the Vize KETEM contact point for information and to schedule an appointment."

2.7. Data analysis

All data were analyzed using the SPSS statistical software. Descriptive statistics (percentages, means, and standard deviations) were used to summarize participants' socio-demographic characteristics. Pearson's Chi-Square test, One-Way ANOVA, Wilcoxon and the Marginal Homogeneity test were performed to compare groups. A significance level of $p < .05$ was considered statistically significant. Effect size was analyzed using the G-Power 3.1.9.2 (11).

2.8. Ethical Consideration

Ethical approval was obtained from the Ethics Committee of the Institute of Health Sciences at Marmara University (Approval date: 20.09.2023; Number:91). Written permission was also obtained from the Kırklareli District National Health Directorate. Before starting the research, information about the study was provided to the participants, and their written consent was obtained.

3. RESULTS

There were no statistically significant differences between the groups in terms of marital status, educational level, income level, or family history of cancer (p<.05). (Table 2).

Table 2. *Sociodemographic characteristics of the participants*

		Remainder Text Message Group	Training Group	Control Group	Test/p
Marital Status		**n (%)**	**n (%)**	**n (%)**	
	Married	37 (80.4)	39 (81.3)	42 (91.3)	2.56*/.278
	Single	9 (19.6)	9 (18.8)	4 (8.7)	
Education level					
	Literature	2 (4.3)	3 (6.3)	3 (6.5)	11.71*/.069
	Primary school	7 (15.2)	13 (27.1)	14 (30.4)	
	Secondary school	14 (30.4)	15 (31.3)	21 (45.7)	
	High school and above	23 (50.0)	17 (35.4)	8 (17.4)	
Income status					
	Income equal to expenses	36 (78.3)	40 (83.3)	37 (80.4)	3.04*/.550
	Income less than expenses	6 (13.0)	3 (6.3)	7 (15.2)	
	Income more than expenses	4 (8.7)	5 (10.4)	2 (4.3)	
Family cancer history					
	Yes	19 (41.3)	25 (52.1)	34 (73.9)	10.30*/.006
	No	27 (58.7)	23 (47.9)	12 (26.1)	
		Mean±Sd	**Mean±Sd**	**Mean±Sd**	
Age (years)		[a]39.7±8.2	[b]42.2±9.3	[c]46.2±9.4	6.05**/.003
		a=b(p=.575***); a<c(p=.002***); b=c (p=.096***)			

Pearson Chi-Square, ** One-way ANOVA, * Bonferroni*

In the post-test, the Attitude Scale for Cancer Screening score was statistically significantly higher in both the reminder text message group and the training group compared to the pre-test (p<.001), whereas no statistically significant difference was observed in the control group (p>.05). The reminder text message group demonstrated a large effect size (Cohen's d = 0.79), whereas the training group showed a small-to-moderate effect size (Cohen's d = 0.36) between pre – and post-test scores. (Table 3).

Table 3. *Comparison of attitude scale for cancer screening scores*

Groups	Pre-test Mean ± Sd	Post-test Mean ± Sd	Test*/p value	Effect size Cohen's d	Power
Remainder text message group (n=46)	109.5 ± 8.4	115.3 ± 4.6	5.2 / .000	.79	.99
Training group (n=48)	109.0 ± 9.1	112.0 ± 6.5	3.4 / .000	.36	.79
Control group (n=46)	111.4 ± 7.5	111.3 ± 7.5	.73 / .461	—-	—-

**Wilcoxon test*

In the post-test, the proportion of participants who underwent a Pap smear test was statistically significantly higher in both the reminder text message group and the training group compared to the pre-test (p<.001), whereas no significant difference was observed in the control group (p>.05). The rate of participants who had a Pap smear test (action + maintenance stages) increased from 30.5% to 76.1% in the reminder text message group and from 41.7% to 70.9% in the education group (Table 4).

The proportion of participants performing breast self-examination was significantly higher in both the reminder text message group and the training group in the post-test compared to the pre-test (p<.001), whereas no significant change was observed in the control group (p>.05). The percentage of participants in the action and maintenance stages increased from 71.8% to 97.8% in the reminder text message group, and from 66.7% to 93.8% in the training group (Table 4).

The proportion of participants undergoing mammography was significantly higher in both the reminder text message group and the training group in the post-test compared to the pre-test (p < .01), while no significant change was observed in the control group (p>.05). The percentage of participants in the action and maintenance stages increased from 23.9% to 41.3% in the reminder text message group, and from 25.1% to 41.7% in the training group (Table 4).

In the post-test, the proportion of participants who underwent a fecal occult blood test was significantly higher in both the reminder text message group and the training group compared to the pre-test (p<.05), whereas no significant change was observed in the control group (p>.05). The percentage of participants in the action and maintenance stages increased from 2.2% to 8.7% in the reminder text message group, and from 0% to 20.9% in the training group (Table 4).

Table 4. *Comparison of pre – and post – tests of participants' cancer screening test status*

Cancer Screening Tests	Groups		Precontemplation n (%)	Contemplation n (%)	Preparation n (%)	Action n (%)	Maintenance n (%)	Test ^{MH} /p value
Pap smear test	Text group	Pre-test	9 (19.6)	21(45.7)	2 (4.3)	5 (10.9)	9 (19.6)	4.9 /**.001**
		Post-test	3 (6.5)	-	8 (17.4)	22 (47.8)	13 (28.3)	
	Training Group	Pre-test	6 (12.5)	21 (43.8)	1 (2.1)	15 (31.3)	5 (10.4)	4.0^{MH}/**.001**
		Post-test	3 (6.3)	5 (10.4)	6 (12.5)	27 (56.3)	7 (14.6)	
	Control Group	Pre-test	-	4 (8.7)	-	27 (58.7)	15 (32.6)	1.00^{MH}/.31
		Post-test	-	4 (8.7)	-	26 (56.5)	16 (34.8)	
Breast self-exam	Text Group	Pre-test	7 (15.2)	6 (13.0)	-	17 (37.0)	16 (34.8)	4.46 ^{MH} /**.001**
		Post-test	1 (2.2)	-	-	12 (26.1)	33 (71.7)	
	Training Group	Pre-test	1 (2.1)	15 (31.3)	-	24 (50)	8 (16.7)	4.67^{MH}/**.001**
		Post-test	3 (6.3)	-	-	20 (41.7)	25 (52.1)	
	Control Group	Pre-test	2 (4.3)	8 (17.4)	5 (10.9)	20 (43.5)	11 (23.9)	1.0^{MH}/.31
		Post-test	2 (4.3)	8 (17.4)	4 (8.7)	21 (45.7)	11 (23.9)	
Mammography	Text Group	Pre-test	14 (30.4)	20 (43.5)	1 (2.2)	5 (10.9)	6 (13)	3.93^{MH} /**.001**
		Post-test	11 (23.9)	8 (17.4)	8 (17.4)	9 (19.6)	10 (21.7)	
	Training Group	Pre-test	9 (18.8)	26 (54.2)	1 (2.1)	9 (18.8)	3 (6.3)	2.74^{MH}/**.006**
		Post-test	8 (16.7)	20 (41.7)	-	17 (35.4)	3 (6.3)	
	Control Group	Pre-test	6 (13)	23 (50)	2 (4.3)	10 (21.7)	5 (10.9)	1.41^{MH}/.157
		Post-test	6 (13)	21 (45.7)	2 (4.3)	12 (26.1)	5 (10.9)	
Fecal occult blood tests	Text Group	Pre-test	32 (69.6)	12 (26.1)	1 (2.2)	1 (2.2)	-	2.26^{MH} /**.023**
		Post-test	29 (63)	6 (19.6)	4 (8.7)	4 (8.7)	-	
	Training Group	Pre-test	13 (27.1)	32 (66.7)	3 (6.3)	-	-	2.94^{MH}/**.003**
		Post-test	12 (25.0)	23 (47.9)	3 (6.3)	9 (18.8)	1 (2.1)	
	Control Group	Pre-test	8 (17.4)	37 (80.4)	-	1 (2.2)	-	0.00^{MH}/1.00
		Post-test	8 (17.4)	37 (80.4)	-	1 (2.2)	-	

Precontemplation: *I haven't had the test, and I don't plan on having one in the next month.*
Contemplation: *I haven't had the test done but I'm thinking of having it done in the next 6 months.*
Preparation: *I haven't had the test done yet but I'm planning to have it done within the next month.*
Action: *I had the test done.*
Maintenance: *I have had the test done and will continue to have it done in the future.*
MH: *Marginal Homogenity Test*

4. DISCUSSIONS

This study aimed to evaluate the impact of reminder text messages and training on cancer early screening behaviors among women aged 30 to 65 years. The results of this study indicate that both the reminder text message group and the training group achieved significant improvements in Attitude Scale for Cancer Screening scores from pre-test to post-test, whereas no significant change was observed in the control group. Notably, the reminder text message intervention produced a large effect size (Cohen's d = 0.79), while the traditional training intervention yielded only a small-to-moderate effect size (Cohen's d = 0.36). These findings suggest that periodic reminders may be more effective in promoting positive attitudes toward cancer screening than a single training session.

The findings demonstrated that both the reminder text message group and the training group were effective in increasing participation in cancer screening tests, including cervical, breast, and colorectal cancer screenings. Notably, reminder text messages were found to be as effective as training in enhancing screening uptake for cervical, breast, and colorectal cancers.

In our study, the proportion of participants who underwent a Pap smear test was found to be higher in both the reminder text message group and the training group. Previous studies that implemented reminder text message interventions for cervical cancer screening have reported positive effects on screening uptake (12,13). Contrary to our findings, however, Bhardwaj et al. (14) found that text messages were not effective in increasing the rate of Pap smear uptake. Similarly, in another study conducted in Sub-Saharan Africa, the use of text message reminders aimed at increasing cervical cancer screening did not lead to improved screening rates among women living in urban areas (15). In our study, the content of the reminder text messages was specifically designed to direct participants to the screening center and appointment system, which may have contributed to the increased uptake of Pap smear testing.

In our study, the proportion of participants who underwent a Pap smear test increased in the training group at the post-test. Similarly, Løkke et al. (13) reported in their study that

training was effective in increasing the rate of Pap smear testing. In another study, an educational intervention focused on cervical cancer in a rural area led to an increase in the screening uptake (16).

In this study, both reminder text messages and training were found to be effective in increasing the proportion of women performing breast self-examination (BSE). Labrague et al. (17) reported in their study that mobile text message reminders increased women's knowledge about BSE, but no significant difference was found in the actual practice of BSE. In studies where BSE training interventions were implemented, it was reported that such training encouraged women to regularly perform BSE and significantly improved their knowledge levels (18,19). In the study conducted by Koirala et al. (20), the BSE training intervention was found to be effective in increasing participants' awareness. In our study, the demonstration of BSE using a model in the training group and the sending of an educational video to participants in the reminder text message group may have enhanced the effectiveness of the intervention.

In our study, the proportion of women who underwent mammography at the post-test was statistically significantly higher in both the reminder text message group and the training group. In studies where reminder text message interventions were used for breast cancer screening, Ntiri et al. (21) reported that text messages sent to encourage mammography among low-income African American women had a positive impact on screening participation. Similarly, in a study conducted by De Jesus et al. (22), among Latina immigrants using a specialized mobile health breast cancer education and text messaging intervention, it was found that participants' knowledge levels regarding breast cancer increased after the intervention, although no behavioral change was observed in mammography screening participation. These findings suggest that reminder text message interventions may yield different outcomes across various populations. In our study, the inclusion of a direct link to the screening center, location details, and appointment scheduling in the messages may have encouraged women to undergo mammography screening.

in the literature, studies involving educational interventions for mammography screening have shown positive outcomes. For instance, Kamyab et al. (23) conducted a study based on the Theory of Planned Behavior, aiming to promote early detection and screening of breast cancer among women living in rural areas. The intervention effectively encouraged women in rural communities to value early cancer screening behaviors and undergo timely breast cancer screening. These findings are consistent with those of Zafar et al. (24), who also reported the effectiveness of community education in increasing breast cancer screening participation.

In light of this evidence, our study similarly found that women placed greater importance on early cancer screening behaviors, and the educational intervention was effective in promoting breast cancer screening. The content delivered to the training group—such as explanations about the mammography procedure and facilitating appointment scheduling at the participants' convenience—may have contributed to increased participation. In addition, studies by Yusuf et al. (25), which utilized mobile applications to deliver health education on breast cancer, and by Cueva et al. (26), which employed webinar-based education for breast, cervical, and colorectal cancer, have reported these methods to be effective in enhancing knowledge and promoting screening behavior. In our study, the group education sessions and distribution of informative brochures containing links to the appointment system may have encouraged women to schedule mammography appointments.

In our study, the proportion of participants who completed the fecal occult blood test (FOBT) at post-test was statistically significantly higher in both the reminder text message group and the training group. In a study by Vives et al. (27) , which compared reminder text messages and postal reminders to improve participation in colorectal cancer screening programs, it was reported that these interventions had a moderate effect on increasing participation. Hirst et al. (28) found that while reminder text messages did not lead to a general increase in colorectal cancer screening in the overall population, they had a positive impact among individuals invited to screening for the first time. In our study, sending an instructional video on how to perform the FOBT to the reminder text message group and supporting the training group with both verbal and visual explanations may have encouraged women to engage in cancer screening behavior.

This study has several limitations that should be considered when interpreting the findings. First, the mean ages of the study groups were not equivalent; the reminder text message group had a mean age of 39.7 years, the training group 42.2 years, and the control group 46.2 years. Although this difference was statistically significant, we do not believe it had a meaningful impact on participants' attitudes or behaviors toward cancer screening tests. Second, post-test measurements were conducted three months after the interventions, with no subsequent follow-up assessments performed beyond this point due to the limited study duration and logistical challenges associated with participant tracking. Finally, due to privacy regulations, it was not possible to access participants' health records; as a result, the determination of whether participants underwent screening tests was based solely on self-reported data, which may be subject to recall or reporting bias.

5. CONCLUSION

The results of this study indicate that both reminder text messages and training interventions significantly improved cancer early screening behaviors among women. Notably, reminder text messages were found to be as effective as training in promoting participation in Pap smear testing, breast self-examination, mammography, and fecal occult blood testing. Given their practicality, cost-effectiveness, and time-saving nature, reminder text messages offer a viable alternative or complement to traditional education-based

interventions. Integrating such digital approaches into public health strategies may enhance accessibility and adherence to cancer screening programs, especially in resource-limited or time-constrained settings. Future research should explore the long-term impact and scalability of these interventions across diverse populations.

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Clinical and Experimental Health Sciences

The Role of The Tissue-Level Yap–Taz Pathway in Glioma Development

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ABSTRACT

Objective:The aim of this research is to evaluate the tissue-level expression of LATS1, YAP1, PI3KCG, and WWTR1 genes, which are key components of the Hippo-YAP/TAZ signaling pathway, in relation to glioma development.

Methods:This research included tissue samples collected from 30 patients aged between 18 and 80 years who underwent neurosurgical resection at the institution's affiliated hospital with a diagnosis of glioma. Tumor tissue samples were processed for total RNA isolation. Complementary DNA (cDNA) synthesis was subsequently performed, followed by quantitative polymerase chain reaction (qPCR) analysis to assess the relative expression levels of the selected genes. All procedures were conducted in compliance with standardized molecular protocols, and data were statistically analyzed using appropriate methods to determine expression differences.

Results:Among the genes analyzed, YAP1 demonstrated a statistically significant 2.6-fold downregulation in glioma tissues compared to adjacent non-tumoral tissues ($p = 0.03$). Expression changes in other genes were observed, but did not reach statistical significance within the scope of this study.

Conclusion:Our findings suggest that YAP1 may play a critical role in glioma pathogenesis. The observed downregulation indicates a potential dysregulation of the Hippo-YAP/TAZ signaling pathway in tumor development. These results underscore the importance of further investigating YAP1 and related signaling components as potential therapeutic targets in glioma and other central nervous system tumors. Future studies with larger patient cohorts and functional analyses are warranted to validate these preliminary findings.

Keywords:Glioma, YAP/TAZ, Hippo signaling pathway, gene expression

1. INTRODUCTION

Gliomas represent the most common and aggressive group of primary brain tumors in adults, accounting for approximately 80% of all malignant brain neoplasms (1,2). Despite advances in treatment strategies, the prognosis for high-grade gliomas remains poor, with a median survival of only 14–16 months following diagnosis (3,4). This reality necessitates a deeper understanding of glioma biology at the molecular level and the development of novel biomarkers (5,6).

A variety of intracellular signaling pathways are known to play a role in glioma development and progression, including PI3K/AKT/mTOR, MAPK/ERK (7), p53 (8), RB (9), the Wnt/β-catenin signaling pathway (10), Hedgehog (11), and NF-κB (12).

In recent years, the Hippo-YAP/TAZ pathway, whose key components include Yes-associated protein (YAP) and PDZ-binding motif (TAZ/WWTR1), has gained attention for its role in glioma development (13–16). In this pathway, YAP and TAZ/WWTR1 are phosphorylated by LATS1/2 and thereby inactivated; when the pathway is suppressed, they translocate into the nucleus and activate genes that promote cell proliferation and survival (17–23). Although aberrant activity of YAP/TAZ has been associated with various cancers, their specific roles in gliomas remain unclear (23–28). The available data are conflicting and are mostly derived from in vitro experiments or animal models (29–35). Therefore, investigating the expression of Hippo pathway-related genes in human glioma tissues may help elucidate the underlying

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molecular mechanisms (33–38). The generation of such new scientific data is essential not only to clarify the biological roles of these genes in glioma pathogenesis, but also to assess their potential as biomarkers for diagnosis, prognosis, and prediction of therapeutic response.

In this context, the aim of our study is to analyze the tissue-level expression of the YAP1, WWTR1, LATS1, and PI3KCG genes in tumor samples surgically resected from adult glioma patients using quantitative PCR, in order to evaluate potential alterations in the Hippo-YAP/TAZ signaling pathway and to elucidate its possible role in glioma development.

Table 1 presents a total of 45 glioma-associated genes retrieved from the Human Phenotype Ontology (HPO), listed in the format of Gene Symbol followed by NCBI Gene ID.

Table 1. *A total of 45 glioma-associated genes listed in the Human Phenotype Ontology (HPO) Gene Symbol (NCBI Gene ID)*

NF2 (4771)	IFNG (3458)	PMS2 (5395)
APC (324)	TSC2 (7249)	TGFBR2 (7048)
ZFTA (65998)	ERBB2 (2064)	KRAS (3845)
TSC1(7248)	IDH1 (3417)	EPCAM (4072)
MDM2 (4193)	NF1 (4763)	PIK3CA (5290)
CHEK2 (11200)	RPS20 (6224)	PMS1 (5378)
CDKN2A (1029)	MUTYH (4595)	PTEN (5728)
TP53 (7157)	POLD1 (5424)	IDH2 (3418)
MLH1 (4292)	SEMA4A (64218)	MSH3 (4437)
CDKN1B (1027)	BMPR1A (657)	FGFR1 (2260)
MEN1(4221)	ATM (472)	NSD1 (64324)
CDKN2B (1030)	BRCA2 (675)	APC2 (10297)
CDKN2C (1031)	POLE (5426)	NBN (4683)
CDKN1A (1026)	MSH6 (2956)	SMO (6608)
SETBP1 (26040)	MSH2 (4436)	SPRED1 (161742)

2. METHODS

2.1.Ethical Approval and Informed Consent

This case-control study was approved by the Ethics Committee for Non-Drug and Medical Device Research of the Marmara University Faculty of Medicine on September 20, 2024 (Protocol No: 09.2024.1069), in accordance with international ethical standards and guidelines. Written informed consent was obtained from all participants prior to enrollment, documented with wet signatures. Participants provided explicit consent for both participation and the use of their data. The anonymity and confidentiality of all study participants were rigorously protected throughout the research process.

2.2. Inclusion and Exclusion Criteria

The study included a total of 30 glioma patients and 20 control individuals. Glioma group samples were obtained from patients aged 18–80 years who underwent neurosurgical resection at the institution's affiliated hospital, had no prior history of recurrence, and had not received chemotherapy or radiotherapy before surgery. Control samples consisted of dura mater tissues collected as surgical waste from patients undergoing neurosurgical procedures for non-neoplastic conditions (e.g., acute subdural hematoma, aneurysm, hemangioblastoma), with no pathological evidence of tumor. Samples with insufficient RNA and/or cDNA quantity or quality, or samples in which the target genes could not be amplified via qPCR, were excluded from the study.

2.3. Tissue Collection

Tumor tissues obtained from patients with a preoperative diagnosis of glioma, later confirmed by histopathological evaluation, were weighed using a precision balance. Specimens were stored at +4°C in RNAlater solution until the RNA isolation step.

2.4. RNA Isolation from Tissue Samples

Total RNA was isolated using RNAzol® RT reagent. Tissue samples were mechanically fragmented, transferred to tubes with ceramic beads, and treated with 1 mL of RNAzol® RT. Homogenization was performed using the MagNA Lyser® system at 7000 rpm for two 15-second cycles. The supernatant was transferred to 2 mL tubes, mixed with 0.4 mL RNase-free water, vortexed, and incubated for another 15 minutes. Following centrifugation at 12,000 × g for 15 minutes to remove DNA and proteins, the supernatant was mixed with 0.4 mL of 75% ethanol and incubated for 10 minutes. mRNA was precipitated by centrifugation at 12,000 × g for 8 minutes. RNA pellets were washed twice with 75% ethanol and centrifuged at 4000 × g for 1 minute. After ethanol evaporation, pellets were dissolved in 30 µL of nuclease-free water. RNA concentration and purity were assessed using a NanoDrop™ 2000/2000c spectrophotometer. Samples were stored at –80°C until cDNA synthesis.

2.5. cDNA Synthesis

Complementary DNA (cDNA) synthesis was performed using a commercial kit (OneScript® Plus cDNA Synthesis Kit, Applied Biological Materials [abm], Canada) with an input concentration of 200 ng/µL of total RNA. Reaction components and their respective volumes are provided in Table 2, and the total reaction volume was adjusted to 20 µL with nuclease-free water. The reverse transcription reaction was carried out in a T100 Thermal Cycler under the following optimized conditions: 15 minutes at 53°C, followed by 5 minutes at 85°C. The concentration and purity of synthesized cDNA were verified using the NanoDrop™ 2000/2000c. Samples were stored at -20°C until use in RT-qPCR.

Table 2. *Component volumes for cDNA synthesis using the OneScript® Plus cDNA Synthesis Kit*

Component	Amount
5× RT Buffer	4 µL
dNTPs	1 µL
Oligo Primer	1 µL
mRNA	Adjusted to 200 ng/µL
OneScript Plus RTase	1 µL
Nuclease-Free Water	To a final volume of 20 µL

2.6 Quantification of Gene Expression by Real-Time PCR (RT-qPCR)

Following cDNA synthesis, the expression levels of LATS1, YAP1, PI3KCG, and WWTR1 genes were quantified using a commercial real-time PCR kit (Blastaq™ 2X qPCR MasterMix, Applied Biological Materials [abm], Canada), in accordance with the manufacturer's protocol. The reaction components and volumes are listed in Table 3. GAPDH was used as the internal reference gene. Template cDNA was diluted to contain 100 ng/µL per reaction. All RT-qPCR analyses were performed on a BIO-RAD® CFX96 Real-Time PCR Detection System. Thermal cycling conditions are detailed in Table 4.

Table 3. *Components and volumes used for gene expression analysis with the Blastaq™ 2X qPCR MasterMix kit*

Component	Amount (per reaction)
BlasTaq™ 2X qPCR MasterMix	10 µL
Forward Primer (10 µM)	0.5 µL
Reverse Primer (10 µM)	0.5 µL
cDNA Template	1 µL
Nuclease-Free Water	Up to 20 µL total volume

Table 4. *RT-qPCR reaction conditions*

Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	3 minutes	1 cycle
Denaturation	95 °C	15 seconds	
Annealing/Extension	60 °C	1 minute	40 cycles
Melt Curve Analysis	65 °C → 95 °C	0.5 °C increment every 5 seconds	1 cycle

2.7 Statistical Analysis

Comparative expression analyses were performed between the glioma and control groups based on cycle threshold (Ct) values for target and reference genes. A Ct cut-off value of 37 was defined; samples exceeding this threshold were repeated to ensure data reliability. For each sample, ΔCt values were calculated by subtracting the Ct of GAPDH from that of the target gene. Then, mean ΔCt values of the control group were used as calibrators to calculate ΔΔCt values for the glioma samples. The ΔΔCt values were further analyzed using the 2^-ΔΔCt method to determine fold changes in gene expression. All calculations were performed using GeneGlobe software, and raw data were recorded in Microsoft Excel for statistical analysis. The statistical significance of the study was evaluated based on p values, while additional measures supporting clinical relevance such as fold regulation and fold change effect sizes, are also presented in Table 5.

Table 5. *Expression analysis results demonstrating significant downregulation of the YAP1 gene in glioma patients*

Gene Symbol	Fold Regulation	Fold Change	p-Value
LATS1	-1.42	0.71	0.970677
YAP1	-2.60	0.39	0.033092
PI3KCG	-2.39	0.42	0.253500
WWTR1	-1.32	0.76	0.669688
GAPDH (Housekeeping)	1.00	1.00	***N/A**

(*) The designation of "N/A" (Not applicable) for the p-value in the GAPDH (Housekeeping) row is due to its role as the reference/normalizing gene. Since GAPDH expression is fixed at 1.00 for both fold regulation and fold change, no statistical test is applicable; therefore, a p-value is not computed.

3. RESULTS

Quantitative gene expression analysis performed using GeneGlobe software revealed a fold change value of 0.39 for the YAP1 gene, indicating an approximate 61% reduction in expression compared to the control group. This substantial decrease supports the downregulation of YAP1 in glioma tissue samples. Furthermore, the corresponding fold regulation value of –2.60 reinforces the interpretation that YAP1 expression is significantly suppressed in glioma patients.

Importantly, the p-value of 0.033092 confirms that this difference is statistically significant, suggesting that the observed downregulation is unlikely to be due to random variation.

The p values for the other genes were calculated as follows: LATS1: 0.970677, PI3KCG: 0.253500, and WWTR1: 0.669688. Collectively, the data summarized in Table 5 demonstrate a significant downregulation of YAP1 gene expression in glioma patients, supporting its potential involvement in glioma pathogenesis.

4. DISCUSSION

In this study, we investigated the role of the Hippo-YAP/TAZ signaling pathway in glioma pathogenesis through targeted gene expression analysis. Our findings demonstrated a statistically significant downregulation of the YAP1 gene in glioma patients. Analysis via GeneGlobe software revealed a fold change of 0.39, a fold regulation of –2.60, and a p-value of 0.033092, collectively indicating a marked and statistically meaningful reduction in YAP1 expression in tumor tissues compared to healthy controls. While YAP1 has been widely recognized for its oncogenic potential in various solid tumors, its function in central nervous system malignancies, particularly gliomas, appears to be more context-dependent. The significant downregulation observed in our cohort supports the hypothesis that YAP1 may exhibit a tumor-suppressive role in glioma biology, in contrast to its well-established oncogenic role in other cancers.

Given the complex interplay between Hippo pathway components and their potential crosstalk with other

oncogenic signaling cascades (e.g., PI3K/AKT, MAPK/ERK), a more comprehensive analysis of the entire pathway and its regulatory networks is warranted. The variability in the expression and function of YAP/TAZ across tumor types and within glioma subtypes highlights the necessity for context-specific investigations rather than generalized assumptions based on other malignancies.

In contrast to our findings, the 2012 study by Ji et al. reported a significant association between reduced LATS1 gene expression and glioma progression; specifically, lower LATS1 levels were observed in high-grade gliomas, highlighting its potential tumor suppressor role (39). Additionally, in the 2016 study by Li et al., TAZ (WWTR1) expression was found to be upregulated in glioma samples and positively correlated with tumor grade (40). In other cancer types, LATS1 has been associated with breast cancer and head and neck squamous cell carcinomas; PI3KCG has been shown to exhibit hyperactivation in solid tumors such as colorectal and gastric cancers; and increased expression of WWTR1 (TAZ) has been linked to metastasis in a wide range of cancers including melanoma, head and neck, breast, and lung cancers.

Our study provides novel, tissue-level evidence regarding YAP1 gene expression in glioma patients**,** contributing to the growing body of literature on Hippo signaling in neuro-oncology. However, to validate and expand upon these preliminary findings, future research should include larger patient cohorts**,** comparative analysis across glioma subtypes**,** and integrative assessment of additional Hippo pathway components**.** Such investigations will be critical to fully elucidate the functional implications of YAP1 dysregulation and its potential utility as a biomarker or therapeutic target in glioma.

5. CONCLUSION

This study examined the expression of YAP1, a central Hippo-YAP/TAZ pathway component, in glioma tissues and found it significantly downregulated compared to healthy controls. These results suggest a potential tumor-suppressive role for YAP1 in glioma, contrasting its established oncogenic role in other solid tumors and indicating its function may vary by tumor subtype and biological context.

The reduced YAP1 expression highlights glioma's molecular heterogeneity and implies that the Hippo pathway may act in a context-dependent manner, modulated by tumor type and microenvironment. Thus, the YAP-TAZ axis should be reconsidered not only as an oncogenic driver but as a modulator of tumor behavior in the CNS.

Though preliminary, these findings offer important insight into glioma pathogenesis. Validation through larger cohorts, subtype-specific analyses, and comprehensive evaluation of Hippo components is essential to clarify the clinical relevance and therapeutic potential of YAP1 dysregulation in neuro-oncology.

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Clinical and Experimental Health Sciences

Effect of Abdominal Massage on Abdominal Distension, Pain, Comfort, and Patient Satisfaction After Colonoscopy: A Randomized Controlled Trial

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ABSTRACT

Objective: This study aimed to determine the effect of abdominal massage on post-colonoscopy abdominal distension, pain, comfort, and patient satisfaction.

Methods: The randomized controlled study was conducted with 166 patients who underwent colonoscopy without sedation. No intervention was applied to the control group (n= 83). The experimental group (n= 83) received an abdominal massage for 15 minutes on the day of colonoscopy (day 0), after the procedure, in the evening on day 0, and in the morning and evening on day 1. Abdominal distension, pain, comfort and satisfaction were assessed with Visual Analogue Scale.

Results: In the experimental group, abdominal distension and pain level decreased and comfort level increased after abdominal massage compared to the pre-massage level ($p<.01$). In addition, compared to the control group, the abdominal distension and pain level of the patients in the experimental group were substantially lower, and their abdominal comfort level and satisfaction with the procedure were substantially higher ($p<.01$).

Conclusion: Abdominal massage after colonoscopy reduced abdominal distension and pain, increased abdominal comfort and patient satisfaction with the procedure.

Keywords: Colonoscopy, abdominal pain, patient satisfaction, randomised controlled trial, nursing

1. INTRODUCTION

Abdominal pain, abdominal discomfort, and bloating are problems that patients frequently experience after colonoscopy (1,2). These problems may be caused by factors such as the experience of the endoscopist, distension caused by air insufflation, the technical difficulty level of the procedure, and the duration of the procedure. These problems, observed in approximately 60% of patients, may persist for several days after the procedure (2,3). A study revealed that 23% of patients experienced these side effects during a one-month follow-up following colonoscopy, and 6% were not able to continue working as a result (1). These minor side effects may lead to mental and physical stress and require the close follow-up of patients after the procedure (1,3). This increases patients' care requirements and observation time, resulting in higher health costs (2,4). Furthermore, patients' satisfaction with the colonoscopy procedure can influence their willingness to adhere to recommended screening programs. Therefore, the acceptance of colonoscopy for colorectal cancer screening can be increased by reducing abdominal distension and pain after colonoscopy and improving abdominal comfort and patient satisfaction with the procedure (2).

Studies conducted to reduce abdominal symptoms after colonoscopy and increase abdominal comfort and patient satisfaction with the procedure have reported varying outcomes. For example, while one study determined that rectal tube application after colonoscopy did not

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affect patients' abdominal distension, pain, comfort, and satisfaction after the procedure and 24 hours after the procedure (5). Another study found that rectal aspiration applied immediately after colonoscopy was more effective in reducing abdominal symptoms than rectal tube placement after the procedure (2). Another study in which total colonic decompression was performed by repeating caecal intubation following colonoscopy revealed an 86.1% decrease in the abdominal pain of the patients and a significant difference in abdominal pain 24-48 hours after the procedure between the patients who did and did not receive the intervention (6). In contrast, there are also researchers indicating no significant difference in pain intensity between the group in which air aspiration was performed from the flexion points of the colon and the group in which it was performed from the rectum alone (4).

The literature reports that massage applied to the abdominal region relaxes the muscles and stimulates bowel movements, thereby promoting elimination (7). Furthermore, it is noted that it provides relief by reducing discomfort and pain in healthy/ill individuals with abdominal pain associated with cramps or flatulence, thereby improving quality of life (8). Abdominal massage is a non-invasive, non-pharmacological, safe method with no known side effects. Furthermore, it does not require any cost (7) and is defined as an intervention that individuals can perform themselves with training (9). Although many invasive (2,3), and non-invasive (10,11) methods have been tried to reduce abdominal symptoms experienced after colonoscopy and to increase comfort and patient satisfaction, there is a scarcity of studies assessing the effect of abdominal massage on colonoscopy-related abdominal symptoms (11). In this context, this study was conducted to evaluate the effect of abdominal massage, one of the independent nursing interventions after colonoscopy, on distension, pain, comfort, and patient satisfaction with the procedure.

2. METHODS

2.1. Study Design

This study was designed as a two-group, randomised, controlled study. The study was conducted from November 2020 to May 2021 at the endoscopy unit of a tertiary public hospital located in Istanbul, Türkiye.

2.2. Sampling

The number of individuals required to be included in the study was calculated by power analysis using G*Power (v3.1.7) software. The power of the study was expressed as 1-β (β = probability of type II error). It is generally accepted that studies should have a power of at least 80%. According to Cohen's effect size coefficients (12), the calculation made assuming that the evaluations between two independent groups would have a medium effect size (d = 0.50) revealed that the minimum number of participants should be 134 (67 in each group) to obtain 80% power at the α = 0.05 level. Considering possible data loss, 90 individuals were included in each group.

Patients who were planned to undergo outpatient colonoscopy without sedation for diagnostic and screening purposes, aged 18 years or older, with a body mass index (BMI)<30 were included in the study. Individuals who had undergone bowel resection and those who underwent colonoscopy for therapeutic purposes were excluded from this study. Furthermore, individuals who underwent colonoscopy under sedation were also excluded because abdominal distension, pain, and comfort could not be objectively assessed due to the effects of sedation (13). In addition, patients whose colonoscopy procedure could not be completed due to insufficient bowel cleansing, those who withdrew from the study, and those who did not comply with the study process and requirements were excluded from the sample.

Patients who met the study inclusion criteria were randomized into experimental and control groups using a random number generator (14). Seven patients in the experimental group (four patients whose colonoscopy procedure was incomplete, one patient at risk of bleeding, and two patients who did not submit the forms) and seven patients in the control group (five patients whose colonoscopy procedure was incomplete and two patients who did not submit the forms) were excluded from the study. A power analysis was performed and the study was completed with 166 patients (83 in each group). Figure 1 presents the CONSORT flow diagram of the study.

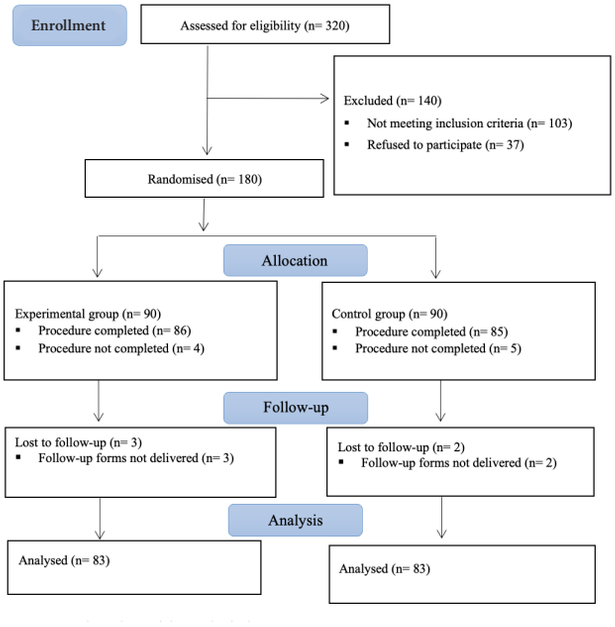

Figure 1. *Flow chart of the study design*

2.3. Blinding

Due to the feasibility and nature of the study, blinding could not be applied to the principal investigator and patients during the allocation process; however, blinding of the evaluator to the groups was ensured.

2.4. Instruments

2.4.1. Descriptive and Clinical Characteristics Form

This form, prepared in accordance with the literature, consists of 26 items. The form included patients' demographic characteristics (gender, age, BMI, marital and educational status) and colonoscopy-related characteristics (colonoscopy history and indication, pre-procedure information status, colonoscopy start and end times, and procedure duration), as well as telephone numbers for communication (2,5).

2.4.2. Visual Analogue Scale (VAS)

The VAS consists of a 10-cm line drawn vertically or horizontally. At either end of this line are the two extreme descriptive words of the subjective category (0= "no feeling at all" and 10= "worst feeling ever experienced"). The patient is instructed to indicate the intensity of their emotion by putting a cross on the line (15).

2.4.3. Follow-up Forms

In the experimental group, abdominal symptoms and comfort levels were recorded on the form after colonoscopy (day 0) both in the unit and at home, as well as before and after abdominal massage on the morning and evening of day 1. In the control group, abdominal symptoms and comfort levels were recorded on the form after colonoscopy (day 0) both in the unit and at home, and in the morning and evening of day 1. In addition, at the end of day 1, the level of satisfaction with the procedure was also recorded for both groups.

2.5. Pre-Implementation

In order to determine the comprehensibility and usability of the forms, a pre-implementation was conducted between 4 November 2020 and 6 November 2020 at the Endoscopy Unit of the hospital where the research was conducted with 18 patients (experimental group: 9, control group: 9) who met the inclusion criteria for the study. No issues were identified in the operation of the data collection forms during this process. The patients included in the pre-application were not included in the study.

2.6. Intervention

The experimental group received an abdominal massage for 15 minutes on the day of colonoscopy (day 0), after the procedure, in the evening on day 0, and in the morning and evening on day 1. Abdominal massage was performed in accordance with the instructions "Abdominal Massage Application Directive" developed by Uysal, Eşer, and Akpınar in Türkiye (16). Massage movements included three basic manoeuvres: effleurage, petrissage, and vibration (17). The incidence of abdominal distension and pain is highest within the first 24–48 hours following colonoscopy (18). Therefore, patients were monitored for two days (the day of the procedure and the day after the procedure) in this study.

2.6.1. Experimental group

The first face-to-face interview with the experimental group was undertaken one hour before the colonoscopy procedure by the researcher, who is the first author. During this interview, the descriptive and clinical characteristics form was completed, and abdominal massage training was given. In addition, the patients and/or their relatives were informed about the use of the VAS and the follow-up forms.

The second face-to-face consultation with the patient and/or their relative was conducted in the observation room immediately after the colonoscopy. During this consultation, patients' abdominal distension, pain, and comfort levels were assessed using the VAS and recorded on the patient follow-up form. The first abdominal massage was performed by the researcher. In order to benefit sufficiently from the decompression effect of abdominal massage, 30 minutes after the massage was completed (19), the patients' abdominal distension, pain and comfort levels were assessed using the VAS and recorded. Additionally, the 'Abdominal Massage Education Form,' 'VAS,' and 'Patient Follow-up Form' were provided to the patient and/or their relative prior to discharge from the unit. The second abdominal massage was performed by the patient and/or their relative at home on the evening of the day the colonoscopy was performed (day 0). The third and fourth massages were performed by the patient and/or their relative on the morning and evening of the first day after colonoscopy. Before each application and 30 minutes after the application, the patient's abdominal distension, pain, and comfort levels, as well as their satisfaction levels at the end of the first day, were marked on the VAS by the patient and/or their relative. On the evening of day 0 after colonoscopy, telephone contact was made with the patient and/or their relative to support the massage training and to collect the forms given on the evening of day 1. The forms were collected on the evening of day 1. The markings made on the VAS by the patient and/or their relative were evaluated by the first author of this study.

2.6.2. Control group

The first face-to-face interview with the control group was held one hour before the colonoscopy. During this meeting, the descriptive and clinical characteristics form was completed, and the patients and/or their relatives were informed about the use of VAS and the patient follow-up forms.

Table 1. *Descriptive and clinical characteristics of the groups (N=166)*

Variable		Control Group (n = 83)	Experimental Group (n = 83)	Test Statistic	p value
Gender	Female	41 (49.4)	40 (48.2)	χ^2: 0.024[a]	.877
	Male	42 (50.6)	43 (51.8)		
Age (years)	Mean ± SD	49.55 ± 13.09	50.45 ± 11.28	Z: – 0.373[c]	.709
	Median (min-max)	50 (18-69)	53 (19-68)		
	Median (min-max)	78 (46-100)	75 (50-100)		
Body mass index BMI (kg/m^2)	Mean ± SD	26.68 ± 2.64	26.48 ± 2.54	Z: – 0.835[c]	.404
	Median (min-max)	27.4 (19.4-29.7)	27.2 (19-29.8)		
Marital status	Married	73 (88.0)	71 (85.5)	χ^2: 0.210[a]	.647
	Single	10 (12.0)	12 (14.5)		
Education level	Primary school	45 (54.2)	49 (59)	χ^2: 2.924[b]	.579
	Middle school	11 (13.3)	10 (12.1)		
	High school	14 (16.9)	17 (20.5)		
	University	13 (15.7)	7 (8.4)		
History of colonoscopy	Absent	58 (69.9)	61 (73.5)	χ^2: 0.382[b]	.930
	Present	25 (30.1)	22 (26.5)		
	Once	19 (22.9)	16 (19.3)		
	Twice or more	6 (7.2)	6 (7.2)		
Information about colonoscopy before procedure	Uninformed	4 (4.8)	4 (4.8)	χ^2: 0.000[e]	1.000
	Informed	79 (95.2)	79 (95.2)		
Colonoscopy indication	Rectal bleeding	23 (27.7)	16 (19.3)	χ^2: 3.943[b]	.737
	Change in bowel habits	15 (18.1)	19 (22.9)		
	Anaemia	15 (18.1)	15 (18.1)		
	Abdominal pain	12 (14.5)	10 (12.0)		
	Weight loss	10 (12.0)	14 (16.9)		
	FOB positivity	8 (9.6)	9 (10.8)		
Purpose of colonoscopy	Diagnosis	74 (89.2)	72 (86.7)	χ^2: 0.227[a]	.633
	Control	9 (10.8)	11 (13.3)		
Caecal intubation time (minute)	Mean ± SD	9.24 ± 2.74	9.02 ± 2.82	t: – 0.503[d]	.616
	Median (min-max)	4 (2-15)	4 (2-10)		
Colonoscope exit time (minute)	Mean ± SD	4.34 ± 2.13	4.29 ± 1.53	Z: – 0.413[c]	.680
	Median (min-max)	4 (2-15)	4 (2-10)		
Total procedure time (minute)	Mean ± SD	13.58 ± 3.19	13.31 ± 3	t: – 0.552[d]	.582
	Median (min-max)	13 (8-22)	13 (7-22)		

[a]Chi-square test, bFisher-Freeman-Halton test, cMann-Whitney U test, dStudent t-test, eFisher's exact test
SD: standard deviation, min: minimum, max: maximum, BMI: body mass index; FOB: faecal occult blood

The second face-to-face interview with patients was conducted in the observation room after colonoscopy. During this interview, the abdominal distension, pain and comfort levels of the experimental group patients were assessed using the VAS 45 minutes after colonoscopy, simultaneously with the control group (15 minutes of massage and 30 minutes of massage effect duration). Before discharge from the unit, patients were given the 'VAS' and 'Patient Follow-up Form' and any questions they had regarding their use were explained. On the evening of the colonoscopy day (day 0) and on the morning and evening of the first day after colonoscopy, abdominal distension, pain and comfort levels were marked on the VAS by the patient and/or their relative, and satisfaction levels were marked at the end of the first day. Patients were contacted by telephone on the evening of day 0 after colonoscopy to provide support and on the evening of day 1 to collect the patient follow-up forms. The forms were collected on the evening of day 1. The markings made on the VAS by the patient and/or their relative were evaluated by the first author of this study.

2.7. Ethical Considerations

Approval for the study was obtained from the Clinical Research Ethics Committee of the Marmara University Faculty of Medicine (date: 24.07.2020, protocol number: 09.2020.885) and the hospital management where the study was conducted (date: 13.07.2020). Informed verbal and written consent were received from the patients participating in the study. The study was conducted by

Table 2. *Abdominal distension scores of the groups (N=166)*

Evaluation time	Abdominal Distension				Test Statistic	p value
	Control Group (n = 83)		Experimental Group (n = 83)			
	Mean ± SD	*Median (Min-max)*	*Mean ± SD*	*Median (Min-max)*		
Day 0 (45 minutes after colonoscopy)	3.31 ± 1.17	3.1 (1-7.2)	1.87 ± 0.72	1.8 (0.5-4)	Z: 8.267[c]	*.001***
Day 0 evening	1.85 ± 0.81	1.8 (0.5-4.4)	0.79 ± 0.48	0.8 (0-2)	Z: 8.371[c]	*.001***
Day 1 morning	1.02 ± 0.53	1 (0-2.7)	0.35 ± 0.24	0.4 (0-1)	Z: 8.706[c]	*.001***
Day 1 evening	0.43 ± 0.31	0.4 (0-1.5)	0.08 ± 0.14	0 (0-0.6)	Z: 8.282[c]	*.001***

[c]*Mann-Whitney U test, **p<.01*
SD: standard deviation, min: minimum, max: maximum

adhering to the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act. The study was registered at ClinicalTrials.gov (ClinicalTrials.gov ID NCT04979351), and no modifications were made to the study protocol after its initiation.

2.8. Statistical Analysis

The Number Cruncher Statistical System (NCSS) 2007 program was used for the statistical analysis of the data collected in the study. Descriptive statistics, independent-samples Student t-test, Mann-Whitney U test, Wilcoxon signed-rank test, Pearson chi-square, Fisher's exact, and Fisher-Freeman-Halton exact tests were used in the analysis of the data. Statistical significance was accepted as p<.05.

3. RESULTS

3.1. Participants

The experimental and control groups were similar in terms of descriptive and clinical characteristics (p>.05) (Table 1).

3.2. Abdominal Distension

In the experimental group, the mean abdominal distension score evaluated by the VAS was 3.6±0.97 after colonoscopy and 1.87±0.72 after the first abdominal massage (p=.001). It was observed that the mean abdominal distension score decreased by 0.61 units after the massage applied in the evening of day 0 (0.79±0.48) compared to the pre-massage score (1.4±0.69) (p=.001). In the same group, the mean abdominal distension score decreased significantly after massage compared to before massage in the morning of day 1 following colonoscopy (0.84±0.39 before massage vs. 0.35±0.24 after massage) and in the evening of day 1 following colonoscopy (0.49±0.28 before massage vs. 0.08±0.14 after massage) (p=.001).

The comparison of the mean abdominal distension scores between the experimental and control groups revealed that the mean abdominal distension scores obtained 45 minutes after colonoscopy, in the evening of day 0, and in the morning and evening of day 1 were significantly lower in the experimental group (p<.001) (Table 2).

3.3. Abdominal Pain

In the experimental group, the mean pain intensity score of the patients evaluated by the VAS was 3.99±1.14 after colonoscopy, decreasing to 1.95±0.69 after the first abdominal massage (p=.001). The mean pain intensity score of this group was also significantly lower after the abdominal massage applied in the evening of day 0 (0.63±0.35) compared to the pre-massage score (1.19±0.43) (p=.001). Similarly, the group's mean pain intensity scores decreased significantly after the massage application compared to before massage in the morning (0.56±0.28 before massage and 0.19±0.22 after massage) and evening (0.2±0.22 before

Table 3. *Pain intensity scores of the groups (N=166)*

Evaluation time	Pain Intensity				Test Statistic	p value
	Control Group (n = 83)		Experimental Group (n = 83)			
	Mean ± SD	*Median (Min-max)*	*Mean ± SD*	*Median (Min-max)*		
Day 0 (45 minutes after colonoscopy)	3.54 ± 1.09	3.4 (1.9-6.7)	1.95 ± 0.69	1.9 (0.7-5.0)	Z: – 9.133[c]	*.001***
Day 0 evening	1.85 ± 0.69	1.8 (0.4-5)	0.63 ± 0.35	0.6 (0-1.4)	Z: 10.125[c]	*.001***
Day 1 morning	0.9 ± 0.43	0.8 (0-2.1)	0.19 ± 0.22	0.2 (0-1.3)	Z: 9.965[c]	*.001***
Day 1 evening	0.29 ± 0.28	0.3 (0-1.3)	0.01 ± 0.04	0 (0-0.3)	Z: 8.514[c]	*.047**

[c]*Mann-Whitney U test, *p<.05, **p<.01*
SD: standard deviation, min: minimum, max: maximum

massage and 0.01±0.04 after massage) on day 1 following colonoscopy (p=.001).

The abdominal pain intensity scores of the experimental group were significantly lower than in the control group at all evaluation times (45 min after colonoscopy, evening of day 0, and morning and evening of day 1) (p<.05) (Table 3).

3.4. Abdominal Comfort

In the experimental group, the mean abdominal comfort score evaluated by VAS was 5.84±0.96 after colonoscopy, which significantly increased after the first abdominal massage (7.11±0.63) (p=.001). Similarly, the group's mean abdominal comfort scores increased significantly after the massage application compared to before massage in the evening on the day of colonoscopy (7.27±0.76 and 7.8±0.7), in the morning on the first day following colonoscopy (8±0.71 and 8.48±0.67), and in the evening on the first day following colonoscopy (8.62±0.72 and 9.28±0.52) (p=.001).

The abdominal comfort scores of the experimental group were significantly higher than those of the control group at all evaluation times (p<.001) (Table 4).

3.5. Satisfaction

When the satisfaction of the groups with the procedure obtained by VAS was investigated in the evening on the first day following colonoscopy, it was determined that the satisfaction level of the experimental group patients (9.10±0.59) was significantly higher than that of the control group patients (8.06±0.74) (p<.001) (Table 5).

Table 4. *Abdominal comfort scores of the groups (N=166)*

	Abdominal Comfort				*Test Statistic*	*p value*
	Control Group (n = 83)		Experimental Group (n = 83)			
Evaluation time	*Mean ± SD*	*Median (Min-max)*	*Mean ± SD*	*Median (Min-max)*		
Day 0 (45 minutes after colonoscopy)	5.65 ± 1.21	5.8 (1.2-8.7)	7.11 ± 0.63	7 (5.9-9.1)	Z: – 8.848[c]	*.001***
Day 0 evening	6.93 ± 0.94	6.7 (4.4-10)	7.8 ± 0.74	7.8 (6-10)	*Z: – 6.374*[c]	*.001***
Day 1 morning	7.52 ± 0.98	7.5 (6-10)	8.48 ± 0.67	8.5 (7-10)	Z: – 6.725[c]	*.001***
Day 1 evening	8.14 ± 0.77	8 (7-10)	9.28 ± 0.52	9.4 (8-10)	Z: – 8.161[c]	*.001***

[c]*Mann-Whitney U test, **p<.01*
SD: standard deviation, min: minimum, max: maximum

Table 5. *Group satisfaction level scores (N=166)*

	Satisfaction				**Test Statistic**	**p value**
	Control Group (n=83)		Experimental Group (n=83)			
Evaluation time	*Mean ± SD*	*Median (Min-max)*	*Mean ± SD*	*Median (Min-max)*		
Day 1 evening	8.06±0.74	8 (5.8-10)	9.10±0.59	9 (7.8-10)	Z:-8.137[c]	*.001***

[c]Mann Whitney U Test **p<.01

4. DISCUSSION

Findings of our study showed that abdominal massage applied to the patients in the experimental group immediately after colonoscopy, in the evening on the day of the procedure, and in the morning and evening on the first day following the procedure significantly reduced their abdominal distension levels. In addition, the abdominal distension levels of the experimental group were significantly lower than those of the control group 45 minutes after colonoscopy, in the evening on the day of the procedure, and in the morning and evening on the first day following the procedure. These findings provide evidence for the efficacy of abdominal massage applied after colonoscopy in reducing abdominal distension. Consistent with our findings, previous studies have reported that abdominal massage reduces abdominal distension. For example, abdominal massage has been reported to minimise abdominal distension in patients who underwent colonoscopy (11), intensive care patients who are enterally fed (8), patients staying in neurology/neurosurgery units who are intermittently fed enterally (20), and those fed with a nasogastric tube (21). In a systematic review and meta-analysis study examining the effect of abdominal massage on enteral nutrition complications in

adult patients receiving intensive care, it was reported that abdominal massage was effective in relieving abdominal symptoms and that there was a significant difference between the massage and non-massage groups in terms of abdominal distension levels (22). The positive effect of abdominal massage in reducing distension has also been shown by studies evaluating elderly patients who complained of constipation (23) and individuals receiving treatment for opioid-induced constipation (24). A meta-analysis study examining the efficacy of abdominal massage in relieving constipation stated that abdominal massage improved pain and distension by increasing the frequency of defecation, improved the quality of life of patients (25), and effectively diminished abdominal distension caused by the accumulation of malignant acid in terminally ill oncology patients (26). In the literature, it is emphasised that abdominal massage increases blood flow, stimulates the absorption of nutrients, and reduces abdominal distension by accelerating evacuation (20). Abdominal massage enhances intestinal peristalsis by altering the intra-abdominal pressure through its mechanical and reflexive effects. By employing this specific mechanism, it accelerates the passage of colon contents through the digestive tract and reduces abdominal distension (8,20,21). Accordingly, it is considered that in the current study, abdominal massage stimulated bowel movements and facilitated the release of air introduced during colonoscopy, thus contributing to the reduction of abdominal distension.

It is known that abdominal massage reduces abdominal distension and associated pain by improving digestive function and bowel movements (8,27). Consistent with the findings of the current study, previous research has demonstrated the efficacy of abdominal massage in reducing pain resulting from abdominal cramps caused by distension. For example, Mutlu et al. (2024) reported that abdominal massage after colonoscopy reduced the distension caused by the introduction of air during the procedure and lowered abdominal pain associated with distension. Randomised controlled studies conducted with individuals with constipation have found that abdominal massage reduces abdominal pain and constipation-related distension and thus improves the quality of life of patients (28,29). Abdominal massage has been shown to be an effective treatment for abdominal distension and gastrointestinal issues, such as abdominal pain, in patients with neurogenic bowel dysfunction resulting from multiple sclerosis (30) and spinal cord injury (27). The findings of the current study revealed that abdominal massage applied at different times after colonoscopy reduced abdominal pain. In addition, the observation that the pain intensity of the patients who received abdominal massage was significantly lower than that of the control group provided evidence for the efficacy of abdominal massage applied after colonoscopy in reducing abdominal pain. This may be a result of the abdominal massage applied after colonoscopy, which increases bowel movements and ensures the release of air introduced to the intestines during the procedure, thus reducing abdominal distension and pain due to distension.

Our findings showed that abdominal massage applied at different times after colonoscopy also significantly increased abdominal comfort. This finding provides evidence for the efficacy of abdominal massage in improving abdominal comfort. This is related to the effects of abdominal massage on relieving abdominal distension and decreasing pain intensity in patients. Similarly, studies conducted with different massage techniques and different patient groups have reported that massage applications increase patient comfort (31,32). For example, it has been determined that back massage reduces pain intensity and increases the comfort level of patients with congestive heart failure (33) and liver transplant patients, which has been attributed to this method increasing patient comfort by relieving pain through the relaxation of muscles and reduction of oedema (31). In addition, preoperative hand massage applied to elderly people living in nursing homes (32) and those scheduled to undergo cataract surgery (34) has been shown to reduce patients' anxiety, positively affect their vital signs, and increase their comfort levels. It has been emphasised that hand massage accelerates blood circulation by reducing the tension in muscles and providing relaxation and increases patient comfort by diminishing pain perception and transmission in the neuromatrix, i.e., brain regions responsible for pain sensation (31,32). Ensuring patient comfort is an important component of the quality of colonoscopy services and significantly contributes to the overall satisfaction of patients after the colonoscopy procedure (35,36). It also reflects the endoscopist's technique and the quality of the procedure. Patient comfort after colonoscopy is related to abdominal pain and distension. As pain and distension decrease, patient comfort increases (35).

In the current study, the patients who received abdominal massage were significantly more satisfied with the procedure than the controls. This provides evidence of the efficacy of abdominal massage applied after colonoscopy in increasing patients' satisfaction with the procedure. Post-colonoscopy abdominal massage increases the abdominal comfort of patients by reducing abdominal distension and pain. Consistent with our findings, previous studies have also shown that various massage treatments increase patient satisfaction. For instance, applying massage therapy to the hand and arm area of orthopaedic patients after surgery (37) or applying massage therapy to the back, shoulder, neck, hand, foot, or leg of patients after cardiac surgery can effectively provide relaxation by reducing pain, anxiety, and muscle tension, thereby enhancing patient satisfaction (38). It has also been reported that hand massage reduces anxiety levels and increase satisfaction levels among outpatients (39). In contrast, there is also a study reporting that despite the significant difference in pain and anxiety levels between the group that received foot massage after open heart surgery and the group that did not receive this intervention, there was no difference in patient satisfaction. Nevertheless, the authors suggested that any interaction

between patients and healthcare professionals for any reason could increase patient satisfaction; therefore, the positive effects of massage on patients should still be acknowledged (40).

Massage creates a physiological and psychological effect on the organism through systematic manipulation by stimulating soft tissues. It allows patients to relax during application and increases physical activity by relieving muscle spasm and pain. In addition, it helps patients relax by redirecting their focus (31,41,42). By means of tactile stimulation, it facilitates the enhancement of positive relationships with patients and improves behaviours and moods (31,41). For these reasons, massage is widely recognised as a practice that increases patient satisfaction by promoting courage, awareness, support, and participation (43).

4.1. Limitations of the Study

This study has certain limitations. The first limitation is that only patients who undergo outpatient colonoscopy without sedation were included in the study, and the effect of abdominal massage on abdominal distension, pain, comfort, and satisfaction with the procedure in patients who underwent colonoscopy under sedation was not investigated. Another limitation of the study is that, although patients and/or their relatives were instructed on abdominal massage before colonoscopy and were asked to perform the second, third, and fourth massage applications during discharge with standardised guidelines, it was not possible to observe whether the applications were performed correctly and on time. A further limitation of the study is that the data were obtained based on participants' self-reports using the VAS.

5. CONCLUSION

The findings of this study showed that abdominal massage applied after outpatient unsedated colonoscopy reduced abdominal distension and pain levels, and increased abdominal comfort and patient satisfaction. In this context, abdominal massage can be incorporated into the nursing care process and used to manage abdominal symptoms developing after colonoscopy. Furthermore, nurses can provide abdominal massage training to patients and their relatives, enabling them to participate in their own care.

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Clinical and Experimental Health Sciences

The Effect of Immunotherapy on Macro-Micronutrient Intake and Its Relationship with Anthropometric Measurements in Patients with Non-Small Cell Lung Cancer

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ABSTRACT

Objective: This study aims to investigate the effect of immunotherapy on macro – micronutrient intake and its relationship with anthropometric measurements in patients with non-small cell lung cancer (NSCLC).

Methods: The 24-hour dietary recall and anthropometric measurements were obtained from patients diagnosed with primary NSCLC receiving immunotherapy treatment at three time points (initial, 2 months, and 6 months) at Adana City Training and Research Hospital. Statistical analyses of the data were performed using SPSS v27.

Results: The study included 47 individuals (M:43, F:4) with a mean age of 64.55±8.19 years; however, the data of 30 individuals (M: 28, F: 2) (due to death/progression) who were able to complete immunotherapy treatment were evaluated. As a result of immunotherapy treatment, the median values of Water (g), Carbohydrate (g), Vitamins (A, B_1, B_2, C, B_{12}), Folate, Zinc, and the mean values of Energy (kcal), Protein (g), Fat (g), Fat (%), Fiber (g), Vitamin E, Potassium, Calcium, Magnesium, Phosphorus, Iron, in the interim and final measurements were found to be higher ($p<.05$) than the initial measurement values. The mean Protein (%) at the interim measurement was found to be statistically higher than the mean of the final and initial measurements ($p<.05$). The mean of the final BMI (kg/m^2), Body Fat Percentage (%) and Lean Body Mass (kg) measurements was found to be statistically higher than the mean of the interim and initial measurements ($p<.05$).

Conclusion: During the immunotherapy treatment process, macro and micronutrient intake and anthropometric measurements increased in individuals diagnosed with NSCLC.

Keywords: Immunotherapy, Nutritional status, Anthropometric measurements, Non-small cell lung cancer

1. INTRODUCTION

Lung cancer is by far the leading cause of cancer-related deaths worldwide. Each year, approximately 2.2 million new cases of lung cancer are diagnosed globally, and an estimated 1.8 million of these cases result in death annually (1). Lung cancer, or bronchogenic carcinoma, is a proliferative malignant neoplasm originating from the primary respiratory epithelium (2). Histologically, lung cancers are classified into two main categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (3).

In lung cancer, the most critical step in determining treatment strategies and predicting prognosis is to determine the stage of the disease (4). Stage III/IV NSCLC represents the locally advanced stage of the tumor and accounts for approximately 80–85% of all lung cancers (5).

According to lung cancer guidelines, treatment approaches are recommended based on the stage of the disease and may include chemotherapy, radiotherapy, chemoradiotherapy, and surgery. American scientist James Allison (University of Texas MD Anderson Cancer Center) and Japanese scientist Tasuku Honjo (Kyoto University School of Medicine) were awarded the 2018 Nobel Prize in Physiology or Medicine for their discoveries that led to new approaches in activating the immune system, based on the belief that the immune system could influence the initiation, growth, and spread of cancer. In addition, together with the use of Programmed death receptor-1 (PD-1) and Programmed death-ligand-1 (PD-L1) immune checkpoint inhibitors, immunotherapy has taken its place in studies as an anti-cancer treatment (6). The current treatment for unresectable stage III/IV NSCLC involves concurrent chemoradiotherapy followed by immunotherapy

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(7). Although these treatments have led to a remarkable increase in survival times, the prevalence of malnutrition ranges from 34.5 % to 69%. Malnutrition is most common in patients with advanced metastatic disease who receive chemotherapy, immunotherapy, and/or radiotherapy (8).

Malnutrition in patients with lung cancer can lead to reduced overall survival, decreased treatment response, increased risk of complications, and impaired physical and cognitive functions (9). Due to the high symptom burden experienced by individuals with lung cancer, the intake of energy, macro and micronutrients that individuals consume through their daily diet may decrease, and therefore the risk of malnutrition may develop (10). Likewise, a study conducted on 50 individuals with advanced-stage NSCLC receiving immunotherapy found that 86% of the participants had energy intake and 60% had protein intake below the recommended levels. This study also reported that individuals with protein intakes greater than 1 g/kg/day had longer overall survival compared to those with intakes lower than those recommended for cancer patients (35.2 vs. 21.9 months) (11).

In an observational study conducted in Spain, 17 individuals (77%) completed a 6-month follow-up of 22 individuals diagnosed with cancer who received immunotherapy. The average daily dietary energy intake was 1813 kcal/day at the beginning of the study, 1831 kcal/day in the 3rd month of immunotherapy, and 1885 kcal/day in the 6th month of immunotherapy. The percentage of energy derived from carbohydrates (CHO) was 46%, 44%, and 43% at the beginning, month 3, and month 6, respectively; the percentage from protein was 16%, 16%, and 17%, respectively; and the percentage from fat was 39%, 39%, and 40%, respectively (12).

In the NutriNet-Santé cohort study (n = 696), which evaluated changes in dietary habits before and after a cancer diagnosis, statistically significant decreases were observed in alcohol, energy, protein, B-group vitamins, iron, potassium, and zinc levels, while increases in lipid, SFA, MUFA, and vitamin E levels were found statistically significant (13).

Metabolic changes resulting from cancer can lead to a decrease in nutrient intake and undesirable changes in body composition. Both findings are considered negative predictors of treatment toxicity, clinical outcomes, quality of life, and survival. Therefore, timely detection and evaluation of malnutrition and body composition measurements are critical for all cancer patients (14).

A cross-sectional study in Greece, in which 82 patients with advanced-stage NSCLC who received chemotherapy, radiotherapy, and immunotherapy were followed for three months, reported that 37 patients (45%) experienced weight loss, 17 patients (20.7%) had weight gain, and 27 patients (32.9%) maintained stable weight (15). A comprehensive review of 27 original research articles by Grundman et al. (16) concluded that BIA-based measurements in cancer patients were beneficial in predicting the outcomes of nutritional interventions and disease prognosis.

In light of all this information, this study is important due to the limited number of studies on this subject in the literature and serves as a reference for future research in this field. This study is particularly important as it examines the nutritional status, changes in anthropometric measurements, and the relationships among research variables in individuals with non-small cell lung cancer during immunotherapy treatment. It provides data that may help reduce mortality, morbidity, and complications, and thus improve quality of life and serve as supportive information during the cancer treatment process, which is an extremely challenging period for patients as well as for their families and social environment.

2. METHODS

2.1. Research Group and Ethics Approval

This study population consisted of all individuals over the age of 18 diagnosed histologically with stage III/IV NSCLC who were receiving outpatient immunotherapy treatment (nivolumab, 3 mg/kg every 14 days, in individuals who developed progression after a prior line of chemotherapy) at the Department of Medical Oncology, Adana City Training and Research Hospital between January 2024 and January 2025.

Ethics committee approval for the research was obtained from Adana Training and City Hospital Clinical Research Ethics Committee (Approval date: 18.01.2024; Decision no: 3112). Prior to participation, informed consent was obtained from all subjects who had volunteered for this study in either written or oral form.

2.2. Sample Size

When the previous period records of the Medical Oncology Department Polyclinics at Adana City Training and Research Hospital were examined, 30 (thirty) individuals diagnosed with NSCLC who received immunotherapy treatment for 6 months and did not develop disease progression or die during the treatment were identified.

The study sample consisted of 47 patients (M: 43, F: 4) diagnosed with NSCLC who were receiving outpatient immunotherapy at the Department of Medical Oncology, Adana City Training and Research Hospital. In the first two months of the study, 14 patients were excluded due to the death of 11 patients and disease progression in 3 patients. Between the second and sixth months of the study, 3 additional patients died (a total of 14 deaths and 3 cases of progression during the study period). Ultimately, 30 individuals (M: 28, F: 2) completed the study.

2.3. Research Design

Data for each individual were collected and evaluated at three time points: the initial measurement on the first day of immunotherapy, the interim measurement at two months after the initiation of immunotherapy, and the final measurement

at six months after the initiation of treatment. In addition, the sociodemographic characteristics of the individuals, their nutritional status (assessed by a 24-hour dietary recall, once at each time), and anthropometric measurements were recorded. Anthropometric measurements were performed during the preparation phase of the immunotherapy treatment protocol, with participants wearing light clothing, without shoes, and without metal items in their pockets. Height (cm) was measured with bare feet positioned together and the head aligned in the Frankfurt plane, using the Tanita DC-360 device (BIA method) available at the hospital.

The 24-hour dietary recall was administered face-to-face by experienced dietitian to determine the daily energy and macronutrient intake of the individuals included in the study. The method involves recording all foods and beverages consumed by the individuals in the previous 24 hours using a recall method (17). The average daily energy and macronutrient intake of the individuals was analyzed using the Nutrition Information System (BeBIS) software, version 9 (18).

2.4. Data Analysis

Descriptive statistics for categorical variables were presented as frequency and percentage. The normality of distribution for numerical variables was assessed using the Shapiro–Wilk test. Descriptive statistics for numerical variables were expressed as mean ± standard deviation (x ± SD) for normally distributed data, and as median (min–max) for non-normally distributed data. Comparisons of more than two related groups with normally distributed data were performed using repeated measures ANOVA, while those with non-normally distributed data were analyzed using the Friedman test. The results of multiple comparison tests were indicated alongside the means and medians using letter notations.

The relationships between numerical variables were analyzed using Pearson's product–moment correlation coefficient for normally distributed data, and Spearman's rank correlation coefficient for non-normally distributed data. The interpretation of the correlation coefficient was based on the following criteria: < 0.2, very weak correlation; 0.2–0.4, weak correlation; 0.4–0.6, moderate correlation; 0.6–0.8, strong correlation; and > 0.8, very strong correlation (Choi et al., 2010) (19). The effects between quantitative variables were examined using multiple regression analysis. For all calculations and interpretations, statistical significance levels of $p < .05$, $p < .01$, and $p < .001$ were considered, and hypotheses were tested in both directions. Statistical analyses were performed using the SPSS v27 software package (IBM Inc., Chicago, IL, USA).

3. RESULTS

The mean age of the participants (n = 47) was 64.55 ± 8.19 years, with 44.7% being between 60–69 years of age. The mean values were as follows: 25.06 ± 5.46 for BMI (kg/m^2), 24.34 ± 8.17 for body fat percentage (%), 52.78 ± 9.47 for lean body mass (kg), 39.00 ± 6.89 for total body water (kg), 1337.19 ± 463.83 for energy (kcal), 1494.68 ± 604.53 for water (g), 58.31 ± 19.85 for protein (g), 17.87 ± 3.39 for protein (%), 53.34 ± 19.59 for fat (g), 36.72 ± 8.13 for fat (%), 150.60 ± 67.95 for CHO (g), 45.00 ± 8.82 for CHO (%), 16.70 ± 8.09 for fiber (g), 752.38 ± 672.39 for vitamin A (µg), 3.87 ± 2.79 for vitamin D (µg), 8.07 ± 3.89 for vitamin E (mg), 0.71 ± 0.30 for vitamin B_1 (mg), 1.31 ± 0.46 for vitamin B_2 (mg), 0.92 ± 0.46 for vitamin B_6 (mg), 229.70 ± 94.19 for total folate (µg), 74.66 ± 71.59 for vitamin C (mg), 3.80 ± 1.85 for vitamin B_{12} (µg), 2881.40 ± 1502.93 for sodium (mg), 1900.42 ± 907.57 for potassium (mg), 691.41 ± 273.87 for calcium (mg), 196.01 ± 84.10 for magnesium (mg), 922.90 ± 349.69 for phosphorus (mg), 7.64 ± 3.17 for iron (mg), and 7.92 ± 3.25 for zinc (mg) (Table 1).

Table 1. *Summary statistics of demographic, anthropometric, energy, macro – micronutrient values of the participants*

Age group (years)	n	%
40–49	2	4.3
50–59	11	23.4
60–69	21	44.7
70–79	12	25.5
80–89	1	2.1
	$\bar{x} \pm SS$	
BMI (kg/m^2)	25.06±5.46	
Body fat percentage (%)	24.34±8.17	
Lean body mass (kg)	52.78±9.47	
Total body water (kg)	39.00±6.89	
Energy (kcal)	1337.19±463.83	
Water (g)	1494.68±604.53	
Protein (g)	58.31±19.85	
Protein (%)	17.87±3.39	
Fat (g)	53.34±19.59	
Fat (%)	36.72±8.13	
CHO (g)	150.60±67.95	
CHO (%)	45.00±8.82	
Fiber (g)	16.70±8.09	
Vitamin A (µg)	752.38±672.39	
Vitamin D (µg)	3.87±2.79	
Vitamin E (mg)	8.07±3.89	
Vitamin B1 Thiamine (mg)	0.71±0.30	
Vitamin B2 Riboflavin (mg)	1.31±0.46	
Vitamin B6 (mg)	0.92±0.46	
Total folate (µg)	229.70±94.19	
Vitamin C (mg)	74.66±71.59	
Vitamin B12 (µg)	3.80±1.85	
Sodium (mg)	2881.40±1502.93	
Potassium (mg)	1900.42±907.57	
Calcium (mg)	691.41±273.87	
Magnesium (mg)	196.01±84.10	
Phosphorus (mg)	922.90±349.69	
Iron (mg)	7.64±3.17	
Zinc (mg)	7.92±3.25	

BMI: Body mass index; CHO: Carbohydrate

The median final measurement values for water (g) [1750 (1000–3500)], CHO (g) [194.4 (95.6–320.1)], vitamin A (µg) [935.2 (420.1–4166)], vitamin B_1 (mg) [1.1 (0.5 – 2.2)], vitamin B_2 (mg) [1.9 (1–2.8)], total folate (µg) [354 (155.6 – 803)], vitamin C (mg) [129.6 (26.3–356)], vitamin B_{12} (µg) [6 (1.4–11.9)], and zinc (mg) [12.1 (6.9–25.4)], as well as the mean values for energy (kcal) (2020.85 ± 382.83), protein (g) (87.44 ± 19.79), fat (g) (92.97 ± 23.08), fat (%) (41.40 ± 7.37), fiber (g) (25.17 ± 7.55), vitamin E (mg) (16.59 ± 6.67), potassium (mg) (3032.52 ± 911.74), calcium (mg) (977.92 ± 283.68), magnesium (mg) (325.85 ± 87.32), phosphorus (mg) (1393.31 ± 322.38), and iron (mg) (11.37 ± 3.27) were higher than the interim measurement values ($p < .05$). An increase of approximately 13% in nutrition intake was observed between the final measurement and the interim measurement.

The median interim measurement values for water (g) [1500 (750–3500)], CHO (g) [170.5 (92.5–317.2)], vitamin A (µg) [933.5 (370.1–5283.3)], vitamin B_1 (mg) [1.0 (0.4–1.6)], vitamin B_2 (mg) [1.8 (1–3.4)], total folate (µg) [313.6 (0.8–572)], vitamin C (mg) [118.3 (25–295.6)], vitamin B_{12} (µg) [5.8 (1.6–11.7)], and zinc (mg) [10.7 (5.8–19.1)], as well as the mean values for energy (kcal) (1783.41 ± 439.51), protein (g) (79.79 ± 21.57), fat (g) (76.84 ± 22.50), fat (%) (38.33 ± 7.03), fiber (g) (21.99 ± 7.57), vitamin E (mg) (14.20 ± 6.80), potassium (mg) (2749.35 ± 943.81), calcium (mg) (909.74 ± 281.58), magnesium (mg) (281.98 ± 86.72), phosphorus (mg) (1243.89 ± 358.05), and iron (mg) (10.34 ± 3.39) were higher than the initial measurement values ($p < .05$). The median initial measurement values for water (g) [1500 (500–3000)], carbohydrate (g) [165.2 (66.4–311.6)], vitamin A (µg) [667.9 (272–4099.3)], vitamin B_1 (mg) [0.8 (0.3–1.4)], vitamin B_2 (mg) [1.4 (0.6–2.2)], total folate (µg) [256 (99.1–434.8)], vitamin C (mg) [71.5 (3.6–338.9)], vitamin B_{12} (µg) [4.3 (2.5–8.1)], and zinc (mg) [7.6 (4.4–15.6)], as well as the mean values for energy (kcal) (1489.36 ± 448.72), protein (g) (62.03 ± 19.35), fat (g) (59.57 ± 18.89), fat (%) (36.67 ± 8.24), fiber (g) (18.30 ± 8.67), vitamin E (mg) (8.60 ± 4.11), potassium (mg) (2146.11 ± 1007.95), calcium (mg) (793.99 ± 271.40), magnesium (mg) (216.02 ± 92.00), phosphorus (mg) (1037.93 ± 349.96), and iron (mg) (8.22 ± 3.26) were significantly lower than both interim and final measurements ($p< .05$).

Table 2. *Comparison of initial, interim, and final measurements of energy, macro – micronutrient values of participants*

Energy and Nutrient Values	Initial Measurement	Interim Measurement	Final Measurement		P
Energy (kcal)	1489.36±448.72[a]	1783.41±439.51[b]	2020.85±382.83[c]	**R=66.118**	**< .001*** **
Water (g)	1500[a] (500-3000)	1500[b] (750-3500)	1750[c] (1000-3500)	**F=23.053**	**< .001*** **
Protein (g)	62.03±19.35[a]	79.79±21.57[b]	87.44±19.79[c]	**R=57.165**	**< .001*** **
Protein (%)	17.07±2.08[a]	18.60±2.50[b]	17.90±2.19[a]	**R=4.180**	**.020***
Fat (g)	59.57±18.89[a]	76.84±22.50[b]	92.97±23.08[c]	**R=39.536**	**< .001*** **
Fat (%)	36.67±8.24[a]	38.33±7.03[a]	41.40±7.37[b]	**R=6.493**	**.003****
CHO (g)	165.2[a] (66.4-311.6)	170.5[b] (92.5-317.2)	194.4[c] (95.6-320.1)	**F=8.867**	**.012***
CHO (%)	46.07±8.09[b]	43.63±7.36[a]	40.73±7.36[a]	**R=7.801**	**< .001*** **
Fiber (g)	18.30±8.67[a]	21.99±7.57[b]	25.17±7.55[c]	**R=19.891**	**< .001*** **
Vitamin A (µg)	667.9[a] (272-4099.3)	933.5[b] (370.1-5283.3)	935.2[b] (420.1-4166)	**F=19.400**	**< .001*** **
Vitamin D (µg)	4.7 (0.8-10.2)	4.6 (1.5-13.5)	5.30 (1.5-10.4)	F=2.229	.328
Vitamin E (mg)	8.60±4.11[a]	14.20±6.80[b]	16.59±6.67[c]	**R=23.064**	**< .001*** **
Vitamin B_1 Thiamine (mg)	0.8[a] (0.3-1.4)	1[b] (0.4-1.6)	1.1[c] (0.5-2.2)	**F=40.542**	**< .001*** **
Vitamin B_2 Riboflavin (mg)	1.4[a] (0.6-2.2)	1.8[b] (1-3.4)	1.9[c] (1-2.8)	**F=42.000**	**< .001*** **
Vitamin B^6 (mg)	1.00±0.52[a]	1.46±0.55[b]	1.62±0.51[c]	**R=37.166**	**< .001*** **
Total Folate (µg)	256[a] (99.1-434.8)	313.6[b] (0.8-572)	354[c] (155.6-803)	**F=26.600**	**< .001*** **
Vitamin C (mg)	71.5[a] (3.6-338.9)	118.3[b] (25-295.6)	129.6[b] (26.3-356)	**F=11.412**	**.003****
Vitamin B_{12} (µg)	4.3[a] (2.5-8.1)	5.8[b] (1.6-11.7)	6[b] (1.4-11.9)	**F=9.252**	**.010***
Sodium (mg)	3056.3 (1300-7356)	3278.8 (1110.87155.2)	3342.3 (1310-7156.6)	F=1.288	.525
Potassium (mg)	2146.11±1007.95[a]	2749.35±943.81[b]	3032.52±911.74[c]	**R=33.237**	**< .001*** **
Calcium (mg)	793.99±271.40[a]	909.74±281.58[b]	977.92±283.68[c]	**R=11.876**	**< .001*** **
Magnesium (mg)	216.02±92.00[a]	281.98±86.72[b]	325.85±87.32[c]	**R=46.854**	**< .001*** **
Phosphorus (mg)	1037.93±349.96[a]	1243.89±358.05[b]	1393.31±322.38[c]	**R=46.600**	**< .001*** **
Iron (mg)	8.22±3.26[a]	10.34±3.39[b]	11.37±3.27[c]	**R=29.658**	**< .001*** **
Zinc (mg)	7.6[a] (4.4-15.6)	10.7[b] (5.8-19.1)	12.1[c] (6.9-25.4)	**F=29.898**	**< .001*** **

: Repeated Measures ANOVA Test; F: Friedman Test

**p< .05; **p<.01; ***p<.001*

a, b, c: Means or medians without common letters are significantly different (p < .05).

The mean interim measurement values for energy (kcal) (1783.41 ± 439.51), protein (g) (79.79 ± 21.57), fat (g) (76.84 ± 22.50), fiber (g) (21.99 ± 7.57), vitamin E (mg) (14.20 ± 6.80), potassium (mg) (2749.35 ± 943.81), calcium (mg) (909.74 ± 281.58), magnesium (mg) (281.98 ± 86.72), phosphorus (mg) (1243.89 ± 358.05), and iron (mg) (10.34 ± 3.39) were higher than the corresponding mean initial measurement values for energy (kcal) (1489.36 ± 448.72), protein (g) (62.03 ± 19.35), fat (g) (59.57 ± 18.89), fiber (g) (18.30 ± 8.67), vitamin E (mg) (8.60 ± 4.11), potassium (mg) (2146.11 ± 1007.95), calcium (mg) (793.99 ± 271.40), magnesium (mg) (216.02 ± 92.00), phosphorus (mg) (1037.93 ± 349.96), and iron (mg) (8.22 ± 3.26) ($p < 0.05$). The median interim measurement values for CHO (g) [170.5 (92.5–317.2)], vitamin B_1 (mg) [1.0 (0.4–1.6)], vitamin B_2 (mg) [1.8 (1–3.4)], total folate (µg) [313.6 (0.8–572)], and zinc (mg) [10.7 (5.8–19.1)] were statistically higher than the median initial values for carbohydrate (g) [165.2 (66.4–311.6)], vitamin B_1 (mg) [0.8 (0.3–1.4)], vitamin B_2 (mg) [1.4 (0.6–2.2)], total folate (µg) [256 (99.1–434.8)], and zinc (mg) [7.6 (4.4–15.6)] ($p < .05$).

The mean interim measurement of protein (%) (18.60 ± 2.50) was statistically higher than both the mean final measurement (17.90 ± 2.19) and the mean initial measurement (17.07 ± 2.08) ($p < .05$). The mean initial measurement of carbohydrate (%) (46.07 ± 8.09) was statistically higher than both the mean interim measurement (43.63 ± 7.36) and the mean final measurement (40.73 ± 7.36) ($p < .05$). An increase of approximately 35.5% in food intake was observed between the initial and final measurements. (Table 2).

Among the participants who completed the study, the mean final measurement of BMI (kg/m^2) (27.33 ± 5.93) was statistically higher than both the mean interim measurement (26.55 ± 6.05) and the mean initial measurement (26.26 ± 6.04). Similarly, the mean final measurement of body fat percentage (%) (26.99 ± 9.05) was statistically higher than both the mean interim measurement (25.90 ± 9.14) and the mean initial measurement (25.83 ± 9.23). The mean final measurement of lean body mass (kg) (55.61 ± 9.76) was also statistically higher than both the mean interim measurement (54.97 ± 9.38) and the mean initial measurement (54.21 ± 9.55) ($p < .05$) (Table 3).

Table 3. *Comparison of initial, interim, and final anthropometric measurements of participants*

	Initial Measurement	Interim Measurement	Final Measurement		
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	R	p
BMI (kg/m^2)	26.26±6.04^a	26.55±6.05^a	27.33±5.93^b	**11.901**	**<.001*** **
Body Fat Percentage (%)	25.83±9.23^a	25.90±9.14^a	26.99±9.05^b	**3.886**	**.026***
Lean Body Mass (kg)	54.21±9.55^a	54.97±9.38^a	55.61±9.76^b	**3.910**	**.026***
Total Body Water (kg)	39.88±6.97	39.80±6.78	39.89±6.97	0.048	.953

R: Repeated Measures ANOVA Test
**p<.05; ***p< .001*
a, b: Means without common letters are significantly different (p<.05)

Table 4. *Correlation coefficients between the difference values of energy, macro – micronutrients, and the difference values of anthropometric measurements*

		BMI	Body Fat Percentage	Lean Body Mass	Total Body Water
Energy (kcal)	**r-s**	**r=0.434**	**s=0.411**	s=0.076	r=0.019
	p	**.017***	**.024***	.692	.922
Water (g)	**s**	s=-0.052	s=0.203	s=0.114	s=0.097
	p	.783	.282	.547	.610
Protein (g)	**r-s**	r=0.304	s=0.319	s=-0.059	r=-0.034
	p	.102	.086	.757	.859
Protein (%)	**r-s**	r=-0.062	s=-0.095	s=-0.141	r=0.020
	p	.745	.617	.459	.915
Fat (g)	**s**	s=0.161	s=0.181	s=-0.108	s=0.029
	p	.396	.338	.570	.877
Fat (%)	**r-s**	r=-0.081	s=0.040	s=-0.071	r=0.114
	p	.672	.834	.710	.550
CHO (g)	**r-s**	r=0.344	s=0.159	s=0.087	r=-0.062
	p	.062	.402	.647	.747
CHO (%)	**r-s**	r=0.101	s=-0.102	s=0.084	r=-0.096
	p	.595	.593	.660	.613
Fiber (g)	**r-s**	r=0.217	s=0.032	s=0.029	r=-0.205
	p	.250	.868	.879	.278
Vitamin A (µg)	**r-s**	r=0.036	s=0.061	s=-0.177	r=0.016
	p	.849	.748	.349	.935
Vitamin D (µg)	**s**	s=0.078	s=0.221	s=-0.123	s=-0.233
	p	.683	.241	.518	.216
Vitamin E (mg)	**r-s**	r=0.123	s=0.168	s=-0.011	r=-0.048
	p	.518	.376	.953	.800
Vitamin B_1 Thiamine (mg)	**s**	s=0.238	s=0.082	s=-0.068	s=-0.215
	p	.205	.667	.723	.255
Vitamin B_2 Riboflavin (mg)	**r-s**	r=0.180	s=0.199	s=-0.066	r=-0.108
	p	.341	.291	.728	.571
Vitamin B_6 (mg)	**r-s**	r=0.200	s=0.237	s=0.012	r=-0.258
	p	.290	.208	.952	.169
Total Folate (µg)	**s**	s=0.208	s=-0.119	s=0.004	**s=-0.405**
	p	.269	.533	.984	**.026***
Vitamin C (mg)	**r-s**	r=0.131	s=-0.038	s=-0.124	r=-0.278
	p	.492	.843	.514	.137
Vitamin B_{12} (µg)	**r-s**	r=0.136	s=0.029	s=0.294	r=0.020
	p	.474	.879	.115	.916
Sodium (mg)	**r-s**	r=-0.120	s=-0.153	s=-0.347	**r=-0.424**
	p	.527	.420	.060	**.020***
Potassium (mg)	**r-s**	**r=0.404**	s=0.242	s=0.041	r=0.043
	p	**.027***	.198	.832	.820
Calcium (mg)	**r-s**	r=0.353	s=0.043	s=0.061	r=0.257
	p	.056	.823	.750	.171
Magnesium (mg)	**r-s**	r=0.275	s=0.262	s=-0.043	r=-0.020
	p	.141	.162	.820	.918
Phosphorus (mg)	**r-s**	**r=0.411**	s=0.189	s=0.054	r=0.130
	p	**.024***	.316	.776	.495
Iron (mg)	**r-s**	r=0.193	s=0.175	s=-0.044	r=-0.264
	p	.306	.354	.817	.158
Zinc (mg)	**s**	s=0.251	s=0.047	s=0.021	s=-0.276
	p	.180	.803	.914	.140

r: Pearson Product-Moment Correlation Coefficient; s: Spearman's Rank Correlation Coefficient
**p<.05*

As the difference values of Energy (kcal) increased, the difference values of BMI (kg/m^2) and Body Fat Percentage (%) increased by 43.4% and 41.1%, respectively (Table 4). As the difference values of Total Folate (µg) increased, the difference values of Total Body Water (kg) decreased by 40.5% (Table 4). As the difference values of Sodium (mg) increased, the difference values of Total Body Water (kg) decreased by 42.4% (Table 4). As the difference values of Potassium (mg) increased, the difference values of BMI (kg/m^2) increased by 40.4%. There was a statistically significant moderate positive correlation between the difference values of Phosphorus (mg) and the difference values of BMI (kg/m^2) ($p < 0.05$). The results indicated that as the difference values of Phosphorus (mg) increased, the difference values of BMI (kg/m^2) increased by 41.1% (Table 4).

4. DISCUSSION

In this study, the interim and final measurement results obtained as a result of immunotherapy treatment of Water (g), CHO (g), Vitamin A (µg), Vitamin B_1 (mg), Vitamin B_2 (mg), Total Folate (µg), Vitamin C (mg), Vitamin B_{12} (µg), and Zinc (mg), as well as the mean values of Energy (kcal), Protein (g), Fat (g), Fat (%), Fiber (g), Vitamin E, Potassium (mg), Calcium (mg), Magnesium (mg), Phosphorus (mg), and Iron (mg) were found to be higher than the initial measurement values. Similarly, studies conducted on cancer patients have reported that energy, protein, and fat intakes increased following immunotherapy treatment (12,20,21).

Lung cancer immunotherapy is a treatment that helps the immune system recognize and destroy cancer cells (22). Nevertheless, patients with lung cancer receiving immunotherapy may still experience side effects such as fatigue and joint pain (23). This may explain why patients diagnosed with NSCLC prefer a healthy dietary pattern to minimize the adverse effects of immunotherapy, strengthen the immune system, and consume foods with higher energy and protein content more frequently (24).

In this study, the mean value of Protein (%) at the interim measurement was found to be statistically higher than the mean values at the final and initial measurements. In contrast, in the study conducted by Higuera-Pulgar et al., the mean Protein (%) value at the interim measurement was found to be statistically lower than the mean value at the final measurement (12). This may be explained by the increased consumption of protein-rich foods during the treatment process by individuals to strengthen the immune system and prevent muscle loss (25).

In this study, the mean value of CHO (%) at the initial measurement was found to be statistically higher than the mean values at the interim and final measurements. Similarly, Higuera-Pulgar et al. found that the percentage of energy coming from carbohydrates in the first measurement values was higher than the average of the intermediate and final measurement values (12). This may be explained by the fact that cancer patients, during diagnosis, treatment, and post-treatment periods, tend to modify their dietary patterns to prevent cancer recurrence, improve overall health, and manage side effects associated with anticancer therapy, particularly by adopting a healthier diet and limiting the consumption of simple sugars containing carbohydrates (26).

In this study, the mean values of BMI (kg/m²), body fat percentage (%), and lean body mass (kg) at the final measurement were statistically higher than the mean values at both the interim and initial measurements. Similarly, in a study analyzing data from four different international centers, BMI values were found to be high during immunotherapy treatment (27). In contrast, a retrospective study conducted by Shijubou et al. (2022) on 38 patients with advanced NSCLC treated with immunotherapy observed a decrease in body weight after the initiation of treatment (28). Likewise, in 82 male patients diagnosed with stage IV NSCLC who received chemotherapy, immunotherapy, or chemotherapy plus immunotherapy, the mean values of BMI (kg/m²) and body fat percentage (%) at the final measurement, taken three months after treatment initiation, were statistically higher than the mean values at the initial measurement (29). Cancer cachexia, which is frequently reported in NSCLC patients along with changes in body composition, metabolism, and nutritional status, has been reported to potentially affect the immune response and impair the efficacy of immunotherapy. This finding in our study can be explained by the fact that the patients included had an initial mean BMI (kg/m²) of 26.26 ± 6.04, indicating they were not cachectic, which may have contributed to the increased efficacy of immunotherapy (30).

As the difference in daily dietary potassium (mg) intake increased among the individuals, the difference in BMI (kg/m²) values also increased; likewise, as the difference in phosphorus (mg) intake increased, the difference in BMI (kg/m²) values also increased. Similarly, in a cross-sectional study involving 404 participants from the healthy Indian population and patients with chronic kidney disease, dietary potassium and phosphorus intake were reported to be higher in individuals with higher BMI compared to those with normal BMI, and a positive correlation between these values was observed (31). This may be explained by the fact that cancer patients, during diagnosis, treatment, and post-treatment periods, tend to make changes in their existing diets to prevent cancer recurrence, improve overall health, and manage side effects associated with anticancer treatments. As part of these adjustments, they may adopt a healthy diet pattern by increasing the consumption of potassium-rich vegetables while limiting processed foods, red meat, foods and beverages high in salt, sugar, and fat, as well as alcohol, in order to maintain a healthy body weight (26).

5. CONCLUSION

Despite all the advances in medicine, lung cancer remains the leading cause of cancer-related deaths. Immunotherapy is currently the most powerful new treatment for lung cancer. Approaches that strengthen and support treatment are highly

important for reducing mortality and morbidity. Following treatment, various physiological changes and alterations in nutrient intake may occur. It is essential for patients and healthcare professionals to be aware of and prepared for these changes, as entering the treatment process with this awareness can make the process shorter, easier, and less burdensome for both patients and healthcare providers

5.1. Strengths and Limitations of The Study

This cross-sectional clinical study was planned and conducted with a multidisciplinary team approach, and the data on this subject in the literature are quite limited. Therefore, this study provides supportive information for future research in this area and represents an original work. It will serve as a pioneering and guiding study; by the end of this research, healthcare professionals will be able to gain insight into the energy, macro – micronutrient intake, anthropometric measurements, and potential changes in individuals with non-small cell lung cancer receiving immunotherapy. They will also be able to take necessary precautions and manage new conditions in a timely manner against possible complications of treatment through early intervention. However, research was conducted in limited sample size due to single-center.

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Clinical and Experimental Health Sciences

Diastema Closure Following Detection and Removal of Residual Resins After Orthodontic Treatment: Case Report with 1-year follow-up

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ABSTRACT

This case report aimed to determine and remove the residual resins after debonding, to restore the upper lateral teeth with a single shade composite to evaluate with follow-ups. An 18-year-old female patient applied to our clinic with the complaint of the presence of diastemas. Following the sond marking technique, MDP (Smile Lite, Smile Line) and mobile phone (iPhone 11, Apple); DSLR camera (Nikon), macro lens and D-Light caries detection mode (GC) before and after the application of plaque disclosing gel (Tri Plaque ID, GC) to detect resin residues on tooth surfaces after bracket removal. The residual resins were removed with finishing and polishing discs (last 3 stages) (OptiDisc, Kerr). One week later, the selective-etch technique, universal adhesive (Gluma Bond Universal, Kulzer), and single shade composite (Charisma Diamond One, Kulzer) were used for diastema closure. The patient was called for follow-up at 3, 6, and 12-months and evaluated FDI criteria. Residual resins were best determined by using a combination of plaque disclosing agent and MDP since the composite used was not a fluorescent light-emitting material. For restorations, FDI 1 and 2 scores were observed in the esthetic, functional, and biological evaluations.

Keywords: Bracket, cross polarizing filter, diastema closure, residual resin, plaque disclosing gel.

1. INTRODUCTION

Many clinical conditions in anterior teeth, such as diastema, peg-shaped teeth, alterations in shape and color, or asymmetry of the smile, may potentially require esthetic treatment. Patients undergoing orthodontic treatment to resolve malposition are a population in which anterior restorations are indicated to complete the esthetic aspect of the therapy (1). There are multiple alternative treatments for anterior esthetic restorations, including direct and indirect methods. A contemporary and more conservative approach to direct restorations without preparing sound tissues demonstrates promising clinical outcomes. If minimally invasive or noninvasive procedures are required on healthy teeth, it may be recommended that the tooth shape be corrected, and the diastema be closed following orthodontic treatment with micro or nano-filled resin composites. Moreover, the recent introduction of single shade universal resin composites has simplified the restorative procedure, allowing for the achievement of esthetic requirements with ease of shade selection (2). Before the restorations, removing residual resin after orthodontic treatment is critical for restoring the enamel surface to its original state before treatment (3). The de-bracketing and removal of residual orthodontic adhesive resin is often a complex task that may involve the mechanical removal of enamel, which poses a risk to the dental structure and may result in irreversible enamel damage. There are studies evaluating different resin removal methods. Among these studies, some studies found no difference in surface properties between removal systems (4) or recommended using a 4-stage finishing and polishing disc system (Sof-Lex, 3M, USA) (5).

Clinical photography has become a standard procedure in daily practice. A variety of photography methods are utilized in dentistry to examine and imitate tooth shade and structure, as well as to follow patients and treatments. DSLR (digital single lens reflex) body, macro lens, and external sources of light remain the golden standard in terms of photographic equipment used in dentistry (6). On the other hand,

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smartphones are constantly being developed in combination with the portable device Mobile Dental Photography (Smile Lite, Smile Line, Switzerland) represents a photographic instrument for the documentation and communication of dental practice. This system is less expensive than other instruments and high-quality images can be taken using this simple and easy smartphone-based system (7). In addition to dental photography, there are various systems, devices, or agents for examining defects, caries, restorations, or dental plaque. Although traditional plaque-disclosing gels are available, 3-tone plaque-disclosing gels (Tri Plaque ID Gel, GC, Japan) have been developed to confirm the presence of caries, plaque, and cariogenic microorganisms, as well as oral hygiene (8). Furthermore, a variety of devices and light sources are utilized to assess dental caries, initial enamel defects, surface texture, and restorations. One such device is the D-light Pro GC (GC, Japan), which uses an LED at near-ultraviolet wavelengths in detect mode to visualize bacteria in plaque, fissures, infected dentin, and the presence of microleakage. It has been reported that this device is not invasive, safe to use, and convenient (9).

In the absence of a detailed examination of residual resins following orthodontic treatment, there is a risk that they might be left on the tooth surface without either the clinician or the patient noticing. Similarly, the removal of residual resins from the tooth surface without a detailed examination might result in damage to the dental hard tissues. In both instances, the long-term consequences of these conditions include increased susceptibility to complications such as discoloration, plaque accumulation, and caries. Furthermore, these factors influence the procedures for planning restorative treatments following orthodontic treatment and the potential lifespan of restorations. Considering this information, this case report examined the diagnosis of residual resins following orthodontic treatment using various methods and technologies. The 4-stage disc system was used to remove residual resins while causing minimal damage to the tooth structure. To meet the patient's esthetic expectations, the diastemas were closed with single shade nanohybrid resin composite, and the patient was followed up.

2. CASE PRESENTATION

2.1. Detection and Removal of Residual Resin

An 18-year-old female patient applied to the Restorative Dentistry Department with a complaint of the presence of diastemas following orthodontic treatment. After clinical and radiological examinations, residual resins were identified in the buccal surface of the lower and upper teeth.

This case report employed a range of imaging techniques to optimally detect residual resins in the patient's teeth. First, residual resins were determined by the sond marking technique. The residual resins were also evaluated with 6 different systems as follows:

I. DSLR Camera (D500, Nikon, USA), Macro Lens (100 mm, Nikon, USA), Twin Flash (MK-MT24IIC, Meike, China).

II. DSLR Camera, Macro Lens, Twin Flash, and plaque-disclosing gel (Tri Plaque ID Gel).

III. DSLR Camera, Macro Lens, and LED light-curing unit (D-light Pro GC).

IV. DSLR Camera, Macro Lens, LED light-curing unit, and plaque-disclosing gel.

V. Cross-polarized mobile dental photography (Smile Lite MDP, Smile Line; iPhone 11, Apple).

VI. Cross-polarized mobile dental photography and plaque-disclosing gel.

The residual resin photographs obtained from the designed systems are presented in Figure 1. Among the imaging systems used, the boundary of residual resins was most clearly discernible in images obtained with "cross-polarized mobile dental photography and plaque-disclosing gel". Despite the DSLR camera's superior resolution, the plaque-disclosing gel is purple, and the mobile dental photography system exhibits a cross-polarized image. In contrast, the resin residues were not clearly discernible in the photographs obtained with the cross-polarized mobile dental photography system alone. The second most effective imaging system for detecting the boundaries of residual resins was the system that combined a "DSLR camera with plaque-disclosing gel". In this instance, the use of plaque-disclosing gel might be considered an appropriate method for the detection of residual resin. In this case report, the caries detection mode of the light-curing unit did not accurately determine the boundaries of the residual resins due to the absence of a fluorescence-emitting material during the orthodontic treatment. The combination of a "light-curing unit and plaque-disclosing gel" resulted in superior visualization of the boundaries of residual resins.

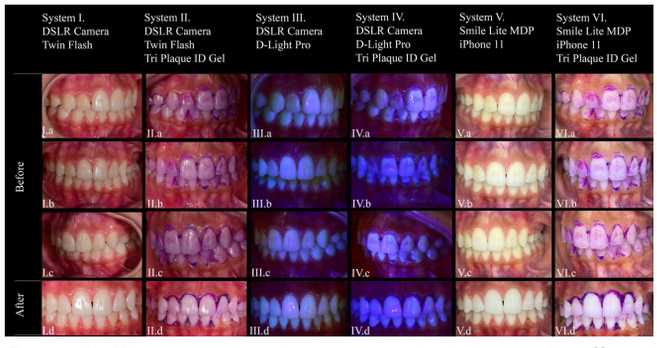

Figure 1. *The residual resins were evaluated with 6 different systems. I. a-d: Nikon DSLR Camera and Twin Flash, II. a-d: DSLR Camera, Twin Flash, and Tri Plaque ID Gel, III. a-d: DSLR Camera and D-Light Pro, IV. a-d: DSLR Camera, D-Light Pro, and Tri Plaque ID Gel, V. a-d: Smile Lite MDP and iPhone 11, VI. a-c: Smile Lite MDP, iPhone 11, and Tri Plaque ID Gel. Images before the removal of the residual resins were presented in I, II, III, IV, V, and VI. a-c. Images after the removal of the residual resins were as follows: I, II, III, IV, V, and VI-d.*

After the determination, the residual resins were removed with the last 3 stages (40, 20, and 10 µm) of finishing and

polishing discs (OptiDisc, Kerr, USA) at 10,000 rpm, following the manufacturer's instructions. The sond marking technique was also used during the removal process (Fig. 2). The coarse disc was not used to facilitate greater control over the removal of resin residues. As the final step in the process, the surface was polished with Opti1step diamond rubber point (Kerr, USA) for 10 seconds. Following the removal of residual resin, the teeth' buccal surfaces were again examined using the mentioned different systems (Fig. 1 and Fig. 3). The patient was scheduled for an appointment 1 week after the resin removal session.

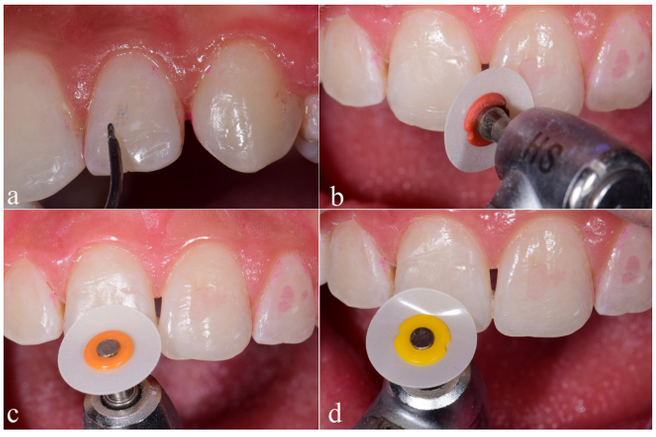

Figure 2. *a. Residual resins were also detected with the sond marking technique. b-d. The last 3-stages of multi-step discs were used to remove the residual resin.*

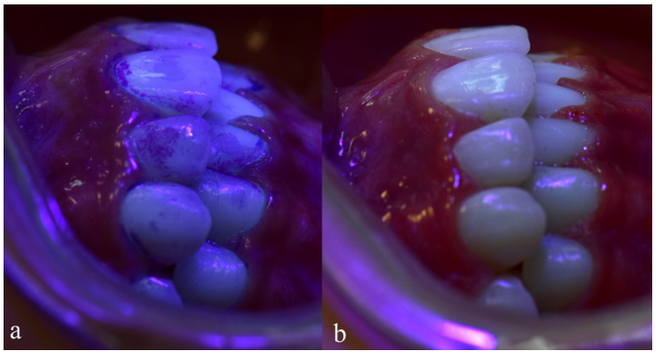

Figure 3. *a. Before the removal of residual resins. b. After the removal of residual resins.*

2.2. Diastema Closure with Single shade Composite and Recalls

One week later, the mesial of the right upper lateral tooth and the distal of the left upper lateral tooth were etched with 37% orthophosphoric acid (Etching Gel, President, Germany) under rubber-dam isolation. One week later, the mesial of the right upper lateral tooth and the distal of the left upper lateral tooth were etched with 37% orthophosphoric acid (Etching Gel, President, Germany) under rubber-dam isolation (Fig. 4). Universal adhesive agent (Gluma Bond Universal, Kulzer, Germany) was applied according to the manufacturer's recommendations. Layering technique (2 mm increments) and single shade composite (Charisma Diamond One, Kulzer, Germany) were preferred. A polywave LED light curing device (Valo Cordless, Ultradent, USA) was used for polymerization with 1000 mW/cm^2 for 20 seconds. Finishing and polishing were completed with discs (OptiDisc) and 2-step diamond spiral rubbers (Diacomp Twist Plus, Eve, Germany). In the 6th month follow-up, a chipping and fracture of the restoration in the right upper lateral tooth was detected in the incisal corner due to eating hazelnuts. A repair procedure was applied with tungsten carbide burs (1.5 mm preparation) and the same materials and protocols as in the first restoration session. The patient was called for follow-up at 3-, 6-, and 12-months and evaluated according to revised FDI criteria (Fig. 5, 6) (10). Restorations received a "clinically very good (score 1)" in all esthetic and biological aspects subcategories at the 3-, 6-, and 12-month follow-ups. At six months, the upper right lateral was fractured due to the patient's consumption of nuts. This resulted in a "clinically unsatisfactory restoration (score 4)" in the fracture of the material and retention criteria.

Figure 4. *a. Rubber dam isolation on the restoration session. b. 37% orthophosphoric acid etching procedure.*

Figure 5. *Six-month follow-up of restorations. Chipping of the restoration in the right upper lateral tooth in the incisal corner (blue arrow).*

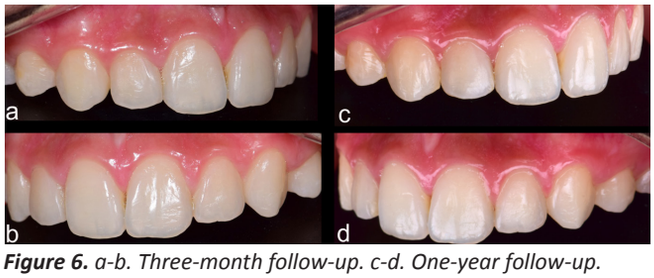

Figure 6. *a-b. Three-month follow-up. c-d. One-year follow-up.*

In addition, restoration in the right upper lateral tooth received a "clinically good restoration (score 2)" in the proximal contact point and form and contour criteria at the 12-month follow-up (Table 1).

Table 1. *The scoring of restorations according to the revised FDI criteria.*

Categories	Subcategories	Upper right lateral			Upper left latral		
		3-months	6-months	12-months	3-months	6-months	12-months
Esthetic Aspects	Surface luster & surface texture	1	2	1	1	1	1
	Marginal staining	1	1	1	1	1	1
	Color match	1	1	1	1	1	1
Functional Aspects	Fracture of material & retention	1	4	1	1	1	1
	Marginal adaptation	1	1	1	1	1	1
	Proximal contact point	1	1	2	1	1	1
	Form & contour	1	1	2	1	1	1
	Occlusion & wear	1	1	1	1	1	1
Biological Aspects	Caries at restoration margins	1	1	1	1	1	1
	Dental hard tissue defects at restoration margin	1	1	1	1	1	1
	Postoperative hypersensitivity & pulp status	1	1	1	1	1	1

1: Clinically very good restoration (sufficient), 2: Clinically good restoration (sufficient), 3: Clinically satisfactory restoration (sufficient), 4: Clinically unsatisfactory restoration (insufficient), 5: Clinically poor restoration (insufficient).

3. DISCUSSION

In the field of dentistry, the role of photography is becoming increasingly significant. The capabilities of smartphones offer advantages that make them valuable tools for digital photography. Some of these include their small size and lightweight design, favorable ISO settings, high resolution, and superior sensor quality. Additionally, they possess the capacity for manual alteration of certain camera values. The light source in smartphones can be provided by the SmileLite MDP device, which also enables the use of a cross-polarizing filter for photographic purposes (7). Prasad et al. conducted a questionnaire study on the evaluation and comparison of images taken with DSLR and smartphone by orthodontists and as a result, no significant difference was found between the two devices (6). In this case report, the use of a cross polarized mobile dental photography device in combination with a plaque staining agent was found to be superior to others, as the aim was the detectability of the residual resin rather than the quality of the photograph.

Leaving residual resin after orthodontic treatment means that rough areas remain on the tooth surface. The increase in the roughness of the surface is one of the most important factors determining the increase in the amount of dental plaque (11). Since the amount of plaque is expected to be high around the residual resin, the use of a plaque staining agent has shown an effect to increase its detectability. On the other hand, it would have been useful to use a composite disclosing agent which can help dentists distinguish resin boundaries from the tooth structure and facilitate its complete removal while avoiding damage to the surrounding sound tooth structures (12). In their study on the removal of adhesive residues under UV light following lingual bracket removal, Albertini et al. found that this method minimized damage to the enamel tissue and was an easier, more accurate, reliable, non-invasive, inexpensive, and time-saving alternative (13). Although a D-light Pro device that reflects UV light was utilized in this case, the UV light imaging method was not superior to others in the detection of residual resin due to the preference for resin material without fluorescent dye for bracket cementation. It has been reported by studies that orthodontic adhesives cannot be completely removed from the enamel surface regardless of the debonding techniques used and enamel damage occurs (14).

Direct resin composite restorations are a common treatment option in dentistry, utilized for restoring both posterior and anterior teeth. In particular, the anterior teeth may require restorative treatment following orthodontic treatment. Surface quality is an important parameter affecting the clinical behavior of dental restorations. Optimal finishing and polishing of resin composites are essential to maintain esthetics and ensure the longevity of resin-containing restorations. Smooth and polished restorations are less prone to plaque accumulation and staining. It can also affect the physical and mechanical properties, and wear resistance of restorations. Ali et al. concluded that the surface smoothness obtained with Optidisc (Kerr) is superior (15). Optidisc was preferred for resin composite restoration sessions due to its superior finishing and polishing properties. In their five-year follow-up study, Comba et al. found that direct composite restorations following orthodontic treatment exhibited satisfactory esthetic outcomes and good or very good clinical performance when combined with conservative procedures. Additionally, the study revealed that many failures were occured, which were easily repaired to achieve the correct shape, function, and esthetic. Furthermore, the study demonstrated a minimal incidence of repairs that were completed with minimal disruption to form, function, and esthetic quality (1). The functional, esthetic, and biological outcomes of diastema closure restorations following orthodontic treatment remained satisfactory during the one-year follow-up period in this case report. The upper right lateral tooth exhibited the only fracture resulting from hazelnut consumption at six months. However, this was easily repairable. It would be beneficial to conduct further studies and case reports on both the removal of residual resin with fluorescent material and the follow-up of single shade resin composite restorations longer than 1 year.

4. CONCLUSION

The residual resins were most accurately identified using a combination of plaque disclosing agent and MDP, as the resin composite was not a fluorescent light-emitting material. For single shade composite restorations, FDI 1 and 2 scores were observed in the esthetic, functional, and biological evaluations. Single shade resin composite restorations may be preferred for the treatment of anterior teeth. It was noted that the incisal fracture detected at the six-month follow-up was cohesive and that the adhesive bond of the cervical part of the composite remained intact.

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Clinical and Experimental Health Sciences

Direct Composite Restoration of Anterior Diastemas with Multidisciplinary Approach

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ABSTRACT

It was aimed to present an illustrative clinical case report with an accurate treatment protocol for the esthetic rehabilitation of a patient through the combination of orthodontic and restorative therapy. After intraoral and radiographic examinations of the patient with no systemic disease, it was revealed that there was a midline spacing between maxillary central incisors. Following orthodontic treatment, direct esthetic partial composite laminate veneers as build-ups for both maxillary incisors were considered. For the restorative procedure, it is planned to restore teeth 12, 13, 22, and 23 with direct universal composite resin. To simulate a natural outlook, the shades DA2, and EA1 (Vittra APS Essential, FGM, Brasil) were decided to be used together as layers. Recalls were arranged for 1 week, 6 months, and 18 months. Restorations were evaluated according to modified USPHS Criteria, 18 months follow – up, there was no evidence of any fracture, surface defect, or discoloration. Composite resin restorations might be considered as a simple treatment option with patient satisfaction in diastema cases. It was thought that the correct positioning of diastemas with orthodontic treatment increases the success of composite restorations.

Keywords: Diastema, composite resin, aesthetic restoration, aesthetics, braces

1. INTRODUCTION

Diastema, the space between two teeth, has a multifactorial etiology. The differences in the size of the teeth are smaller compared to the space in the arch, as well as tooth loss, impacted teeth, and congenital tooth deficiency can cause diastema. Abnormal labial frenulum attachment is the leading cause of midline diastema (1-4).

With the increasing importance given to aesthetics, the number of patients who apply to dentists with aesthetic complaints such as diastema and deformities in the teeth is increasing day by day. Depending on the width of the diastema, the patient's expectation, socioeconomic level, and living standards, treatment options in these cases can be listed as orthodontic treatment, minimally invasive direct composite resin applications, porcelain lamina restorations, and full ceramic crowns (5).

Orthodontic tooth movement should be the first treatment option for teeth with misalignment as a more conservative option (6, 7). However, teeth with have defects in color or shape will already be subject to a more invasive restorative treatment for cosmetic aesthetic rehabilitation (8). Restorative and orthodontic treatments can be combined to obtain a better aesthetic and conservative result. In some cases, short-time orthodontic treatment with the use of brackets has allowed the repositioning of the teeth for minimally invasive preparation and better adhesive bonding (9, 10).

Patients with midline diastema on central incisors are usually restored with direct or indirect restorations. However, in the presence of a wide major diastema, a multidisciplinary approach including orthodontic short-term treatment followed by direct restorative treatment may support the restorative treatment planning by providing more ideal tooth proportions and emergence profiles regarding the smile aesthetics (11). Therefore, it is always better to think in a multidisciplinary way and taking the pre-restorative treatment options into account during the restorative treatment planning (12). This case report presents the direct aesthetic restoration of a wide major diastema between the central incisors in a conservative and multidisciplinary way.

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2. CASE PRESENTATION

A 37-years-old male patient was admitted to the Marmara University, Faculty of Dentistry, Department of Restorative Dentistry with the complaint of esthetic disappointment in the upper front tooth region. As a result of intraoral and radiographic examinations of the patient with no systemic disease, it was revealed that there was a midline diastema between maxillary central incisors (3 mm) (Figure 1).

***Figure 1** . Preoperative intraoral view of the patient and the midline diastema*

After orthodontic treatment, direct composite restorations as build-ups for maxillary lateral incisors and canines were planned.

Since the patient presented with a Class I dental relationship and there is no crowding on the lower teeth, orthodontic treatment is planned for just the upper teeth. Aim for upper orthodontic treatment is closing midline diastema and distributing 3 mm spaces mesial to upper lateral teeth. Treatment began by bonding standard edgewise brackets (Mini Master Series Diagonal Twin .022 Bracket; American Orthodontics, Sheboygan, WI, USA) from 24 to 14 and alignment start with 0.012" Nitinol wire (Figure 2). The patient was checked every 4 weeks and the wire changed to in sequence 0.018" Nitinol and 016" × 0.022" stainless-steel wire. Closing midline diastema was started by putting elastic chain to 11 and 21 on the stainless-steel wire. The elastic chain was renewed every 4 weeks and after closing spaces between 11 and 21. After root parallelism was checked by panoramic x-ray and consultation was done by the Restorative Department, braces were removed and fixed by 0.0215-inch five-stranded lingual retainer wire (PentaOne; Carlsbad, CA, USA) applied to 11 and 21 (Figure 3).

The restorative procedure was planned to restore the mesial surfaces of teeth 12, 13, 22, and 23 with direct universal composite resin without surface treatment (Figure 4). Wax-up models were created on a stone cast and a silicone guide was generated before the restoration appointment. At the restorative appointment, the shade selection was considered with the button technique. To select the dentinal shade, round button composite samples, in different shades, are placed on the middle third of the right upper central incisor, and to select the enamel shade, round enamel composite shades placed on the incisal third of the same tooth, and cured with LED light source (Smart Lite Focus; Dentsply Sirona, Charlotte, NC, USA). The shade selection was performed in 2 minutes to keep teeth hydrated. To simulate a natural outlook, the shades DA2, and EA1 (Vittra APS Essential; FGM, Joinville, SC, Brasil) were decided to be used together with polychromatic layering. Following the shade selection, the teeth were isolated with a rubber-dam (NicTone; Zapopan, Jalisco, Mexico), and upper incisors were retracted by using the floss tie retraction. No surface treatment was performed before the restorative procedure. 37% phosphoric acid (Condac 37; FGM, Joinville, SC, Brasil) was applied for 15 seconds to the enamel surfaces, rinsed for 20 seconds, and dried with air slightly. Universal adhesive (Ambar APS; FGM, Joinville, SC, Brasil) was applied and rubbed for 10 seconds, treated with a gentle air stream for 10 seconds to evaporate the solvent, and polymerized with LED light source with an irradiance of 1250 mW/cm^2 (Smart Lite Focus; Dentsply Sirona, Charlotte, NC, USA) for 10 seconds. A thin layer of EA1 shade composite resin was used palatinally as enamel by using the silicon guide. After removing the silicon guide, proximal walls were restored with a thin layer of EA1 shade composite resin. A thin layer of DA2 shade was placed as the dentin layer. Finally, a thin layer of EA1 shade was used as the top layer.

***Figure 2.** First session of orthodontic treatment, placing brackets with 0.012" Nitinol wire*

A 12-bladed flame-shape carbide bur (Dia-Tessin; Gordevio, Switzerland), polishing discs (Soflex; 3M, St. Paul, MN, USA), interdental strips (Kerr Dental; Brea, CA, USA), and spiral wheels (Twist DiaComp Plus; EVE, Keltern, Germany) were used for detailed finishing and polishing procedures respectively from coarse to superfine grits (Figure 5). After restorations were completed, the patient's upper teeth were fixed with a clear aligner. Control appointment was arranged for 1 week and recall appointments were arranged for every 6 months. The final restorations were evaluated according to the Modified United States Public Health Service (USPHS) criteria for 6th and 18th month-recalls (Table 1). While no recurrens were observed in the midline diastema in the 6th month recall, it was observed that the recurrens in the midline diastema began in the 18th month follow-up session (Figures 6, 7).

Table 1. *Modified USPHS (Modified United States Public Health Service) scores of 6 and 18 month recalls*

Category	Score	Criteria	Recall (Month)	
			6	18
RESTORATION	Alpha (A) Bravo (B) Charlie (C)	Restoration is present Restoration is partially lost Restoration absent	A	A
MARGINAL INTEGRITY	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Resin-enamel interface is excellent No crevice is visible at margins Crevice at margin, enamel exposed Restoration is mobile, fractured or missing	A	A
MARGINAL DISCOLORATION	Alpha (A) Bravo (B) Charlie (C) Delta (D)	No discoloration on the margin between restoration and the tooth surface Slight staining can be polished away Obvious staining can not be polished away Gross staining	A	A
ANATOMICAL FORM	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Restoration continuous with existinganatomical form and margins Restoration is slightly overcontoured or uncontoured Restoration is undercontoured, dentin or base exposed Restoration is missing	A	A
SECONDARY CARIES	Alpha (A) Bravo (B)	No evidence of carious contagious with the margin of the restoration Caries evident contagious with the margin of the restoration	A	A
SURFACE TEXTURE	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Smooth surface Slightly rough or pitted Rough, cannot be refinished Surface deeply pitted, irregular grooves	A	A
SHADE MATCH	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Restorations matches the shade and translucency of adjacent tooth structure Discoloration between restoration and tooth structure within the normal range of tooth Discoloration between restoration tooth structure outside the normal range of tooth Unacceptable color, shade and translucency	A	A
POSTOPERATIF SENSITIVITY	Alpha (A) Bravo (B) Charlie (C)	No postoperative sensitivity Postoperative sensitivity Postoperative sensitivity with treatment need	A	A
RESTORATION	Alpha (A) Bravo (B) Charlie (C)	Restoration is present Restoration is partially lost Restoration absent	A	A
MARGINAL INTEGRITY	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Resin-enamel interface is excellent No crevice is visible at margins Crevice at margin, enamel exposed Restoration is mobile, fractured or missing	A	A
MARGINAL DISCOLORATION	Alpha (A) Bravo (B) Charlie (C) Delta (D)	No discoloration on the margin between restoration and the tooth surface Slight staining can be polished away Obvious staining can not be polished away Gross staining	A	A
ANATOMICAL FORM	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Restoration continuous with existinganatomical form and margins Restoration is slightly overcontoured or uncontoured Restoration is undercontoured, dentin or base exposed Restoration is missing	A	A
SECONDARY CARIES	Alpha (A) Bravo (B)	No evidence of carious contagious with the margin of the restoration Caries evident contagious with the margin of the restoration	A	A
SURFACE TEXTURE	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Smooth surface Slightly rough or pitted Rough, cannot be refinished Surface deeply pitted, irregular grooves	A	A
SHADE MATCH	Alpha (A) Bravo (B) Charlie (C) Delta (D)	Restorations matches the shade and translucency of adjacent tooth structure Discoloration between restoration and tooth structure within the normal range of tooth Discoloration between restoration tooth structure outside the normal range of tooth Unacceptable color, shade and translucency	A	A
POSTOPERATIF SENSITIVITY	Alpha (A) Bravo (B) Charlie (C)	No postoperative sensitivity Postoperative sensitivity Postoperative sensitivity with treatment need	A	A

Figure 3 *. After alignment and spaces were organizated mesial to upper lateral teeth.*

Figure 4. *Prerestorative intraoral view of the patient after orthodontic treatment*

Figure 5. *Final restoration*

Figure 6. *The view of the restorations at 6 months recall*

Figure 7. *The view of restorations at 18 months recall*

3. DISCUSSION

Many adult patients who expect an aesthetic appearance on their teeth may experience several problems. The size differences between the teeth can negatively affect the patients in terms of function and speech, and cause aesthetic problems, especially when it causes crowding or diastema (13-15). Also, sometimes the lateral teeth can be very small or peg-shaped due to genetics (16). Restoration of such deformities provides long-term retention when performed by evaluating the patient's remaining growth potential and occlusion.

Aesthetic rehabilitation of these cases, clinical crown length and size, and the position of the teeth should be evaluated. The ideal treatment of these cases involves an interdisciplinary approach (17-19). Restorative treatment specialists and orthodontists should work together to achieve the desired clinical result, especially in cases of diastema due to arch length and tooth size mismatch or in cases where the anterior teeth are crowded (8, 20). In the presence of large diastema, closure of the space with direct restoration may change the normal length-width relationship of the teeth, causing the anterior teeth to look too wide and unnatural. Restoration of large diastemas may result in an overly contoured outlet profile in the interproximal areas. This can lead to dental plaque retention and inflammation of the gums (7). A combined treatment with orthodontics allows, small tooth movements, to distribute large diastema spaces, repositioning teeth and creating optimal spaces for correct restorations (11, 21, 22). In this case report, with orthodontic treatment, the large midline diastema was distributed between the lateral and central teeth, and the small lateral teeth were restored to provide a normal size and close the diastema spaces. Although orthodontic treatment prolonged the treatment period, teeth axes were corrected, teeth were restored in the proper form, and smile symmetry was achieved.

Adhesive dentistry ensures the preservation of healthy tooth tissue while creating aesthetics. Composite resins

allow a long-term aesthetic restoration when applied by the application protocol of adhesive resins (23).

Restorations were evaluated according to Modified USPHS Criteria, at the 6th and 18th months of recall, there was no evidence of any fracture, surface defect, and discoloration on the teeth and the restorations. The general outlook of the restored teeth was considered natural and aesthetical. The restorations were scored as "Alpha", based on clinical examination, and there was no diastema between the restorations and adjacent teeth at the 6th and 18th month recall session (Figures 6, 7). There were no recurrences of midline diastema at the 6th month recall. At the 18th-month follow-up session, it was observed a recurrence of midline diastema despite the patient still wearing the fixed retainer. However, it was learned from the patient's history that he did not use his clear aligner regularly. The patient was motivated to use the clear aligner and 24-month follow-up appointment was arranged.

Closure of diastemas with direct composite resin restorations is a very common practice in today's dentistry. One of the most important advantages of composite restoration is the correction of the shape, color, and position of the teeth by performing the restoration in a single session. Compared to laboratory-produced indirect restorations, direct composite restorations are advantageous as they are minimally invasive. This treatment option is inexpensive for patients as it does not require laboratory work (24, 25). The fact that there is a repair option in composite restorations makes this restoration type superior to alternative applications such as porcelain lamina and ceramic crowns.

In conclusion, the patient's aesthetic problem was resolved with a conservative way including direct composite restorations after orthodontic treatment. At 18 month recall the modified USPHS scores of the restorations were considered as acceptable. Multidisciplinary approach in diastema cases increases the aesthetics and durability of restorations.

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Design of the study: BK, DT
Acquisition of data for the study: SM, SE
Analysis of data for the study: BK, DT
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