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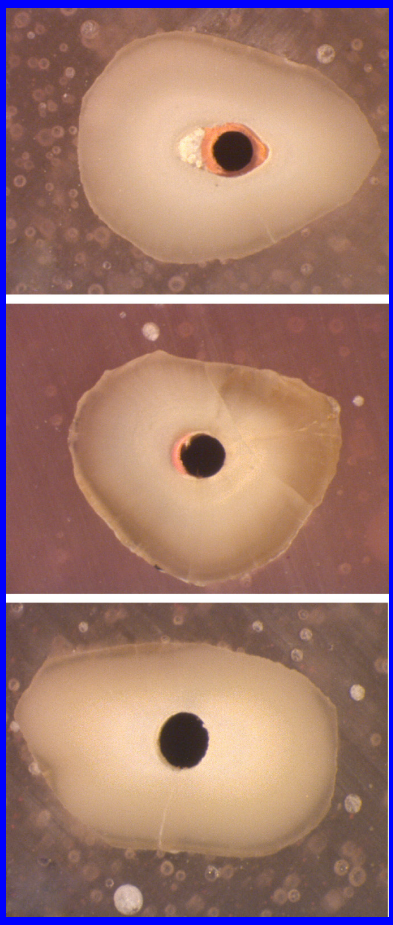

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Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (530) 177 30 97
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
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Original Articles

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EDITORIAL

Dear Readers;

We are with you again in the 3rd issue of 2024 with interesting studies from different branches.

In this issue, we chose the cover image from İlkgelen and İriboz's study titled "Investigation of the Effect of Sodium Hypochlorite, EDTA, Propolis, Boric Acid and Citric Acid Irrigation Solutions on Push-out Bond Strengths of Root Canal Sealers". In this study, the authors investigated the effect of different canal irrigation solutions, including propolis and boric acid (BA), on the bond strengths of epoxy-resin-based AH Plus and bioceramics-based Bioserra canal sealers on root dentin. After 100 single-root-single-canal extracted human teeth were shaped with a rotary tool, they were divided into 2 groups according to the canal filling material (AH Plus and Bioserra) and each group was divided into 5 subgroups containing 10 samples according to washing solutions (5.25% NaOCl, 17% EDTA, 10% citric acid, 10% BA, 10% propolis). The teeth, of which canals were filled, were incubated at 37 °C for a week and then embedded in acrylic resin. Horizontal sections of 2 mm thickness were taken from the midline of the root of each tooth, and vertical compression force was applied until the connection broke, and bond strength values were calculated. Additionally, the samples were examined with a binocular microscope (the picture reflects this). You can find the details of this study, which we found interesting, in our journal.

Other articles selected by our editorial board are:

1- In the article of Başar et al. titled "Factors Affecting the Attitude of Medical Doctors in Türkiye towards Using Artificial Intelligence Applications in Healthcare Services", the impact of artificial intelligence (AI), which enters our lives every day, on healthcare services, medical doctors' acceptance of AI applications and the factors that determine their intention to use are examined. This research is based on the evaluation of 275 medical doctors in Türkiye. It reveals that trust, perceived usefulness, and perceived ease of use are the main positive factors in the acceptance and use of AI by medical doctors. Ultimately, more knowledge and experience about artificial intelligence will lead to greater trust and ease of use.

2- The article of Yılmaz et al. titled "Comparison of Free Gingival Graft and Modified Apical Repositioned Flap Techniques to Create Attached Gingiva: Long-Term (2 Years) Retrospective Study" compares two methods to protect gum health.

3- In the article of Comba et al. titled "Comparison of the Results of Conventional and Laparoscopic Methods of Lymph Node Dissection Performed in Endometrial Cancer Surgery", it was aimed to determine a current approach to 3-en bloc paraaortic lymphadenectomy and to compare the results of this new approach performed by laparotomy and endoscopically.

4- In the article of Altunova et al. titled "Investigating the Frequency of Stent Fracture and Its Impact on In-Stent Restenosis in Patients Undergoing Coronary Artery Stenting", it was aimed to evaluate the incidence and predictors of carotid artery Xact stent fractures and their impact on in-stent restenosis during long-term follow-up.

The most important problem experienced in peer-reviewed journals today is the difficulty in finding referees to evaluate. We have updated the list and contact information of our referees to reduce these difficulties experienced by our editors. Here, I recommend you, our valued readers, to be on the referee list of our journal. Let's work together to take our Bezmialem Science journal to better places.

I am sure you will find an article related to your own branch in this issue. I would like to thank my assistant editors, our referees, our publishing house for their efforts and our valued readers who constantly support us.

May everything be as you wish,

Kind regards,

Prof. Dr. Adem AKÇAKAYA
Chief Editor

Original Article

DOI: 10.14235/bas.galenos.2024.56933
Bezmialem Science 2024;12(3):280-7

Determination of Factors Affecting Lactating Women's Perceptions of Insufficient Milk and the Foods They Use to Increase Breast Milk

Laktasyon Dönemindeki Kadınların Yetersiz Süt Algılarını Etkileyen Faktörler ve Anne Sütünü Artırmak için Kullandıkları Besinlerin Belirlenmesi

Emine KOÇ, Şebnem RÜZGAR, Serap ÖZTÜRK ALTINAYAK

Ondokuz Mayıs University Faculty of Health Sciences, Department of Midwifery, Samsun, Türkiye

ABSTRACT

Objective: Many factors affect breastfeeding rates. One of these factors is the mother's perception of insufficient milk. This study was conducted to determine the factors affecting lactating women's perceptions of having insufficient milk and the foods they used to increase the breast milk.

Methods: This descriptive study was conducted with 356 women. The data were collected using the "sociodemographic information form", the "form for determining the foods used by women to increase breast milk", and "perceived insufficient milk supply (PIMS)" scale. One-way analysis of variance, the independent samples t-test, the Bonferroni test, and Pearson correlation analysis were applied in the evaluation of the data.

Results: The mean PIMS score was 42.39±7.48, and 80.3% of the women perceived their milk to be sufficient. A significant relationship was found between the mean PIMS score of the participants and employment status (p<0.000), time of first breastfeeding (p<0.000), milk being produced immediately after birth (p<0.000), and having enough milk to meet the needs of the baby (p<0.000). It was found that women consumed it and found it beneficial for increasing breast milk by consuming herbs: 30.6% used fennel, 12.6% linden; beverages: 71.3% used water, 50.6% compote, and 28.7% soups; foods: 31.2% used bulgur wheat, 24.2% fruit.

ÖZ

Amaç: Emzirme oranlarını birçok faktör etkilemektedir. Bu faktörlerden biri de annenin yetersiz süt algısıdır. Bu çalışma laktasyon dönemindeki kadınların yetersiz süt algılarını etkileyen faktörler ve anne sütünü artırmak için kullandıkları besinlerin belirlenmesi amacıyla yürütülmüştür.

Yöntemler: Bu tanımlayıcı çalışma 356 kadın ile yürütülmüştür. Araştırma verileri, "sosyodemografik bilgi formu", "kadınların anne sütünü artırmak için kullandıkları besinleri belirleme formu" ve "yetersiz süt algısı ölçeği (PIMS)" ile toplanmıştır. Verilerin değerlendirilmesinde tek yönlü varyans analizi, bağımsız örneklem t-testi, Bonferroni testi, Pearson korelayon analizi uygulanmıştır.

Bulgular: Kadınların PIMS puan ortalamasının 42,39±7,48 olup, %80,3'ünün sütünü yeterli algıladığı belirlendi. Katılımcıların PIMS puan ortalaması ile çalışma durumu (p<0,000), ilk emzirme zamanı (p<0,000), doğumdan hemen sonra sütün gelmesi (p<0,000), bebeğin ihtiyacını karşılayacak kadar sütün olması (p<0,000) arasında anlamlı ilişki bulunmuştur. Kadınların anne sütünü artırmak için; bitkilerden %30,6 rezene, %12,6 ıhlamur, %6,2 maydanoz; içeceklerden %71,3 su, %50,6 komposto, %28,7 çorbalar; yiyeceklerden %31,2 bulgur pilavı, %24,2 meyve tükettikleri ve anne sütünü artırmada faydalı buldukları saptandı.

Address for Correspondence: Emine Koç, Ondokuz Mayıs University Faculty of Health Sciences, Department of Midwifery, Samsun, Türkiye
E-mail: emine_koc555@hotmail.com **ORCID ID:** orcid.org/0000-0003-4333-6797

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This study was presented as an oral presentation at the 6th International 7th National Midwifery Congress on 27-27 September 2023 in Ankara/Türkiye.

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ABSTRACT

Conclusion: It was observed that most mothers perceived their milk supply as sufficient. It was determined that PIMS score was affected by working status, time of milk coming in after birth, and breastfeeding status immediately after birth. It was determined that mothers consumed the most fennel, linden, parsley, water, compote, soup, fruit juice, bulgur pilaf, fruit, raisins, green vegetables and molasses to increase their milk supply.

Keywords: Breast milk, breastfeeding, galactogogues, lactation

ÖZ

Sonuç: Annelerin çoğunun sütünü yeterli algıladığı görüldü. PIMS puanını çalışma durumu, doğumdan sonra sütün gelme zamanı ve doğumdan sonra hemen emzirme durumunun etkilediği saptandı. Annelerin sütünü artırmak için en fazla rezene, ıhlamur, maydanoz, su, komposto, çorba, meyve suyu, bulgur pilavı, meyve, kuru üzüm, yeşil sebzeler, pekmez tükettikleri belirlendi.

Anahtar Sözcükler: Anne sütü, emzirme, galaktogog, laktasyon

Introduction

Breast milk is the most important source of nutrients for maintaining neonatal health. In addition to providing the nutrients the newborn needs, it has many benefits for both mother and baby, such as protecting the newborn from various infections, reducing the risk of allergies, accelerating the mother's involution process, protecting against breast cancer, and aiding mother-child bondin (1). The United Nations Children's Fund and the World Health Organization (WHO) recommend that every infant should be exclusively breastfed for the first six months of life and then continue breastfeeding with supplementary food until the age of 2 (2,3). According to WHO, worldwide between 2015 and 2022 only approximately 44% of babies aged 0-6 months reported exclusive breastfeeding (3). It is worth mentioning that this rate is similar in Türkiye (40.7%) (4). Despite the numerous benefits of breast milk and the constant emphasis on its importance, one of the most prevalent reasons breastfeeding rates are not at the desired level is the perception that the mother's milk is insufficient (5). This stems from mothers' concerns that the quality and quantity of their milk is not adequate for their baby and that their baby is not fully fed (6,7). Results show that during the lactation period, many women immediately start formula or early supplementary food with the thought that their milk is insufficient, and some women terminate breastfeeding early (8,9). Therefore, it is crucial to identify the factors that cause this perception (5). However, the prevalence of the perception of producing insufficient milk has not been sufficiently addressed in the literature, and most studies have been conducted in Western countries (7).

In many societies throughout history, women have traditionally used herbs and nutrients to increase their milk (10,11). Although the foods used vary geographically, it is known that malt beverages, herbs such as fenugreek, goat psoriasis, bull thistle, anise, basil, and fennel seeds, and sweets such as confectionery similar to Turkish delight are generally used (10,12,13). Although the mechanisms of many foods that are thought to increase breast milk during the lactation period are unknown, they are widely used (14). Therefore, it has been recommended to increase the number of studies determining the nutrients used to increase breast milk in the literature (14).

Midwives, who spend the most time with women during pregnancy and after birth, should continue to support and maintain breastfeeding from the antenatal period until the point at which the baby is weaned. They should also take into consideration the perception of having insufficient milk, the foods that mothers consume to increase breast milk, and their attitudes towards, and knowledge and experiences of breastfeeding while developing women's breastfeeding behaviors (11,14).

The literature shows that studies on the perception of having insufficient milk and the nutrients used for increasing breast milk are quite limited (15,16). For this reason, this study was conducted to determine the factors affecting the perception of insufficient milk and the nutrients used by lactating women to increase breast milk.

Methods

Study Design

This study had a descriptive design. The population of the study consisted of mothers with infants aged 0-24 months who came to the Obstetrics and Gynecology and Pediatric Outpatient Clinics of a Maternity Hospital in the north of Türkiye for follow-up. The research was conducted between April 10, 2022, and April 10, 2023. The sample size was calculated using the Epi Info StatCalc program and data from the previous year (2021) (n=4656). The sample size was determined as 355 with a 95% confidence interval, 5% type-1 error level, 50% prevalence, "design effect", and "cluster" 1. The study was completed with 356 women using the random sampling method.

This study was conducted in compliance with the Helsinki Declaration.And informed consent was obtained from all participants before starting the study.

Criteria for inclusion in the study;

- 18 years and over,

- Not having any problems that prevent breastfeeding (such as neurological disease, medication use, psychological illness, mastitis),

- Not having any problem that would affect breastfeeding (anatomical, physiological and neurological disorders, etc.),

- Having a baby aged 0-24 months,

- No problems with vision or hearing,

- Breastfeeding and volunteer mothers were included in the study.

Exclusion criteria from the study;

- Under 18 years old,

- Having any problems that prevent breastfeeding (such as neurological disease, medication use, psychological illness, mastitis),

- Having a problem that affects breastfeeding (anatomical, physiological and neurological disorders, etc.),

- Having a baby over 24 months,

- Having problems with vision or hearing,

- Mothers who did not breastfeed and did not volunteer were excluded from the study.

Procedure

The research data were obtained by the researchers through face-to-face interviews with the women. Before the data collection, each woman participating in the research was given the necessary information about the purpose of the study and the research method of the research, and the data collection then started and lasted an average of 10-15 minutes.

Data Collection

The research data were collected with the sociodemographic information form prepared by the researchers in line with the literature, the form for determining the foods women use to increase breast milk, which is a checklist of foods that increase breast milk, and the perceived insufficient milk supply (PIMS) scale (9-11,13,17).

Sociodemographic Information Form: This form consisted of 16 questions inquiring about socio-demographic (age, education level, income level, etc.), obstetric (number of living children, mode of delivery, etc.), and breastfeeding characteristics (breastfeeding time, breastfeeding education, etc.).

Form for Determining the Foods Women Use to Increase Breast Milk: Herbs, liquid foods, and solid foods consumed by women to increase breast milk were listed. This form contained information about the mothers' use of these and the benefits from them.

Perceived Insufficient Milk Supply Scale: This scale, which was developed by McCarter-Spaulding in 2001 to determine perceptions of having insufficient breast milk, is a form consisting of six questions. The first question asks whether the mother perceives her milk to be sufficient. The mother answers this question as "yes" or "no". The other questions aim to measure the degree of perception of having insufficient milk. The mother is asked to score these questions between 0-10. Zero indicates that milk is perceived as completely insufficient, and 10 indicates that the milk is perceived as completely adequate. A minimum score of 0 and a maximum score of 50 can be obtained from the scale. A higher total score indicates the milk is perceived to be more sufficient. In the original scale, the Cronbach's α value was determined as 0.81 (18). The Turkish validity and reliability study of the scale was conducted by Gökçeoğlu (19) and Küçükoğlu and the Cronbach's α value was found to be 0.82. In the current study, the Cronbach's α value was determined as 0.80.

Ethical Principles of the Research: Ethical permission for the research was obtained from the Social and Human Sciences Research Ethics Committee of Ondokuz Mayıs University (decision no: 2021-803, date: 22.10.2021). The data collection process was initiated after the ethics committee and institutional permission was obtained.

Statistical Analysis

The data were analyzed with the SPSS 25.0 (IBM SPSS Statistics for Windows, Version 25.0) program after the researchers had checked for errors. In the evaluation of the data, number, percentage, mean, and standard deviation were used as descriptive statistical analyses, and their distribution was tested by the Kolmogorov-Smirnov test. One-way analysis of variance and the independent samples t-test were used for intergroup comparisons. Bonferroni test was used for within-group comparisons. Pearson correlation analysis was used to determine the relationship between numerical data and the scale scores. The statistical significance level was accepted as $p<0.05$ in the evaluation of the results.

Results

In Table 1, the minimum and maximum scores obtainable from the PIMS scale and the mean scores for the scale are given. The results showed that women had a minimum score of 16 and a maximum score of 50, and the mean score of the scale was 42.39±7.48, indicating that they had a strong perception that their milk was sufficient. It was determined that 80.3% of the participants perceived their milk to be sufficient (Table 1).

Table 2 shows that there was a significant correlation between the mean total score for the PIMS scale and employment status ($p<0.000$), whereas no significant correlation was found between maternal age ($p=0.865$), birth week ($p=0.435$), the number of living children ($p=0.297$), educational level ($p=0.268$), income level ($p=0.459$), planned pregnancy status ($p=0.129$), and mode of delivery ($p=0.806$) (Table 2).

As seen in Table 3, there was a significant relationship between the mothers' total mean score for the PIMS scale and the time of first breastfeeding ($p<0.000$), milk coming immediately after birth ($p<0.000$) and having enough milk to meet the baby's needs ($p<0.000$), while breastfeeding experience ($p<0.000$), the first food given to the baby ($p=0.303$) and the status of receiving breastfeeding education ($p=0.05$) were not found to be significant (Table 3).

As seen in Table 4, the women found it beneficial to increase breast milk by consuming herbs: fennel was used by 30.6%,

linden by 12.6%, and parsley by 6.2%; beverages: water was used by 71.3%, compote by 50.6%, soups by 28.7%, and fruit juice by 21.1%; foods: bulgur wheat was used by 31.2%, fruit by 24.2%, raisins by 23.3%, green vegetables by 20.5%, and molasses by 19.9% (Table 4).

Discussion

The findings of this study, which examined the factors affecting lactating women's perceptions of having insufficient milk and the foods they used to increase breast milk, were discussed here in relation to the relevant literature. In this study, more than three-quarters of the participants perceived their milk to be sufficient, which is the highest value in the literature in terms of similar studies. When other studies conducted in the literature using the same scale (PIMS) were examined, these rates were 73.1% in Japan (20), 60.2% in Pakistan (21), 50-55.7% in Australia (22), 41.1% in China (23), 37.3% in Singapore (24), 18.4% in the USA (25), 14.9-23.6% in Mexico (26), and 7.5-18% in Canada (27). The fact that the value in this study is higher than in other studies suggests that studies conducted to increase breast milk have started to yield results, and women have started to see their milk as sufficient since the data are recent.

According to a comprehensive systematic review of 27 studies on mothers' perceptions of insufficient milk between 2000 and 2021, factors such as birth week, educational status, income level, sex of the newborn, breastfeeding experience, parity, mode of delivery, planned pregnancy, the psychological status of the mother, breastfeeding education, skin-to-skin contact, and the crying of the newborn all affect PIMS (5). In addition, in a

Table 1. Minimum and maximum scores obtainable from the PIMS scale and the mean scores for the scale (n=356)

Scale	Min-max score obtainable	Min	Max	Mean ± SD
PIMS scale	0-50	16	50	42.39±7.48
Perception of milk as sufficient	**Yes**	**No**		
	286 (80.3%)	70 (19.7%)		

PIMS: Perceived insufficient milk supply, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2. Comparison of sociodemographic and obstetric characteristics of the mothers with the mean total scores for the PIMS scale (n=356)

Variables	Average	Min-max	Applied test, p-value
Age	28.67±5.12	18-43	r=-0.009, p=0.865
Birth week	38.16±2.13	26-42	r=0.42, p=0.435
Number of living children	1.84±0.87	1-5	r=0.55, p=0.297
Variables	n (%)	PIMS scale mean score	
Educational level			F=1.322, p=0.268
Primary education	123 (34.5)	41.78±7.83	
High school	119 (33.4)	42.20±8.29	
University and above	114 (32)	43.32±6.05	
Income status			F=0.907, p=0.459
Income lower than expenditure	108 (30.3)	41.62±7.77	
Income equal to expenditure	216 (60.7)	42.80±7.17	
Income higher than expenditure	32 (9)	42.28±8.54	
Employment status			F=3.757 p<0.000
Employed	34 (9.6)	40.50±7.90[a]	
Unemployed	261 (73.3)	42.14±7.74[a]	
Not working due to childbirth	61 (17.1)	44.52±5.51[b]	
Planned pregnancy status			t=1.520 p=0.129
Planned	245 (68.8)	42.80±6.99	
Unplanned	111 (31.2)	41.50±8.42	
Mode of delivery			t=-0.246 p=0.806
Normal birth	116 (32.6)	42.25±7.41	
Cesarean section	240 (67.4)	42.46±7.50	

X ± SD: Mean ± standard deviation
r: Pearson correlation analysis, [a,b]: Letters indicating statistically significant differences between groups according to the Bonferroni test. There is no statistically significant difference between groups with the same letter. F: One-way analysis of variance, t: Independent samples t-test, min-max: Minimum-maximum

recent study conducted with 370 mothers in the USA, it was found that age and planned breastfeeding duration affected PIMS (28). In the current study, in addition to the studies in the literature, it was determined that employment status, breastfeeding immediately after birth, and breastfeeding the baby as much as the baby needed negatively affected PIMS. That is, these women believed that their milk was more sufficient. This suggests that the factors that may affect the perception of having insufficient milk may vary from woman to woman, which may be due to the difference in the selected samples and the number of participants. Therefore, it is very important for health professionals to take a detailed history from the mothers to whom they provide care and counseling and to identify these factors to maintain breastfeeding.

Maternal nutrition is considered by breastfeeding mothers to be an important factor affecting the amount of breast milk (29,30). Mothers therefore consume various foods to increase their milk

Table 3. Comparison of the breastfeeding characteristics of the mothers and the mean total scores for the PIMS scale (n=356)

Variable	n (%)	PIMS scale mean score	Test, p-value
Breastfeeding experience			t=1.794, p=0.74
Yes	210 (32.6)	42.99±7.24	
No	146 (67.4)	41.54±7.76	
Time of first breastfeeding			F=4.432, p<0.000
Immediately after birth	188 (52.5)	43.14±6.70[a]	
1-2 hours after birth	105 (29.5)	40.59±8.46[b]	
3 or more hours after birth	63 (17.7)	43.19±7.56[a]	
First food given to the baby			t=1.031 p=0.303
Breast milk	276 (77.5)	42.61±7.16	
Baby food	79 (22.5)	41.63±8.51	
Did you produce milk immediately after giving birth?			F=13.753, p<0.000
Milk had come	190 (53.4)	44.16±5.87[a]	
Milk didn't come	56 (15.7)	38.96±8.87[b]	
Had very little milk	110 (30.9)	41.10±7.48[b]	
Breastfeeding education status			t=2.821, p=0.05
Yes	88 (24.7)	44.17±6.45	
No	268 (75.3)	41.81±7.71	
Did you have enough milk to meet your baby's needs?			
Yes	273 (76.7)	44.39±5.65	t=10.274, p<0.000
No	83 (23.4)	35.83±9.03	
Who helped you increase your milk supply?*			
Older family members	159 (44.7)		
Health personnel	87 (24.4)		
My friends	61 (17.1)		
Internet sources	124 (34.8)		
What increases your milk?*			
Herbs and teas	253 (71.1)		
Desserts and similar foods	170 (47.8)		
Sleep	239 (67.1)		
Shower	245 (68.8)		
Rest	198 (55.6)		
Medicines	19 (5.3)		
Social support	87 (24.4)		
Exercise	38 (10.7)		
Midwife/nurse support	76 (21.3)		

X ± SD: Mean ± standard deviation
[a,b]: Letters indicating statistically significant differences between groups according to the Bonferroni test. There is no statistically significant difference between groups with the same letter.
F: One-way analysis of variance, t: Independent samples t-test.*: Participants chose more than one option

(14). In this study, it was found that the mothers benefited the most by consuming herbs such as fennel, linden, and parsley; beverages such as water, compote, soups, and fruit juice; and foods such as bulgur wheat, fruit, raisins, green vegetables, and molasses. Other studies conducted in Türkiye also showed that mothers consumed similar herbs (fennel, linden, and parsley etc.), foods (bulgur wheat, fruit, raisins, green vegetables, molasses, etc.) and beverages water, compote, soups, fruit juice, etc.) (31-34). However, it has been determined that different foods are consumed in different countries to increase breast milk (15-17). In a scoping review of clinical studies on plant galactagogues worldwide 13 studies were found, and it was determined that mothers consumed fenugreek, goat's rue, milk thistle, carduus, stinging nettles, melissa, caraway, anise, fennel, lemongrass, banana flower, ginger, malunggay and Asparagus racemosus (17). A study conducted in New Zealand found that mothers consumed commercially-available lactation cookies, lactation teas, and lactation blends to increase milk (15). Australian mothers consumed lactation cookies, brewer's yeast, fenugreek, and domperidone (16). In a study conducted in Malaysia, lactogenic biscuits made of banana flower extract of Musa x paradisiaca were used (35). Considering that each country has different eating habits, it is normal that the foods consumed by mothers vary from country to country. This may be the reason why the results of different studies conducted in our country are similar. This shows that there are differences

Table 4. Foods consumed/not consumed by mothers to increase milk (n=356)

Plants consumed/not consumed by mothers*	Consumed with no benefit % (n)	Consumed and benefited % (n)	Not consumed % (n)
Fennel	16.3 (58)	30.6 (109)	53.1 (189)
Linden	9.6 (34)	12.6 (45)	77.8 (277)
Parsley	5.9 (21)	6.2 (22)	87.9 (313)
Aniseed	1.7 (6)	4.5 (16)	93.8 (334)
Nettle	2 (7)	2 (7)	96.1 (342)
Beverages consumed/not consumed by mothers*			
Water	11.8 (42)	71.3 (254)	16.9 (60)
Compote	14.6 (52)	50.6 (180)	34.8 (124)
Soups	14.6 (52)	28.7 (102)	56.7 (202)
Fruit juice	8.1 (29)	21.1 (75)	70.8 (252)
Puerperium sherbet	9.6 (34)	17.7 (63)	72.8 (259)
Buttermilk	6.2 (22)	14 (50)	79.8 (284)
Tea	7.6 (27)	12.6 (45)	79.8 (284)
Milk-forming instant teas	2 (7)	12.1 (43)	86 (306)
Sugar water	4.5 (16)	9.8 (35)	85.7 (305)
Cow's milk	6.7 (24)	3.9 (14)	89.3 (318)
Zamzam water	1.7 (6)	3.9 (14)	94.4 (336)
Foods consumed/not consumed by mothers*			
Bulgur wheat	12.9 (46)	31.2 (111)	55.9 (199)
Fruit	12.4 (44)	24.2 (86)	63.5 (226)
Raisins	8.1 (29)	23.3 (83)	68.5 (244)
Green vegetables	12.1 (43)	20.5 (73)	67.4 (240)
Molasses	12.9 (46)	19.9 (71)	67.1 (239)
Tahini halva	8.7 (31)	16.9 (60)	74.4 (265)
Meat/chicken/fish	12.1 (43)	14.6 (52)	73.3 (261)
Salad	11.5 (41)	13.5 (48)	75 (267)
Hazelnuts/pistachios	11 (39)	18 (64)	71.1 (253)
Desserts	8.7 (31)	16.3 (58)	75 (267)
Figs	7 (25)	13.5 (48)	79.5 (283)
Onions	5.6 (20)	8.4 (30)	86 (306)
Dried beans/chickpeas	2.5 (9)	5.1 (18)	92.4 (329)
Potatoes	5.3 (19)	4.2 (15)	90.4 (322)

*: Participants chose more than one option

in the foods consumed due to the cultural and geographical differences between countries. The lack of studies on the side effects of the plants used in the literature is also noteworthy (17). For midwives who provide breastfeeding counseling to mothers to be better informed about the side effects of the herbs used, studies with adequate levels of evidence are required.

Study Limitations

The study data were collected based on self-reporting by participants, and the information they provided was assumed to be correct. At the same time, since the study sample consisted only of women admitted to the abovementioned hospital, the fact that the results obtained cannot be generalized to all women in Türkiye is a limitation of the study.

Conclusions

The results showed that most of the mothers included in the study perceived their milk to be sufficient. The hope is that this will contribute to the literature in terms of being the study in which the rates of having the perception of producing sufficient milk are at their highest. Factors affecting the perception of insufficient milk were found to include employment status, the time that milk was first produced after birth, and breastfeeding immediately after birth. It was concluded that mothers benefited the most by consuming herbs such as fennel, linden, and parsley, beverages such as water, compote, soups, and fruit juice, and foods such as bulgur wheat, fruit, raisins, green vegetables, and molasses to increase their milk. When all the results were taken into consideration, it was seen that there were, nevertheless, mothers who perceived their milk to be insufficient, and that the mothers consumed different plants and foods to increase their milk. However, the mechanism of action of these nutrients is not well known. Therefore, it is recommended to conduct randomized controlled studies to understand the benefits and harms of the foods, herbs, and beverages consumed by mothers. In addition, midwives can contribute to preventing the mother's perception of inadequacy after birth by providing breastfeeding training to expectant mothers before birth. At the same time, midwives can use their care and consultancy role to identify factors that may cause mothers to perceive insufficient milk and be effective in eliminating the problems.

Acknowledgments: We thank the womens who gave up their time to participate in this study.

Ethics

Ethics Committee Approval: Ethical permission for the research was obtained from the Social and Human Sciences Research Ethics Committee of Ondokuz Mayıs University (decision no: 2021-803, date: 22.10.2021).

Informed Consent: This study was conducted in compliance with the Helsinki Declaration.And informed consent was obtained from all participants before starting the study.

Authorship Contributions

Concept: E.K., Ş.R., S.Ö.A., Design: E.K., Ş.R., Data Collection or Processing: E.K., S.Ö.A., Analysis or Interpretation: E.K., Ş.R., Literature Search: E.K., Writing: E.K., Ş.R., S.Ö.A.

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Original Article

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Evaluation of a Clinical Decision Support System for the Identification of Inappropriate Prescription Patterns in Elderly in the Community Pharmacy Setting

Toplum Eczanelerinde Yaşlılarda Uygun Olmayan Reçete Kalıplarının Belirlenmesi için Geliştirilmiş Bir Klinik Karar Destek Sisteminin Değerlendirilmesi

Beyza TORUN[1], Şule APİKOĞLU[2]

[1]Marmara University Institute of Health Sciences, Department of Clinical Pharmacy, İstanbul, Türkiye
[2]Marmara University Faculty of Pharmacy, Department of Clinical Pharmacy, İstanbul, Türkiye

ABSTRACT

Objective: This study aimed to design and evaluate a clinical decision support system (CDSS) identifying inappropriate prescription patterns in the elderly to be used at community pharmacies.

Methods: The study was carried out in 20 community pharmacies during a 6-month period on patients ≥65 years. A CDSS was developed and integrated into the pharmacy automation systems to automatically check the medications of the patients for the presence of any potentially inappropriate medications (PIMs). Depending on the preference of the pharmacist the recommendations were communicated with the prescriber or not. The number and characteristics of the PIMs, prescribers' acceptance status of the recommendations, and usability of the CDSS were recorded.

Results: During the 6-month period 1250 prescriptions each from an individual patient were evaluated. The median (interquartile range) age of the patients was 73 (63-81) years. The total number of PIMs was 1359 and 59% of the patients had at least one PIM. The most frequently identified PIMs involved proton pump inhibitors (16%) and selective beta-blockers (11.9%). The pharmacists communicated with the prescribers regarding 24.4% of the PIM-involving prescriptions and 85.8% of the prescribers accepted the recommendations. The usability of the CDSS was found to be good.

ÖZ

Amaç: Bu çalışmada toplum eczanelerinde yaşlılarda uygunsuz reçete kalıplarının belirlenmesine olanak sağlayan bir klinik karar destek sistemi (KKDS) tasarlanması ve değerlendirilmesi amaçlanmıştır.

Yöntemler: Çalışma, 6 aylık bir süre boyunca 20 toplum eczanesinde 65 yaş ve üstü hastalarda yürütülmüştür. Yaşlı hastaların reçetelerinin potansiyel uygunsuz ilaçlar (PUİ) varlığı açısından otomatik olarak kontrol edilmesine olanak sağlayan bir KKDS geliştirilmiş ve eczane otomasyon sistemlerine entegre edilmiştir. Eczacının tercihine bağlı olarak öneriler reçeteyi yazan doktora iletilmiş ya da iletilmemiştir. PUİ'lerin sayısı ve özellikleri, reçete yazan doktorların önerileri kabul etme durumu ve KKDS'nin kullanılabilirliği kaydedilmiştir.

Bulgular: Altı aylık dönem boyunca her biri ayrı bir hastaya ait 1250 reçete değerlendirilmiştir. Hastaların medyan (çeyrekler arası aralık) yaşı 73 (63-81) idi. Hastaların %59'unun en az bir PUİ'ye sahip olduğu ve toplam PUİ sayısının 1359 olduğu tespit edilmiştir. En sık karşılaşılan PUİ'ler proton pompası inhibitörleri (%16) ve selektif beta-blokerlerdir (%11,9). Eczacılar, PUİ içeren reçetelerin %24,4'ü ile ilgili olarak reçeteyi yazan doktorla iletişim kurmuş ve önerilerin %85,8'i doktorlar tarafından kabul etmiştir. KKDS'nin kullanılabilirliği iyi olarak bulunmuştur.

Address for Correspondence: Beyza Torun, Marmara University Institute of Health Sciences, Department of Clinical Pharmacy, İstanbul, Türkiye
E-mail: beyzatorun@gmail.com **ORCID ID:** orcid.org/0000-0002-7915-2933

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ABSTRACT

Conclusion: It is anticipated that the widespread use of this product would prevent drug-related adverse events, hospitalizations, morbidities, and mortalities; thus, would improve patients' health and quality of life, as well as lead to better clinical, humanistic, and economic outcomes.

Keywords: Clinical decision support system, community pharmacy, elderly, geriatric, potentially inappropriate medication

ÖZ

Sonuç: Bu ürünün yaygın kullanımının ilaca bağlı advers olayları, hastaneye yatışları, morbiditeleri ve mortaliteleri önleyeceği; böylece hem hastaların sağlık ve yaşam kalitesinde, hem de sağlık çıktılarının klinik, insani ve ekonomik düzeylerinde iyileşmelere yol açacağı öngörülmektedir.

Anahtar Sözcükler: Klinik karar destek sistemi, toplum eczanesi, yaşlı, geriatrik, potansiyel uygunsuz ilaç

Introduction

The number of elderly people is increasing worldwide. In Europe, the rate of elderly people over 65 years old, which was 17.4% in 2010 is expected to increase to 29.5% in 2060 (1). Türkiye's population is aging, too. In Türkiye, the rate of the elderly population, which was 9.9% in 2022, is expected to be 12.9% in 2030, 16.3% in 2040, 22.6% in 2060, and 25.6% in 2080 (2).

Increasing life expectancy brings new challenges for effective patient care. Factors such as multimorbidity, polypharmacy, and frailty challenge the provision of safe and effective drug therapy for older adults. Multimorbidity, which is the presence of at least two chronic health conditions (3), results in polypharmacy, which is often defined as the routine use of at least five medications. The prevalence of prescription drug use increases with age; 36% of older adults regularly take at least five prescription drugs (4).

Polypharmacy increases the medication error rate as reported by Avery et al. (5), who found that the medication error rate was 30.1% in patients taking five or more medications and 47% in patients receiving 10 or more medications.

Polypharmacy is also associated with a higher risk of "potentially inappropriate prescribing (PIP)" (6). PIP is defined as prescribing medication therapies that do not comply with accepted medical standards and, therefore, may cause significant harm to elderly patients. PIP can either be in the form of "potentially inappropriate medications (PIMs)", which is prescribing a medication that may not produce benefit relative to its harm, or "potential prescription omissions", which is, not prescribing recommended medications.

Inappropriate prescribing can cause adverse drug events in the elderly (7). With a prevalence ranging from 22.6% for community-dwelling older persons (8) to 43.2% for nursing home residents (9), PIPs were associated with lower quality of life as well as increased adverse drug events, hospitalizations, and healthcare costs (7).

PIMs are classified as a category of drug-related problems (DRPs), and elderly patients have a high risk of DRPs (10). While clinical medication review, including patient interviews, is an important tool in identifying and resolving DRPs (10), this process seems to be quite time-consuming for the elderly due to polypharmacy. Therefore, it is necessary to standardize and facilitate the clinical medication review process to use the limited workforce, time, and other resources in the most effective way (10).

Various tools such as the medication appropriateness index, the American Geriatrics Society (AGS) Beers Criteria® (AGS Beers Criteria®) for PIM Use in Older Adults, Screening Tool of Older People's Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) Criteria for PIP in older people (STOPP/START criteria), and Turkish Inappropriate Medication Use in the Elderly (TIME) Criteria to Improve Prescribing in Older Adults: TIME-to-STOP and TIME-to-START have been developed to make the identification of DRPs easier during the medication review process. These tools can be integrated into clinical decision support systems (CDSSs) which are computer programs that generate alerts aimed at helping healthcare professionals improve the quality and safety the of medication therapy they provide (11-14). CDSSs can provide automated, near-real-time monitoring, alerting, analysis, and reporting (15). PIPs can be prevented by the use of CDSSs at the time of prescribing.

CDSSs are generally designed to support physicians when prescribing (16). Only a small number of studies evaluated the use of CDSSs in pharmacy practice (17-21), helping to increase the DRP identification rate during the medication evaluation process (17).

Currently, there isn't any CDSS developed particularly to be used for elderly patients in the community pharmacy setting in Türkiye. Community pharmacists' knowledge of medications, including over-the-counter medications, combined with the availability of electronic medication registration systems puts pharmacists in an ideal position to identify PIPs.

This study aimed to design a CDSSs to identify inappropriate prescription patterns in the elderly and to evaluate the use of this digital system at community pharmacies.

Methods

This study was granted ethical approval by the Ethics Committee of Marmara University (approval no: 115, date: 15.04.2019).

Development of the CDSS

A CDSS to be used in community pharmacies aiming to detect inappropriate medication prescriptions in elderly patients and provide relevant solutions was developed by the authors who

were clinical pharmacy scholars (Ş.A., B.T.) in collaboration with a software developer.

While establishing CDSS algorithms, three criterion sets were taken into consideration: the 2019 Updated AGS Beers Criteria®, the STOPP/START Version 2 Criteria, and the TIME Criteria. Among these criterion sets the most appropriate criteria that could be used in the community pharmacy setting was chosen by the authors and included in the CDSS.

The CDSS consisted of 78 criteria. Fortythree from the 2019 Updated AGS Beers Criteria®, 43 from the STOPP/START Version 2 Criteria, and 56 from the TIME Criteria. Similar criteria from these three criterion sets were merged accordingly.

This CDSS was structured to work as follows:

- First, the CDSS was integrated into the pharmacy automation systems of pharmacies that volunteered to participate in the study.

- The "pharmacy automation system" prompts the CDSS whenever the pharmacist attempts to process a prescription of an elderly (≥65 years) patient.

- The CDSS processes not only the medications listed in the most recent prescription to be filled but also all of the recorded medications the patient seems to be currently using and checks for the presence of any inappropriate prescription pattern.

- If any inappropriate prescription pattern is encountered, the CDSS generates an "alert" through a pop-up screen. An "alert" contains at least one item of warning regarding inappropriate prescription pattern(s) identified for that specific patient. Multiple warnings are generated when more than one inappropriate prescription pattern (hereafter referred to as "PIM") is encountered.

- The warning also includes detailed information about the rationale for the inappropriateness and/or recommendations for appropriate approaches (Figure 1).

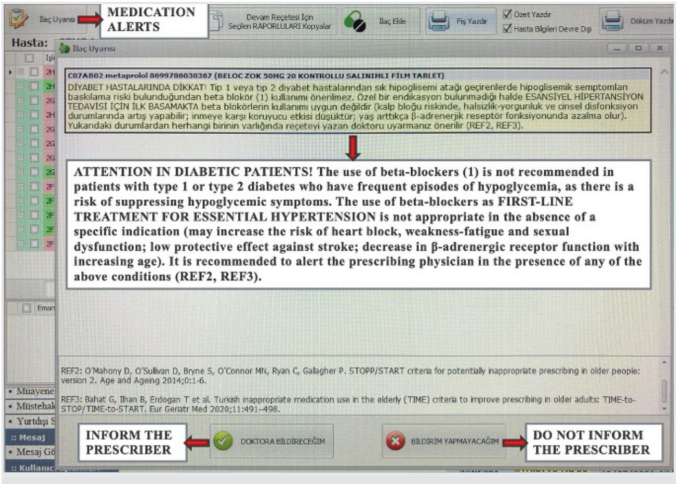

Figure 1. Example of an alert pop-up screen

- At the bottom of the pop-up screen two decision buttons are located, guiding the pharmacist on whether or not to inform the prescriber about the recommendations.

- Pharmacists are considered to decide between two choices: "to inform" or "not to inform" the prescriber. This decision was entirely at the discretion of the pharmacist.

Implementation

The study was conducted in 20 community pharmacies run by qualified pharmacists who had received clinical pharmacy training through MSc studies or certified courses. Patients aged 65 and over who visited the study pharmacies to have their prescriptions filled throughout the study period (1st March-1st September 2021) were invited to the study. The study was conducted on patients who agreed to participate.

First, the CDSS was installed on the computers of the pharmacies and integrated with the pharmacy automation systems. Pharmacists were authorized to sign in to the system using their confidential passwords. Entering the social security ID number of the patient in the system allowed the pharmacist access to the list of currently registered medications of that individual patient.

The pharmacy automation system prompted the CDSS if the patient's age was ≥65 years, and the CDSS checked the medications of the patient for any inappropriate prescription patterns.

Depending on the preference of the pharmacist the recommendations were communicated with the prescriber or not. While communicating with the prescriber, pharmacists explained the rationale for and recommendations about the identified PIM. No information about the presence and/or structure of any inappropriate prescription pattern was shared with the patient.

Usability of the CDSS

The usability of the CDSS was assessed by the system usability scale (SUS), which is a ten-item scale reflecting a global view of subjective assessment of usability. SUS is scored using a 5-point Likert scale (from 1 to 5) ranging from "strongly disagree" to "strongly agree". SUS scores are calculated as described in the work of Brooke (22). The overall SUS score ranges from 0 to 100. The SUS score of 68 represents the 50th percentile (median) corresponding to the center of the range for an average grade (C), while a SUS score between 77.2-78.8 represents the 80th-84th percentile corresponding to a grade of B+, and a SUS score between 84.1-100 represents the 96th-100th percentile corresponding to a grade of A+. A SUS score above 68 indicates average performance, while a score below 68 is considered below average (23).

Other Data

Data regarding the pharmacies (location, number of prescriptions filled per month), the pharmacists (age, gender, experience), patients (age, gender, number of medications, number of chronic diseases, number of PIMs, presence of polypharmacy, i.e.,

simultaneous use of ≥5 medications), and the alerts (number of alerts communicated with the prescribers, the prescribers' reply) were also collected.

The main outcome measures were as follows:

- Descriptive features of inappropriate prescription patterns detected by the CDSS,

- Descriptive features of prescribers' acceptance status of the CDSS-generated recommendations communicated with them,

- Opinions and attitudes of pharmacists regarding CDSS use.

Statistical Analysis

The SPSS 11.5 (SPSS Inc., Chicago, IL) was used for the statistical analysis. Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to determine the distribution characteristics of the data. Frequency data was expressed as n (%), while data with non-normal distribution were expressed as the median and interquartile range (IQR). Statistical significance was considered as $p<0.05$.

Results

The characteristics of the study pharmacies and the pharmacists (n=20) who participated in the study are presented in Table 1. The typical study pharmacist was a 35-year-old woman with 9.5 years of professional experience. The most common (50%) pharmacy location was "near a local general practitioner (GP) office".

Table 1. Characteristics of the study pharmacies and pharmacists (n=20)

Pharmacist	n (%)
Age (year); median (IQR)	35.00 (30.00-52.75)
Experience (year); median (IQR)	9.50 (5.25-29.00)
Female/male	14 (70)/6 (30)
Location of the pharmacy	
Near a local GP office	10 (50)
Pharmacy in a residential area	6 (30)
Near hospital	3 (15)
On a shopping street	1 (5)
Number of monthly prescriptions	
301-600	4 (20)
601-900	2 (10)
901-1200	4 (20)
1201-1500	4 (20)
>1500	6 (30)
Number of monthly geriatric prescriptions	
0-100	3 (15)
101-200	4 (20)
201-300	6 (30)
301-400	2 (10)
401-500	3 (15)
>500	2 (10)

GP: General practitioner, IQR: Interquartile range

From 20 pharmacies, 1250 patients participated in the study. The median (IQR) age of the patients was 73 (68-81) years, and 60.2% of them were female; 660 (52.8%) lived with their spouses and 24.2% with their children. The median (IQR) number of chronic diseases and number of medications for individual patients were 3 (2-4) and 5 (3-8), respectively. Polypharmacy was present in 56.6% of the patients. Detailed patient characteristics are presented in Table 2.

The prescriptions of more than half of the patients (59.04%; n=738/1250) resulted in inappropriate prescription pattern alerts containing a total of 1359 PIMs. The most frequently encountered PIMs involved medications listed in Table 3.

The pharmacists decided "not to inform the prescriber" for 75.6% of the 738 alert-generated prescriptions. Pharmacists' reasons for not communicating the warnings and recommendations with the prescriber are shown in Table 4. Only 10% (n=2/20) of the pharmacists made referrals to the prescribers. These two pharmacists communicated with the prescribers regarding 317 PIMs in 180 prescriptions, while no recommendation was made to the prescriber regarding 1042 PIMs. Prescribers' acceptance status of the recommendations made to them (n=317) was as shown in Table 5.

As the patient's age increased, the number of diseases ($r=0.116$, $p<0.01$), the number of medications used ($r=0.079$, $p<0.01$), and the number of PIMs ($r=0.155$, $p<0.01$) increased significantly.

The number of PIMs increased significantly as the number of chronic diseases ($r=0.401$, $p<0.01$) and the number of medications used ($r=0.612$, $p<0.01$) increased.

The number of medications used increased as the number of chronic diseases increased ($r=0.564$, $p<0.01$); patients with polypharmacy were older ($p=0.025$), had a higher number of chronic diseases ($p<0.001$) and had a higher number of PIMs in their prescriptions ($p<0.001$).

The overall median SUS score of the study CDSS was 77.25. SUS item scores are shown in Table 6.

Discussion

Twenty pharmacists who received clinical pharmacy training through MSc studies or certified courses participated in this study. The typical pharmacist in the study was a 35-year-old woman with 9.5 years of professional experience. The most common location (50%) of pharmacies was "near a local GP office".

The study included 1250 patients from 20 pharmacies. The patients' median age was 73 years, and 60.2% were female. Studies on geriatric patients in the literature included patients of similar age, with a median age ranging from 73.3 to 78.7 years (10,20,24-26).

The median number of chronic diseases and medications per patient was 3 and 5, respectively, in accordance with those reported from similar other studies where patients' median number of diseases and medications ranged from 2 to 11 (10,20,24-27), and from 3 to 10 (10,24-27), respectively.

The most prevalent chronic condition in our study was cardiovascular diseases, followed by diabetes. The pattern of chronic diseases aligned with the local data reported in the study of Zoghi et al. (28) which was conducted on more than 5,000 geriatric patients in Türkiye and with the global data indicating that cardiovascular conditions, arthritis, and diabetes were the most prevalent long-term conditions among geriatric patients (20,29).

More than half (56.6%) of our patients had polypharmacy as anticipated from the geriatric population, where polypharmacy prevalence was reported to range from 4% to 96.5% (30).

The CDSS used in this study was developed based on the 2019 Updated AGS Beers Criteria®, STOPP/START Version 2 Criteria, and TIME Criteria. Only the most appropriate criteria to be used in the community pharmacy setting were chosen; therefore, the START criteria, which are to be used by the prescribers, were not included in the study. The studies in the literature commonly used different versions of Beers criteria (10,20,24,31), STOPP/START criteria (10,20,31-33), and PRISCUS list (20) to detect PIMs in older patients. In addition to these criterion sets, we also used a local criterion set (TIME Criteria) to include the local expert opinion.

In this study, at least one PIM at more than half (59%, n=738/1250) of the prescriptions was identified by the CDSS. The mean number of PIMs per patient was 1.09 (n=1359/1250). Different PIM prevalence rates were reported from both local and international studies depending on the study design, sample size, setting, the criterion sets used, and patient characteristics. Studies on elderly patients in Türkiye revealed a prevalence of PIM ranging from 10.9% to 80.6% (34-41), while similar rates ranging from 7.87% to 57.6% (24,26,42,43) and even higher rates up to 81% for patients residing at residential care facilities (44,45) were recorded in international studies.

A local study using the TIME, Beers 2019, and STOPPv2 criteria revealed a PIM prevalence rate of 46.1%, 30.6%, and 26.2%, respectively, while a higher (46.9%) PIM rate was reported

Table 2. Characteristics of the patients (n=1250)

Patients	n (%)
Age; median (IQR)	73.00 (68.00-81.00)
Female/male	753 (60.2)/497 (39.8)
Chronic diseases	
Hypertension	955 (76.4)
Diabetes	486 (38.9)
Coronary artery disease	357 (28.6)
Dyslipidemia	414 (33.1)
Arrhythmia	151 (12.1)
With whom does the patient live?	
At a nursing home	145 (11.6)
Alone	129 (10.3)
Spouse	660 (52.8)
Children	302 (24.2)
Caretaker	7 (0.6)
Other	7 (0.6)
Number of medications; median (IQR)	5.00 (3.00-8.00)
Polypharmacy	707 (56.6)

IQR: Interquartile range

Table 3. The most frequently encountered potentially inappropriate medications

PIMs	n (%)
Proton pump inhibitors	217 (16.0)
Selective beta-blockers	162 (11.9)
Betahistine, trimetazidine, dimenhydrinate	75 (5.5)
Non-steroidal anti-inflammatory drugs	72 (5.3)
Acetylcholinesterase inhibitors	70 (5.2)

PIM: Potentially inappropriate medication

Table 4. Pharmacists' reasons for not communicating the recommendations with the prescriber (n=20)

Comments	n (%)
The pharmacist thought that the prescriber would not care	10 (50.0)
The pharmacist thought that the prescriber would not trust the information provided by the pharmacist	11 (55.0)
The pharmacist hesitated to contact the prescriber as he/she did not know him/her in person	15 (75.0)
Communication issues	18 (90.0)
Lack of time	19 (95.0)

The question might have more than one answer

Table 5. Acceptance status of the recommendations (n=317)

	n (%)
The recommendation was accepted	
The recommendation was accepted and implemented	158 (49.8)
The recommendation was accepted but not implemented	77 (24.3)
The recommendation was accepted, not implemented, but followed up	37 (11.7)
The recommendation was not accepted	
The recommendation was not accepted; no agreement was reached	3 (0.9)
The recommendation was not accepted; reason is unknown	24 (7.6)
Other	
Recommendation made, acceptance status unknown	18 (5.7)

when all three criterion sets were considered (36). In another study, number of patients with at least one PIM identified by the TIME-to-STOP criteria and Beers 2019 was 33% and 10.9%, respectively (34). Different rates were also reported from another study depending on the criteria set used: as 80.6% (Beers 2019), 59.7% (STOPPv2), and 48.2% [EU(7)-PIM] (35). The PIM rate according to TIME-to-STOP criteria among the elderly attending geriatric outpatient clinics was between 21.5-38%, whereas this rate was 11.7% for those receiving palliative care (34,39-41), and 48.2% for intensive care patients (35). Other studies from Türkiye reported similar rates of patients who had at least one PIM as 45.1% (37) and 41.4% (38).

In the systematic review and meta-analysis of 132 studies, including more than 370 million geriatric patients from 17 countries, conducted by Tian et al. (46), PIM prevalence was reported to be 36.7%. Sub-group analysis of that study showed that PIM prevalence in Türkiye was 39.6% depending on the six studies included and 56.3% in low-income countries (according to the World Bank classification) (46).

OPERAM (OPtimising thERapy to prevent Avoidable hospital admissions in the Multimorbid elderly) study, aiming to optimize the existing therapy among the elderly population aged ≥75 years by the use of a CDSS reported a rate of inappropriate prescribing for 86.1% of the participants (33).

The rate (59%) of patients with at least one PIM in our study was higher than many of those reported in the literature. The reasons for that might be that unlike the other studies we used a combination of three criterion sets instead of one, and as the intervention we used a CDSS instead of medication review; both approaches together might have prevented missing PIMs and helped identify a higher rate of PIMs.

In this study, PIM alerts were mostly generated for PPIs. Similarly, in the systematic review and meta-analysis of Tian et al. (46), benzodiazepines, non-steroidal anti-inflammatory drugs, and proton pump inhibitors were the first three medication classes that generated PIM alerts. The high prevalence of PPI-related PIMs may be due to several factors. As PPIs are known to be gastroprotective agents, most patients tend to continue these medications infinitely, and they might put pressure on their GPs to prescribe a PPI. Also, in elderly patients, bleeding problems may be more prevalent due to the increased number of medications and be more detrimental due to the existing comorbidities. Therefore, prescribers might have preferred a defensive approach and continued PPIs beyond the indicated period. Another factor might be the prescribers' lack of knowledge regarding the adverse effects of PPIs on the elderly.

The pharmacists in our study communicated with the prescribers regarding 24.4% of the prescriptions. The majority (85.8%) of the prescribers accepted the recommendations generated through the CDSS and communicated by the pharmacist. The prescriber acceptance rate of the pharmacist's interventions was similar to the higher rates reported in the literature, which ranged from 61.8% to 93.2% (47-50). The acceptance rate was high because only the pharmacists who felt comfortable communicating with the specific prescriber with whom they already had strong professional communication proposed interventions to the prescribers. Therefore, due to the mutual professional trust, the acceptance rate was high. The rate of pharmacists who felt comfortable communicating with the prescribers was very low (10%). Lack of time and communication issues were the major reasons for almost all of the pharmacists who did not communicate with the prescribers. A professional relationship between a pharmacist and a physician is crucial for the success of a CDSS (18) as demonstrated in our study, where only the pharmacists with a good relationship with the prescribers communicated with them and this communication yielded a high pharmacist intervention acceptance rate.

Table 6. System usability scale item scores (n=20)

System usability scale items	Strongly disagree, n (%)	Disagree, n (%)	Neither agree nor disagree, n (%)	Agree, n (%)	Strongly agree, n (%)
I think that I would like to use this system frequently	0 (0)	0 (0)	3 (15)	9 (45)	8 (40)
I found the system unnecessarily complex	5 (25)	14 (70)	0 (0)	1 (5)	0 (0)
I thought the system was easy to use	0 (0)	0 (0)	2 (10)	8 (40)	10 (50)
I think that I would need the support of a technical person tobe able to use this system	1 (5)	14 (70)	3 (15)	0 (0)	2 (10)
I found the various functions in this system were well integrated	0 (0)	0 (0)	8 (40)	9 (45)	3 (15)
I thought there was too much inconsistency in this system	3 (15)	15 (75)	2 (10)	0 (0)	0 (0)
I would imagine that most people would learn to use this systemvery quickly	0 (0)	0 (0)	2 (10)	14 (70)	4 (20)
I found the system very cumbersome to use	8 (40)	12 (60)	0 (0)	0 (0)	0 (0)
I felt very confident using the system	0 (0)	0 (0)	1 (5)	11 (55)	8 (40)
I needed to learn a lot of things before I could get going with this system	0 (0)	17 (85)	3 (15)	0 (0)	0 (0)

The CDSS used in this study had a SUS score corresponding to a grade of B+ (23) indicating the good usability of the system. Poor usability may increase workload and cause alert fatigue while decreasing the system's acceptability and effectiveness.

Study Limitations

While being a novel research on the use of a CDSS in the community pharmacy setting, our study has several limitations. One of the limitations was that only pharmacists with an established good relationship with individual prescribers communicated with them regarding the PIMs. Therefore, this raised a bias resulting in a high recommendation acceptance rate, which might not be the case in daily practice. Another limitation was that although the calculated SUS score indicated good usability of the system, due to the small sample size, it is hard to anticipate the real usability in daily practice.

Conclusion

This study showed that inappropriate medication prescription patterns in the elderly can be identified comprehensively in the community pharmacy setting by the use of a diligently developed clinical decision support system. It is anticipated that the widespread use of this product would prevent medication-related adverse events and related hospitalizations, morbidities, and mortalities, thus help improving patients' health and quality of life as well as leading to better clinical, humanistic, and economic outcomes.

Ethics

Ethics Committee Approval: This study was granted ethical approval by the Ethics Committee of Marmara University (approval no: 115, date: 15.04.2019).

Informed Consent: The patients included in this study were those who granted their consent after being informed about and invited to the study.

Authorship Contributions

Concept: Ş.A., Design: B.T., Ş.A., Data Collection or Processing: B.T., Analysis or Interpretation: B.T., Ş.A., Literature Search: B.T., Ş.A., Writing: B.T., Ş.A.

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Original Article

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Factors Affecting the Attitude of Medical Doctors in Türkiye towards Using Artificial Intelligence Applications in Healthcare Services

Türkiye'de Tıp Doktorlarının Sağlık Hizmetlerinde Yapay Zeka Uygulamalarını Kullanmaya Yönelik Tutumlarını Etkileyen Faktörler

Enes Emre BAŞAR[1], Aysu KES ERKUL[2]

[1]Anadolu University Faculty of Business, Department of Marketing, Eskişehir, Türkiye
[2]Ankara Science University Faculty of Humanities and Social Sciences, Department of Political Science and Public Administration, Ankara, Türkiye

ABSTRACT

Objective: Artificial intelligence (AI) is transforming various sectors, including healthcare. The aim of this research was to examine the factors that determined acceptance of and intention to use AI applications by medical doctors.

Methods: This research was based on an online survey conducted with 275 medical doctors in Türkiye. The survey was prepared in English and was later translated into Turkish by the researchers. The study employed a convenience sampling technique. The partial least squares-structural equation modeling was employed to ascertain causal relationships for theory confirmation. The data analysis utilized SmartPLS 3. Descriptive statistics were calculated with the SPSS 25 software.

Results: According to the findings, trust (β=0.651; t=25.876; p<0.01) was the strongest positive factor for increased intention to use AI applications. Perceived usefulness (β=0.613; t=22.851; p<0.01) and perceived ease of use (PEOU) (β=0.644; t=14.577; p<0.01), significantly predicted ıntension to use. Technological anxiety was not a significant predictor for intension to use (β=0.067; t=1.014; p=0.093) as well as facilitating conditions (β=0.071; t=1.041; p=0.102).

Conclusion: This research reveals that trust, perceived usefulness, and PEOU are the major positive factors for AI to be accepted and used by medical doctors. The greater trust and ease of use that

ÖZ

Amaç: Yapay zeka (AI), sağlık hizmetleri de dahil olmak üzere çeşitli sektörleri dönüştürmektedir. Bu araştırmanın amacı, tıp doktorlarının AI uygulamalarına yönelik kabullerini ve kullanım niyetlerini belirleyen faktörlerin incelenmesidir.

Yöntemler: Bu araştırma, Türkiye'deki 275 tıp doktoru ile yapılan çevrimiçi ankete dayanmaktadır. Anket İngilizce olarak hazırlanmış ve daha sonra araştırmacılar tarafından Türkçeye çevrilmiştir. Araştırmada kolayda örnekleme tekniği kullanılmıştır. Kısmi en küçük kareler-yapısal denklem modellemesi, teorinin doğrulanması amacıyla nedensel ilişkileri tespit etmek için kullanılmıştır. Veri analizinde SmartPLS 3 kullanılmıştır. Betimleyici istatistikler SPSS 25 yazılımıyla hesaplanmıştır.

Bulgular: Bulgulara göre, güven (β=0,651; t=25,876; p<0,01), AI uygulamalarını kullanma niyetinin artmasında en güçlü pozitif faktördür. Algılanan fayda (β=0,613; t=22,851; p<0,01) ve algılanan kullanım kolaylığı (β=0,644; t=14,577; p<0,01), kullanma niyetini anlamlı şekilde öngörmektedir. Teknolojik kaygı (β=0,067; t=1,014; p=0,093) ve kolaylaştırıcı koşullar (β=0,071; t=1,041; p=0,102) kullanım niyeti için anlamlı bir yordayıcı değildir.

Sonuç: Bu araştırma, AI'nın tıp doktorları tarafından kabul edilmesi ve kullanılmasında güven, algılanan fayda ve algılanan kullanım kolaylığının başlıca olumlu faktörler olduğunu ortaya

Address for Correspondence: Aysu Kes Erkul, Ankara Science University Faculty of Humanities and Social Sciences, Department of Political Science and Public Administration, Ankara, Türkiye
E-mail: aysu.erkul@ankarabilim.edu.tr **ORCID ID:** orcid.org/0000-0001-6283-0606

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ABSTRACT

comes with more knowledge and experience about AI may lead to more action to be taken to benefit from AI in the healthcare sector.

Keywords: AI in healthcare service, extended TAM, AI acceptance, PLS-SEM

ÖZ

koymaktadır. AI hakkında daha fazla bilgi ve deneyimin getirdiği daha fazla güven ve kullanım kolaylığı, sağlık sektöründe AI'dan yararlanmak için daha fazla adım atılmasını sağlayabilir.

Anahtar Sözcükler: Sağlık hizmetlerinde yapay zeka, E-TAM, yapay zeka kabulü, PLS-SEM

Introduction

Artificial intelligence (AI) is undoubtedly the most debated technological development of today. Aside from the technical improvements in AI, the expansion of AI implementations is the major reason for this debate in business world, media, and science community. AI which entered our lives with automation, production systems and sales/marketing applications is now being used in essential sectors such as education and health. It is frequently suggested that in a global scale, AI is affecting individual as well as industrial activities (1). Therefore, it became a transforming element for the society as a whole.

It can be said that there is a clear relationship between a series of developments in recent years and the widening use of AI. The most important of these is the increasing volume of recorded data due to digitalization. These digital records, known as big data, constitute an almost endless resource that can be processed by AI algorithms through machine learning. Today, all transactions occurring in the digital environment, such as sounds, images, numbers and those that can be referred to as behaviors, have become data that can be used by AI. Another feature of big data that can be accessed, stored, and processed is that it can be collected in real time, too. This offers exceptional opportunities for the development of AI.

On the other hand, the coronavirus disease-2019 pandemic has accelerated digitalization in all areas of life. In line with the increasing digitalization, developments in AI gained momentum. The spread of AI in different sectors and its new functions also followed these developments. AI technology provides a base for continued innovation in various sectors (2). In recent years, as one of the sectors where digitalization and datafication are advancing most rapidly, medicine and health sector have become one of the prominent areas in terms of the use of AI. It is also predicted that the opportunities provided by these technologies will create new industries and roles (3). While AI offers great opportunities, it will also pose a threat, especially to manufacturing and process-oriented sectors; and health sector is among them (3).

Apparently, there is an increasing interest in technology to solve problems of today's society such as global warming, sustainable use of resources and public health. Among these, the most pressing societal issues regarding health are increasing workload of the health sector, high costs, and the scarcity of trained personnel due to the increasing and aging population (4). Recent developments in machine learning and AI accelerated efforts to mitigate these problems using technological solutions. Policy makers and politicians are also eager to introduce more state-of-the-art tools to the health system (5). In this context, it is also possible to talk about the digitalization of health data which paved the way to applications that adopt a data-driven approach started to be implemented (6,7). In this context, the healthcare sector has become one of the areas where AI is rapidly developing from managerial, clinical, and patient perspectives. Most importantly, AI is already being applied to clinical tasks normally performed by doctors which draws attention to the positions of them in an AI supported health system (8). Thus, it is possible to suggest that the circumstances for both doctors and patients have been transformed by the introduction of AI (9). In the future, if/when AI becomes a routine part of clinical practice, the self-image of doctors will also be affected (10). From the vantage point of patients, on the other hand, receiving health services is also likely to be converted into a completely new experience. It is also important to note the fact that use of AI should be considered different from the use of AI in other sectors, given the highly sensitive nature of health data and vulnerability of the consumers (2).

The current literature on AI in health sector covers various aspects of the subject, however it does not sufficiently explain the factors shaping the attitudes and willingness of medical doctors (MDs) to use AI applications. Therefore, this research aims to examine the attitudes of MDs towards AI applications and the factors that determine their intention to use. In this context, it is believed that the extended technology acceptance model (E-TAM) will provide a distinctive perspective in explaining doctors' intentions to use AI tools.

Methods

This study was approved by the Anadolu University Social and Human Sciences Scientific Research and Publication Ethics Board (approval number: 70/78, date: 28.12.2023).

Artificial Intelligence in Health

AI is basically composed of machine learning, algorithms, and (big) data. In the context of these components, AI can be described as a smart machine-based system that recognizes patterns in data which can also apply these patterns to new data for particular tasks and purposes (11,12). AI has the ability to replace many human tasks and activities in various industries which is likely to have impact in terms of productivity and performance (2). Since health has become one of the major fields which produces big data, the utilization of AI has been foreseeable; especially

through machine learning and deep learning (13,14). It can be suggested that AI has already started to cause a paradigm change in the field of healthcare (15).

It is possible to consider the use of AI in the field of healthcare in three different dimensions. The first of these is public health applications, while the other two are in the field of medicine, that is, clinical applications for doctors and applications for the use of patients and/or for doctor- patient communication (16,17). AI can be used for diagnosis, treatment, and patient monitoring, as well as image processing and analysis (14,18). In this context, when we look at the AI applications developed for the use of doctors, it can be said that the developments are shaped in line with the promises of data and technology. Different fields of medical research and practice are aimed at by tech companies in terms of helping doctors find all the information and accurate data at once to make precise diagnosis and establish better plans for treatment (19,20). For example, thanks to the developments in AI and image processing technology, AI applications in radiology stand out among other specializations (13,14,17,21). This distinction between areas of expertise is also seen in various studies on the relationship between health and AI in the literature. However, it is possible to suggest that other specialties are catching up quite rapidly. Moreover, AI is becoming increasingly capable at clinical tasks aside from diagnosis and early detection (15).

Risks and Concerns Regarding AI in Health

It is unclear if the doctors will adopt AI technology in clinical practice at some point in the future (15). This is very much related to the perception of risks and concerns as well as the potential benefits that are comprehended by the doctors. Like every technological innovation, there are risks and benefits associated with the use of AI. The concerns and arguments regarding potential risks and drawbacks of the use of AI in health sector are various. Data privacy and security, bias, black-box effect, the question of liability, accountability, and doctors' lack of AI knowledge are among the most significant concerns (8,10,13,22). It is clear that some of these problematic areas are related to the technology itself, while others arise from the utilization and the approach of the users.

Data privacy and security are among major topics of debate when it comes to proliferating digital technologies that utilize data. For machine learning and AI, digital health records of all kinds are essential to achieve desired results. The data used for machine learning is assumed to be flawless by the developers. However, personal health records may be destroyed, stolen, or altered, if there is a security problem with the system. On the other hand, the data is recorded by humans, and they can make mistakes when collecting, classifying, and categorizing the gathered data (22). In these stages implemented by humans (not machines) mostly, it is vital to establish appropriate guidelines and apply them correctly.

AI and machine learning are criticized for giving biased outcomes that may lead to imperfect decisions. The bias may be resulting from bias in training data and/or flawed algorithms. In this sense, the decisions relying on AI supported systems that have social impact are mostly accused of reproducing social inequalities, which also exist in public health. For example, the training data may only represent a certain ethnic or geographical population, while excluding other groups, or some of the critical data about the patients can be in a format that cannot be entered into the system (10). In such cases, the data may not be comprehensive and inclusive enough to give accurate results.

Black-box effect refers to the opacity of algorithms used in AI systems. If the AI system is too complex for any one or more of the stakeholders, then it is considered problematic (23). Some models may lack transparency in terms of interpretable processes including parameters and criteria on which "deep learning" is mostly based on (22). This "black-box" type of AI systems may not be trustworthy both for doctors and patients, since the outcome/diagnosis cannot be explained (22). This issue also raises debates regarding accountability. In an opaque AI system, it is impossible to reduce the risks through verification (23), since the doctor cannot follow the process of decision making. In that context, the allocation of responsibility or liability also becomes an issue in case of unexpected/unwanted effects of decisions made by AI systems and applied by the doctors who have followed these decisions. The question of whether AI systems can be hold responsible for their decisions is beyond the scope of this article. However, it is widely suggested that the doctors who make the final decisions should be hold responsible (23). From the viewpoint of doctors, this may be an obstacle in terms of building trust and willingness to use AI support.

Level of knowledge about AI is another concern from the standpoint of doctors and medical students alike. The efficient and accurate use of AI tools requires knowledge of AI processes starting with data collection and including deep learning. Only with a certain level of knowledge doctors can adopt AI systems to their routines with confidence. On the other hand, if they know the AI processes well, they can support the developers to optimize the system (10).

Questions such as whether AI will replace doctors, whether it will remain only as a decision support technology, and whether the role of doctors (and other healthcare professionals) will change have begun to be asked frequently in recent years and have become one of the popular research topics in the field of AI (8). Aside from the answers to these questions, it is already possible to argue that required skillsets and attitudes for being a good doctor will be redefined in the new era of AI (20). The speed and the extent to which AI will affect the professional practices of doctors is largely related to the answers to the questions above.

Previous Research on Perception and Acceptance of AI among MDs

In recent years, with the increase in the use of tools and applications with AI technology in health sector, various studies have been conducted to examine the knowledge, opinions or attitudes of various professional groups working in this sector. These studies mainly focus on AI in general and on the way in which members of the sector are approaching the use of AI in

the field of health and medicine. The results of such research, which analyze the subject in different contexts and for various research purposes, are noteworthy. Despite the fact that most AI tools for clinical use are still at the research or pilot study phase, the members of health sector are already more or less aware of the imminent change. Therefore, the evolution of the perception and attitude of doctors is an important input for the penetration of AI into the healthcare sector. The effective human (doctor) - AI collaboration is especially very vital for successful applications, so recent research also aims at finding out the relevant factors (24). The literature suggests that the factors such as trust in AI and hesitancy to accept the use of AI tools are slowing down the adoption (7,25).

Doctors' attitudes and perceptions towards AI are related to several variables.

However, when the relevant literature is examined, it is understood that the relationship between healthcare and AI is questioned by both doctors and researchers in certain areas of discussion.

For example, a study conducted with doctors in the Netherlands, Portugal and the United States focused on ethics. This research revealed that MDs did not have enough insight into AI issues such as bias and social inequalities in health, but they had concerns about the involvement of the private sector and large companies in the process since they were believed to be profit-oriented and unaware of the core values of healthcare. Additionally, in this research doctors expressed their interest in learning AI technology in order to be able to explain the outcomes (6).

Another research on the perceptions of trainee doctors in London found that 58% believed AI would have a positive impact on their training, mainly in terms of research and quality as well as time - effectiveness for allocating more time for other educational activities. The findings showed that trainee doctors were optimistic about clinical AI to keep them updated about latest literature and latest evidence to improve their practice. On the other hand, as for the negative opinions about clinical AI, the respondents were concerned about the development of their practical skills and clinical judgement because of the reduced opportunities to train, which in turn could harm accountability (8).

According to a survey on the opinions of Pakistani medical students and doctors more than one-third of the respondents agreed and strongly agreed the statement that AI would reduce errors in diagnosis, which showed a partial trust in this technology. Plus, despite limited trust in AI, they did not consider it as a threat to their occupation (13).

Another research in which data was collected via an online questionnaire revealed contradicting results about knowledge of AI among MDs. The level of knowledge of AI elements such as deep learning and neural networks were more familiar for the respondents who had a clear understanding of AI, whereas they did not have much idea about supervised and unsupervised learning. However, they were concerned about the safe use of AI in health, even though they had little awareness about the lack of transparency. Other results of this study showed that both medical students and doctors feared deskilling as well as doctors becoming redundant (18). This may be because the knowledge about AI is related to personal interest in technology and innovation.

The findings of another survey conducted in UK revealed that half of the 411 radiographers did not feel confident about understanding AI terminology and 64% told they did not develop any skills regarding use of AI in their field. The overall results of the survey indicated a willingness among the radiographers to receive training about AI applications in their field of expertise (26).

Of 297 participants from England 13.8% who responded to another online questionnaire about AI use in clinical practice indicated that they were aware of the use of AI technology. When they were asked to rate their level of knowledge about AI use in healthcare specifically, the mean rating was 3.68 out of 10, which showed an insufficient level of knowledge (27).

According to the findings of a survey conducted in Italy among 1032 radiologist members of The Italian Society of Medical and Interventional Radiology, most radiologists expected AI to improve their workflow. Even though they had a positive attitude towards use of AI, they were concerned about their possibly reduced reputation (28).

As understood from a bird's eye view of previous research on the topic, the subject of AI acceptance and use by MDs is a multifaceted issue. These are several factors from the perspective of doctors that determine their level of acceptance and most of these factors are based on their perceptions, rather than lived experience.

Extended Technology Acceptance Model

Technology acceptance can be expressed as the choice of individuals to voluntarily accept new technologies. The primary aim of the TAM is to prognosticate the adoption of novel technologies among end-users and illuminate challenges prior to their ubiquitous integration within the general populace (29). In recent decades, researchers have formulated diverse models aimed at comprehending the dynamics of user acceptance toward technology. However, Davis's TAM represents the most fundamental and significant foundation for technology acceptance to date. TAM consists of two primary constructs commonly employed in various technological contexts: perceived usefulness and perceived ease of use (PEOU) (30). However, some researchers have expressed concerns about the inadequacy of the original frameworks of the TAM in elucidating users' intentions towards the adoption of healthcare technologies (29). In specific user contexts, such as the acceptance of AI tools, participants' intentions to use are contingent upon many social and behavioral factors that remain unaddressed within the confines of the TAM model. Therefore, the current research has focused on incorporating additional social and behavioral variables into the TAM model and how these variables may affect

perception of users. Accordingly, variables such as "facilitating conditions (FC)", "trust", "perceived risk", "technological anxiety", and "resistance to use (RC)" were included in the scope of the research in order to better understand the user's perception.

Research Methodology

The objective of this study is to scrutinize and elucidate the determinants that mold and impact the attitudes of MDs towards AI applications. The research model, with "AI intention to use (IU)" identified as the dependent variable, is shown in Figure 1. The targeted population for this research comprises doctors working in hospitals and clinics in Türkiye. To ensure the validity of the measurements, the details of the identified determinant structures were adopted from previous studies, as outlined in Appendix A. Proposed hypotheses are shown in Table 1.

Data Collection Method and Measurements

This research was based on an online survey. The survey was conducted with 275 MDs in Türkiye. The study employed a convenience sampling technique. The survey created using Google Forms and participants were contacted via email. All participants were briefed on the research and their explicit consent was obtained. Initial survey for this study was developed in English and was later translated into Turkish by the researchers. Both surveys were carefully crafted to convey the same meaning in terms of perception. The survey was pilot tested for clarity on ten MDs. Data collection was carried out between September 1, 2023, and October 28, 2023.

The survey was divided into two parts. In the first part, there was a brief introduction and five questions related to the profile of participants such as gender, age, institution, clinical specialization, and professional experience. Second part consisted of 21 questions for various constructs shown in Figure 1. The five-point Likert-type scale with a range from 1 (strongly disagree) to 5 (strongly agree) was employed was used to measures for responses.

The survey instruments for each of the constructs were designed to gather exhaustive details and adapted from the literature, including IU -three items (31); PU -two items (32,33); PEOU -three items (34,35); RC - three items (36); T -two items (37); TA -two items (31); FC -three items (38) and PR -three items (37,39).

Statistical Analysis

In this study, the partial least squares-structural equation modeling (PLS-SEM) was employed to ascertain causal relationships for theory confirmation. The data analysis utilized SmartPLS 3. Descriptive statistics were calculated with the SPSS 25 software. The PLS-SEM analysis is divided into two sections for analysis. Part one is based on an evaluation of the outer model's reliability and validity. The second part is based on a model evaluation within which hypotheses were evaluated.

Results

Demographics

A total of 275 MDs responded to the survey. The sample was slightly skewed toward females (52%). The mean age for the entire sample was 48,3 (standard deviation =12.7). The participants were distributed among different clinical specialties

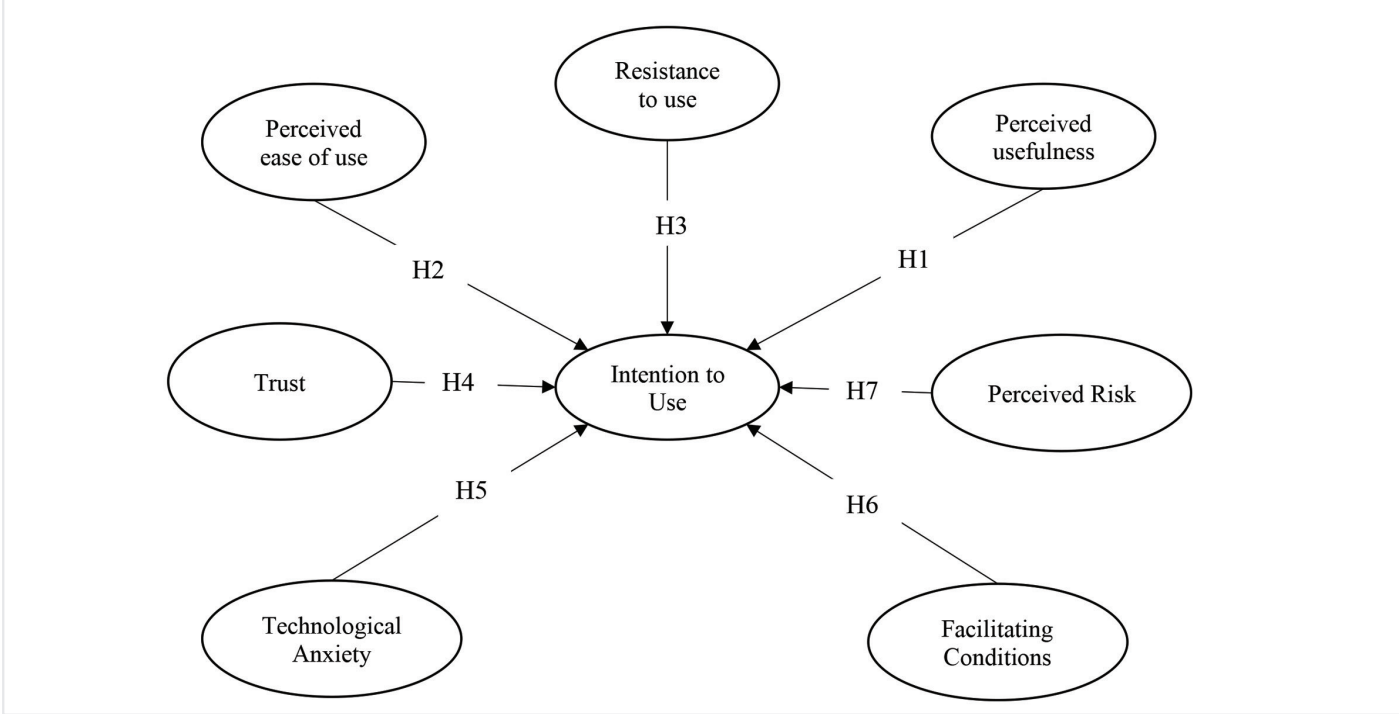

Figure 1. Research Model: extension TAM with social and behavioral factors

as follows: internal medicine (n=82, 30%), pediatrics: (n=55, 20%, general surgery (n=41, %15), obstetrics and gynecology (n=41, 15%), orthopedics: (n=28, 10%), psychiatry (n=14, 5%), other specialties (n=14, 5%). The survey revealed that 31% of the participants had 15-20 years of work experience and 28% had less than 10 years of work experience.

Measurement Model

The measurement model encompasses assessment procedures for testing the reliability and validity of the measures. The current study followed three measurements suggested by Hair et al. (40); 1) indicator loadings and internal consistency reliability, 2) convergent validity, and 3) discriminant validity.

In this study, the item loads for each construct were obtained through PLS-SEM analysis. Table 2 shows the detail of loadings. All items achieved the recommended loading values of >0.700 (40). Internal consistency reliability refers to the evaluation findings for the statistical consistency across indicators and it was assessed using Cronbach's alpha (CA) and composite reliability (CR). The values of CA and CR in this study adhered to the threshold set by Hair et al. (40); CA and CR >0.700. It can be seen from the Table 2 that both CA and CR values for all construct shave good internal consistencies. The reliability ranging from 0.732 to 0.816 for the CA and 0.813 to 0.880 for the CR (Table 2).

Table 1. Proposed hypotheses

Factors	Hypothesis
Perceived usefulness	H1. Perceived usefulness positively effects the intention to use AI applications
Perceived ease of use	H2. Perceived ease of use positively effects the intention to use AI applications
Resistance to use	H3. Resistance to use negatively effects the intention to use AI applications
Trust	H4. Trust positively effects the intention to use AI applications.
Technological anxiety	H5. Technological anxiety negatively effects the intention to use AI applications
Facilitating conditions	H6. Facilitating conditions positively effects the intention to use AI applications
Perceived risk	H7. Perceived risk negatively effects the intention to use AI applications

AI: Artificial intelligence

Table 2. Measurement model, construct reliability and validity values

	Items	Loadings	CA	CR	AVE
Intention to use	IU1	0.832	0.801	0.871	0.696
	IU2	0.855			
	IU3	0.815			
Perceived usefulness	PU1	0.748	0.730	0.855	0.751
	PU2	0.972			
Perceived ease of use	PEOU1	0.876	0.799	0.878	0.710
	PEOU2	0.888			
	PEOU3	0.870			
Resistance to use	RC1	0.814	0.781	0.840	0.637
	RC2	0.832			
	RC3	0.840			
Trust	T1	0.891	0.754	0.880	0.803
	T2	0.900			
Technological anxiety	TA1	0.946	0.816	0.813	0.843
	TA2	0.905			
Facilitating conditions	FC1	0.840	0.732	0.873	0.778
	FC2	0.832			
	FC3	0.813			
Perceived risk	PR1	0.826	0.752	0.850	0.728
	PR2	0.935			
	PR3	0.973			

CA: Cronbach's alpha, CR: Composite reliability, AVE: Average variance extracted

Convergent validity is a statistical concern associated with the concept of construct validity. The average variance extracted (AVE) is an attempt to determine convergent validity. If the AVE is greater than 0.500; it explains 50% or more of the variance (40). In this research, it is known that all constructs have an AVE score that is greater than 0.500 that explains more than 50% of the variance (Table 2).

According to Hair et al. (40), discriminant validity refers to the degree to which a construct differs from other constructs. Through the implementation of the Fornell-Larcker criterion, it is expected that the square root of the AVEs of all constructs should be greater than the highest correlation value for other constructs in the measurement model. Based on the study findings, the square root of the AVEs for each construct are greater than that it's shared variance. Thus, discriminant validity is confirmed through the assessment of the Fornell-Larcker criterion (Table 3).

On the one hand, heterotrait-monotrait ratio (HTMT) values exceeding 0.900 appear discriminant validity problems. It is observed that all HTMT values are below 0.900 (Table 4).

Further, discriminant validity can be assessed by examining cross-loadings. Discriminant validity is demonstrated when the loading value on a construct bigger than all of its cross-loading values on other constructs (Table 5).

It has been determined that the values of all indicators for each construct's outer loading exceed all cross-loading values on other constructs (Table 5). In this context, it can be said that discriminant validity has been emerged.

Following the evaluation of the measurement model, it is evident that the construct is deemed suitable for use. This is evidenced by meeting the criteria for loading indicators, internal consistency reliability, convergent validity, and discriminant validity. Thus, the model can proceed to undergo inspection in the structural model.

Structural Model

The structural model assessment process began with the determination of whether there was collinearity problem. Reporting variance inflation factor (VIF) values is a good indicator of collinearity. The VIF value of each indicator >3.000 indicates the existing of collinearity problem. PU (VIF=1.233), PEOU (VIF=1.890), RC (VIF=1.567), T (VIF=1.241), Technological anxiety (TA) (VIF=1.712), FC (VIF=1.213) and PR (VIF=1.663) are the predictor of IU (VIF=1.000). As seen all values of VIF were below three. Therefore, collinearity problem did not exist in the current research.

Structural model evaluation is also known as an inner model evaluation, as it examines the relationship between latent variables (41). In reflective models, recent studies suggest conducting an evaluation that includes determination coefficients, path

Table 3. The Fornell-Larcker criterion (latent variable correlation and square root of AVE)

	IU	PU	PEOU	RC	T	TA	FC	PR
IU	0.834							
PU	0.467	0.866						
PEOU	0.453	0.611	0.842					
RC	0.391	0.569	0.386	0.798				
T	0.601	0.346	0.512	0.465	0.896			
TA	0.513	0.430	0.647	0.342	0.421	0.918		
FC	0.431	0.444	0.543	0.431	0.545	0.567	0.882	
PR	0.348	0.565	0.465	0.673	0.341	0.603	0.564	0.853

AVE: Average variance extracted, IU: Intention to use, PU: Perceived usefulness, PEOU: Perceived ease of use, RC: Resistance to use, T: Trust, TA: Technological anxiety, FC: Facilitating conditions, PR: Perceived risk

Table 4. Heterotrait-monotrait ratio values

	IU	PU	PEOU	RC	T	TA	FC	PR
IU								
PU	0.677							
PEOU	0.832	0.669						
RC	0.476	0.693	0.728					
T	0.601	0.510	0.721	0.712				
TA	0.527	0.765	0.480	0.526	0.632			
FC	0.398	0.428	0.635	0.469	0.564	0.784		
PR	0.803	0.586	0.482	0.657	0.498	0.414	0.593	

IU: Intention to use, PU: Perceived usefulness, PEOU: Perceived ease of use, RC: Resistance to use, T: Trust, TA: Technological anxiety, FC: Facilitating conditions, PR: Perceived risk

coefficients, and predictability (40,42). For this purpose, the sample was performed a complete bootstrap analysis with 5000 subsample parameters. At a 5% significance level, the majority of hypotheses were supported, except for H5 and H6.

The TA was not a significant predictor for IU (β=0.067; t=1.014; p=0.093). FC was also reported to have not a significant effect on IU (β=0.071; t=1.041; p=0.102). PU (β=0.613; t=22.851; p<0.01), PEOU (β=0.644; t=14.577; p<0.01), RC (β=-0.416; t=7.150; p<0.01), T (β=0.651; t=25.876; p<0.01), PR (β=-0.530; t=14.771; p<0.01) significantly predicted IU. Final results are shown in Table 6.

The explanatory power of the model was determined by measuring R^2 value. The R^2 value represents the proportion of variance in the endogenous variable explained by the exogenous variable. From the results of the PLS-SEM analysis, R^2 IU was 0.615, implying that PU, PEOU, RC, T, TA, FC, and PR could explain 61.5% of the variance in IU. Hair et al. (40) categorized R^2 into the following groups: 0.25 falls into the weak category, 0.50 into the moderate category, and 0.75 into the substantial category. Based on the analysis results, the R^2 value could be classified within the moderate category.

Next, f^2 is reported to assess the effect sizes of endogenous structures. This involves assessing the change in R^2 between a full model and iterations where each time a distinct exogenous construct is excluded (40,43). According to Hair et al. (40), the f^2 value of 0.02 is considered a small effect, 0.15 indicates a medium effect, and 0.35 is characterized as a large effect. The effect sizes of endogenous structures are shown in Table 7.

Table 5. The item loadings and cross-loadings

	IU	PU	PEOU	RC	T	TA	FC	PR
IU1	0.832	0.365	0.365	0.324	0.367	0.287	0.498	0.389
IU2	0.855	0.454	0.552	0.378	0.228	0.443	0.563	0.413
IU3	0.815	0.288	0.288	0.437	0.415	0.369	0.317	0.515
PU1	0.489	0.748	0.417	0.269	0.294	0.414	0.489	0.446
PU2	0.374	0.972	0.469	0.412	0.487	0.352	0.431	0.362
PEOU1	0.312	0.417	0.876	0.321	0.311	0.318	0.365	0.243
PEOU2	0.363	0.469	0.888	0.245	0.253	0.498	0.578	0.405
PEOU3	0.398	0.335	0.870	0.198	0.192	0.265	0.422	0.297
RC1	0.577	0.249	0.335	0.814	0.425	0.497	0.399	0.474
RC2	0.392	0.218	0.249	0.832	0.459	0.432	0.687	0.421
RC3	0.468	0.385	0.218	0.840	0.315	0.375	0.347	0.388
T1	0.401	0.292	0.385	0.387	0.891	0.246	0.418	0.374
T2	0.385	0.197	0.292	0.473	0.900	0.589	0.254	0.298
TA1	0.596	0.468	0.197	0.292	0.402	0.946	0.572	0.435
TA2	0.578	0.298	0.468	0.421	0.356	0.905	0.433	0.192
FC1	0.567	0.176	0.298	0.356	0.267	0.411	0.840	0.359
FC2	0.494	0.369	0.176	0.298	0.183	0.337	0.832	0.389
FC3	0.389	0.422	0.369	0.175	0.391	0.298	0.813	0.313
PR1	0.401	0.316	0.422	0.389	0.475	0.461	0.675	0.826
PR2	0.396	0.417	0.316	0.469	0.402	0.281	0.541	0.935
PR3	0.482	0.469	0.245	0.377	0.336	0.376	0.438	0.973

IU: Intention to use, PU: Perceived usefulness, PEOU: Perceived ease of use, RC: Resistance to use, T: Trust, TA: Technological anxiety, FC: Facilitating conditions, PR: Perceived risk

Table 6. Final results

Hypothesis	Path coefficient (β)	t	Conclusion
H1	0.613	22.851	Supported
H2	0.644	14.577	Supported
H3	-0.416	7.150	Supported
H4	0.651	25.876	Supported
H5	0.067	1.014	Rejected
H6	0.071	1.041	Rejected
H7	-0.530	14.771	Supported

PR (f^2 =0.345) gained the smallest effect while Trust (T) (f^2 =0.648) obtained the largest f^2 while TA and T had no effect size.

Finally, we calculated Stone-Geisser's Q^2 value to assess the predictive relevance of the model for each endogenous variable (given that our model included only one endogenous variable, the assessment of predictive relevance focused on the model's ability to predict IU). When the model undergoes predictive relevance, it demonstrates the accuracy of predicting data points for items in the study (40). The obtained Q^2 value for this variable was 0.41, indicating substantial predictive relevance.

Discussion

According to the findings of our research which aims to reveal the factors determining the IU the AI applications among MDs in Türkiye, T is the strongest determinant. As the trust to AI technology increases, the MDs are more likely to use its applications. Same strong relation between trust and (behavioral) intention was also found in a previous study in China (25). Another research in China found direct and indirect positive effects of trust on AI acceptance among doctors, indicating that increased trust escalated likelihood of acceptance by positively effecting the performance expectancy (44). PEOU was the second most effective factor. PEOU was also found to be a strong factor that led to positive attitude to use AI in research undertaken in UEA (45). Additionally, the more the MDs perceive AI technology and AI applications as useful for healthcare purposes (PU), their intentions to use them also increase. In a previous survey by Pan et al. (46) PU was found the most effective factor in determining attitudes of doctors towards smart healthcare services in Japan. The results of an online survey with 669 Korean MDs and medical students also found that %83.4 of the respondents considered AI useful for medical field and this contributed to the positive attitude of medical community in Korea (47).

Technological anxiety is not a factor in determining the IU AI in healthcare practices for MDs in Türkiye, according to the results of our research. FC were also found to be not affecting IU AI. When it comes to factors that negatively impact the intention of use, perceived risk comes first, which may explain the strength of trust as the main positive factor that is the opposite of risk. This result supports the findings of a previous qualitative study on doctors' resistance of AI in Tunisia. In this study, performance risk of AI applications was found to be one of the barriers in terms of acceptance in healthcare, even though it was perceived beneficial to the medical field (48). RC, also negatively impacts IU AI applications among MDs in Türkiye. As the individual is more resistant towards using new technology the IU AI declines. It can be suggested that the general attitude towards using new technology and/or tools determines the attitude towards AI applications in healthcare as well.

Table 7. Effect sizes

	f^2	Effect size
PU	0.621	Large
PEOU	0.642	Large
RC	0.398	Large
T	0.648	Large
TA	0.011	No effect
FC	0.009	No effect
PR	0.345	Medium

PU: Perceived usefulness, PEOU: Perceived ease of use, RC: Resistance to use, T: Trust, TA: Technological anxiety, FC: Facilitating conditions, PR: Perceived risk

Study Limitations

It should be noted that there will be large variations within the population of MDs working in hospitals and clinics; therefore, the results cannot be considered generalizable. Additionally, the possible support of AI in the treatment of one patient may differ significantly from others. The attitude towards specific AI applications may vary.

Conclusion

Artificial intelligence continues to develop at an astonishing speed and touches our entire lives, including healthcare. In the context of this major transformation, the position of doctors becomes very crucial in terms of applying the opportunities of AI technology to medical practice, while eliminating the risks and side effects. The perceptions and attitudes of them will define the trajectory for acceptance and adoption of AI applications in various aspects medical field. This research reveals the fact that trust, perceived usefulness, and perceived ease of use are the major positive factors for AI to be accepted and adopted by MDs. Although it requires further research, this result can be related to the increasing knowledge and experience of doctors regarding artificial intelligence. Providing more trust and ease of use in this way can enable more action to be taken to benefit from AI in the healthcare sector.

Ethics

Ethics Committee Approval: This study was approved by the Anadolu University Social and Human Sciences Scientific Research and Publication Ethics Board (approval number: 70/78, date: 28.12.2023).

Informed Consent: All participants were briefed on the research and their explicit consent was obtained.

Authorship Contributions

Concept: A.K.E., Design: E.E.B., Data Collection or Processing: E.E.B., Analysis or Interpretation: E.E.B., Literature Search: A.K.E., Writing: A.K.E.

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Appendix A.	
Constructs	**Definitions**
Perceived usefulness	PU1: Using AI applications would improve the quality of my treats.
	PU1: Using AI applications would improve the quality of my treats.
Perceived ease of use	PEOU1: I would find learning to use AI applications would not be very difficult for me.
	PEOU2: I would find it easy for myself to use AI applications.
	PEOU3: Using AI applications would be clear and understandable for me.
Resistance to use	RC1: I wouldn't want AI applications to alter my traditional way of treatments.
	RC2: I wouldn't want AI applications to interfere or change the way my treatments routine.
	RC3: I don't want AI applications to change the way I deal with treatment problems and choices.
Trust	T1: AI applications would be trustworthy for improving my treatment routine.
	T2: I feel satisfied and confident that I will be able to rely on the benefits of AI applications.
Technology anxiety	TA1: Using AI applications would make me feel nervous.
	TA2: Using AI applications would make me confused and uncomfortable.
Facilitating conditions	FC1: I would be able to have all the necessary resources for using AI applications.
	FC2: I would acquire sufficient knowledge for using AI applications.
	FC3: AI applications will suite well with my treatment routine.
Perceived risk	PR1: Learning how to use AI applications and adapting it would be a loss of my time.
	PR2: Using AI applications would be a loss of money and resources.
	PR3: Using AI applications wouldn't be compatible with my moral values and image.
Intention to use	IU1: Assuming that I was given the chance to AI applications, I intend to use AI applications.
	IU2: I would gladly use AI applications.
	IU3: I intend on informing my colleagues and friends about AI applications.
The five-point Likert-type scale with a range from 1 (strongly disagree) to 5 (strongly agree) was employed	

Original Article

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The Effects of Orthosis and Exercise on The Median Nerve Morphology and Functional Status of Patients with Carpal Tunnel Syndrome: A Randomized Pilot Study

Karpal Tünel Sendromlu Hastalarda Ortez ve Egzersizin Medyan Sinir Morfolojisi ve Fonksiyonel Durum Üzerine Etkisi: Randomize Pilot Çalışma

Hakan AKKAN[1], Meltem IŞINTAŞ ARIK[2], Mehmet KORKMAZ[3], Sibel CANBAZ KABAY[4], Hasan Hüseyin GÖKPINAR[5]

[1]Kütahya Health Sciences University, Tavşanlı Vocational School of Health Services, Department of Therapy and Rehabilitation, Kütahya, Türkiye
[2]Kütahya Health Sciences University Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Kütahya, Türkiye
[3]Kütahya Health Sciences University Faculty of Medicine, Department of Radiology, Kütahya, Türkiye
[4]Dokuz Eylül University Faculty of Medicine, Department of Neurology, İzmir, Türkiye
[5]Kütahya Health Sciences University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Kütahya, Türkiye

ABSTRACT

Objective: This study aimed to investigate the effects of orthosis, and additional nerve and tendon gliding exercises (NTGE) on median nerve morphology and functional status in patients with mild-to-moderate carpal tunnel syndrome (CTS) and to compare the effectiveness of proposed treatments.

Methods: Twenty-seven wrists of 19 patients with mild-to-moderate CTS were included in the study. They were randomized into three groups: neutral wrist orthosis alone (n=8), the combination of the orthosis with nerve gliding exercises (NGE) (n=10), and the combination of the orthosis with nerve/tendon gliding exercises (n=9). The cross-sectional area (CSA) of the median nerve (ultrasonography) and functional status (Boston carpal tunnel syndrome questionnaire and patient-specific functional scale) were evaluated at baseline and after six weeks of treatment.

Results: While the functional status of all groups improved significantly ($p<0.05$), the CSA of the median nerve significantly decreased in the combination of the orthosis with NGE group ($p<0.05$). However, there were no significant differences between the treatment groups in relation to the improvement in the intraneural edema and the functional status ($p<0.05$).

ÖZ

Amaç: Bu çalışmanın amacı, hafif-orta şiddette karpal tünel sendromlu (KTS) hastalarda ortez ile orteze ek sinir ve tendon kayma egzersizlerinin (STKE) medyan sinir morfolojisi ve fonksiyonel durum üzerindeki etkilerini araştırmak ve önerilen tedavilerin etkinliğini karşılaştırmaktır.

Yöntemler: Hafif-orta derecede KTS'li 19 hastanın 27 el bileği çalışmaya dahil edildi. Hastalar üç gruba ayrıldı: nötral el bileği ortezi grubu (n=8), ortez ile SKE grubu (n=10) ve ortez ile STKE (n=9). Medyan sinirin enine kesit alanı (ultrasonografi) ve hastaların fonksiyonel durumu (Boston karpal tünel sendromu anketi ve hastaya özgü fonksiyonel ölçek) tedavi öncesinde ve altı haftalık tedavi sonunda değerlendirildi.

Bulgular: Tüm grupların fonksiyonel durumu anlamlı şekilde iyileşirken ($p<0,05$), medyan sinirin kesit alanı ortez ile NGE kombinasyonu grubunda anlamlı şekilde azaldı ($p<0,05$). Ancak, intranöral ödem ve fonksiyonel durumdaki iyileşme açısından tedavi grupları arasında anlamlı bir fark yoktu ($p>0,05$).

Sonuç: Altı haftalık ortez tedavisine ek olarak verilen STKE KTS'li hastaların semptomlarını ve fonksiyonel durumunu iyileştirmede

Address for Correspondence: Hakan Akkan, Kütahya Health Sciences University, Tavşanlı Vocational School of Health Services, Department of Therapy and Rehabilitation, Kütahya, Türkiye
E-mail: hakan.akkan@ksbu.edu.tr **ORCID ID:** orcid.org/0000-0001-7920-1128

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ABSTRACT

Conclusion: The 6-week orthotic treatment and additional NTGE could be effective in improving the patient's symptoms and functional status. However, the combination of a 6-week orthotic treatment and NGE could be effective in reducing intraneural edema.

Keywords: Carpal tunnel syndrome, diagnostic imaging, exercise therapy, ultrasonography, splints

ÖZ

etkili olabileceği bulunmuştur. Bununla birlikte, 6 haftalık ortez tedavisi ve SKE kombinasyonu intranöral ödemi azaltmada olabileceği tespit edilmiştir.

Anahtar Sözcükler: Karpal tünel sendromu, tanısal görüntüleme, egzersiz tedavisi, ultrasonografi, splintler

Introduction

Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy in the upper extremity (1). Compression and/or irritation of the median nerve at the carpal tunnel level causes CTS. Elevated carpal tunnel pressure is responsible for the nerve's compression and/or irritation. High pressure inside the tunnel causes ischemia by affecting intraneural blood circulation (2). Prolonged ischemia leads to intraneural edema and ultimately causes intra/extra-neural fibrotic changes (3). Therefore, the median nerve can adhere to surrounding tissues, and the excursion of the nerve can reduce (4). Long-lasting ischemia also results in focal demyelination and axonal degeneration in the later stages of the disease. These pathophysiological processes are accepted as possible mechanisms for developing CTS symptoms (3).

The goals of CTS treatment are decreasing symptoms and improving hand function. Surgical treatment is superior to non-surgical treatments in improving function and reducing symptoms in six months. However, no strong evidence is found for the 12-month results (5). Surgery costs are high, and non-surgical treatments' side effects are low. Therefore, non-surgical treatments stand out as the first treatment option (6). Non-surgical treatments include patient education, orthosis (customized volar or dorsal thermoplastic orthoses, prefabricated off-the-shelf orthoses), therapeutic exercise, biophysical agents, and manual therapy techniques (7). Nocturnal orthosis is the most frequently used intervention for CTS by hand therapists. Besides, hand therapists often prefer tendon gliding exercises and nerve mobilization (8). These exercises can help reduce tenosynovial edema, improve venous return, and relieve pressure inside the carpal tunnel (9). Generally, combinations of these interventions can be prescribed in clinical practice. However, a limited number of studies have investigated the effects of these treatments on median nerve morphology (10-12). Most of the studies focused on the effects of orthosis and exercises on the function and symptoms of CTS patients (13-15). Moreover, the results are controversial. Because of these controversies and the lack of studies investigating changes in the intraneural edema of the median nerve in patients with CTS, further studies seem necessary. Therefore, this randomized study examined the short-term effects of orthosis when used alone or along with nerve gliding exercises (NGE) or nerve and tendon gliding exercises (NTGE) on the morphology of the median nerve and the functional status of CTS patients.

Methods

A pilot randomized trial was conducted in the electrodiagnostic medicine laboratory of Kütahya Health Sciences University from May 2017 to December 2018. The clinical research Ethics Committee of Kütahya Dumlupınar University approved the study (decision no: 2017-4/2, date: 22.03.2017).

Randomization was performed using an online computer program (http://www.randomizer.org accessed: April 23, 2017). Participants were randomly allocated into either orthosis (control) or orthosis + NGE, or orthosis + NTGE groups at a 1:1 allocation ratio. Both hands were included in the same treatment group for those with bilateral symptoms. Participants and the physiotherapist who delivered the treatment couldn't be blinded to the intervention. When the study was conducted, no previous study provided data on the cross-sectional area (CSA) of the median nerve after exercise and orthotic device. Therefore, a convenient sample was used. Participants signed written informed consent before their inclusion. All measurements were performed at baseline and after six weeks of intervention.

Inclusion criteria were as follows; being between the ages of 18-65 years; having clinical diagnosis (electrophysiologically confirmed) of mild-to-moderate CTS with a minimum of 2 months duration of symptoms and referred to physical therapy. Exclusion criteria were as follows; severe electrodiagnostic findings; pregnant women; comorbidities associated with CTS (rheumatoid arthritis, thyroid disease, renal insufficiency, and diabetes); diagnosis of cervical radiculopathy or osteoarthritis of the wrist or hand; history of any trauma to wrist or hand; previous surgery or injection to affected hand for CTS; prior conservative therapy for CTS in the past three months (splints or exercises or any electrophysical modalities); clinical diagnostic test score <12 (CTS-6) (16); the CSA of the median nerve at carpal tunnel inlet <10 mm^2 (17); unable to understand and speak Turkish.

Outcome Measurement

A technician (specialized in electrophysiologic study) performed NCS with the Nicolet Viking Select system device (Nicolet Biomedical Inc. Madison, USA). All hands below a temperature of 32 °C were warmed before electrodiagnostic testing. Sensory and motor NCS of the median and ulnar nerves were performed. The severity of CTS was graded as mild, moderate, and severe, according to NCS. Prolonged sensory latencies (<50 m/s) with normal motor studies were considered mild CTS. Abnormal

median sensory latencies and prolongation of median motor distal latency (≥4.2 ms) were considered moderate CTS. Severe CTS was defined as an absent or low-amplitude sensory nerve action potential and a low-amplitude or absent thenar compound muscle action potential (18). The same technician performed all NCS.

After NCS, a radiologist with over ten years of experience in musculoskeletal ultrasound measured the CSA of the median nerve with a 7-12 MHz linear-array probe (Aplio 500, Toshiba, Otawara, Japan). The patients sat in a chair with their forearms on the table and facing the radiologist during the ultrasonographic measurements. The forearm of the patient was in supination position while the wrist was in neutral position, and the fingers were in the resting position (metacarpophalangeal and interphalangeal joints in semi-flexion). The probe was placed at the distal palmar wrist crease just proximal to the level of the pisiform to obtain the cross-sectional images of the median nerve. To collect reliable images, a single radiologist took a single image at the pisiform level and analyzed this image once (19). The same radiologist performed all ultrasonographic measurements.

Then, a physiotherapist with ten years of experience in musculoskeletal physiotherapy performed a clinical evaluation with the CTS-6 diagnostic instrument. CTS-6 is a diagnostic tool that helps make a clinical diagnosis by combining history and physical examination findings. A total score of ≥12 points is considered positive-CTS (16).

The patient's functional status and symptoms were assessed using the Boston carpal tunnel syndrome questionnaire (BCTSQ), a self-reported questionnaire (20), and validated for the Turkish language (21). It consists of symptom severity scale (SSS) and functional status scale (FSS). The SSS, which includes 11 questions, evaluates symptoms. The FSS, which contains eight items, assesses difficulties in the activities of daily living. Each scale uses a 5-point Likert scale to generate a final score in the range of 1-5 (the sum of the individual scores divided by the number of items). The higher the score, the greater the disability.

The patient-specific functional scale (PSFS) was developed to evaluate the patient's functional limitations with activities determined by them (22). It allows the patients to identify their difficulty performing activities and rate their difficulty on a numerical scale. PSFS is valid and reliable in upper extremity nerve injuries (23).

All participants included in the study took part in a brief patient education session lasting ~20 minutes after clinical evaluation. The physiotherapist gave this educational session, which included the pathophysiology of CTS, symptoms, causes, diagnosis of CTS, treatment options (surgery and conservative management), and activity modification principles during daily living.

After the education session, all participants were provided a custom-made orthosis with volar support that holds the wrist in a neutral position. Patients were educated to wear the orthosis at night for six weeks. Patients were asked to log their orthotic use to assess their adherence to the prescribed treatment plan. They marked the daily hours when the splint was inserted and removed for six weeks. Patients performed NTGE in the exercise groups as a home program besides the orthosis. The physiotherapist taught the patients how to do the exercises correctly and asked them to do them five times daily, ten repetitions separated by at least 2 hours. The patients were informed that exercises should not worsen their symptoms and were instructed to keep a log of their exercise routine to monitor their progress. Patients performed NGE that provided maximum excursion and did not create tension in the median nerve (24). Patients completed NGE approximately 10 min/day. Elbow flexion and wrist extension alternated with elbow extension and wrist flexion as a first nerve gliding exercise. After completion of this movement, finger flexion and wrist extension alternated with finger extension and wrist flexion as a second nerve gliding exercise. Tendon gliding exercises were taught to the patients as suggested by Wehbé (25). Patients completed tendon gliding exercises approximately 20 min/day.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 25 (IBM Corp, Armonk, NY). Mean ($\overline{\chi}$), standard deviation, number (n), and percentage (%) values were calculated for descriptive variables. Kolmogorov-Smirnov and Shapiro-Wilk's tests were used to assess the distribution of data. The groups were compared with the Kruskal-Wallis test because the data didn't show a normal distribution. The Wilcoxon test was used to compare the change in variables between baseline and week 6. The Kruskal-Wallis H and z values were used to calculate Cohen's d (d), which was used to estimate effect sizes. These are interpreted as follows: "negligible" ≤0.19, "small" 0.2≤d≤0.49, "medium" 0.5≤d≤0.79, and "large" ≥0.8 (26). A p-value of less than 0.05 was considered a statistically significant result.

Results

Forty individuals diagnosed with mild-to-moderate CTS were included in the study. However, data from 19 patients (27 hands; 8 bilateral, 11 unilateral) were included in the data analysis. This study is reported according to the Consolidated Standards of Reporting Trials guidelines (27), and the flowchart is reported in Figure 1. In the baseline characteristics, there was no statistically significant difference between groups (Table 1).

The measurement of the CSA of the median nerve, SSS and FSS scores of BCTSQ, and PSFS scores at baseline were homogeneous for the three groups (Table 2). There were no significant differences between groups for the measurement of the CSA of the median nerve (p=0.238, d=0.388), SSS (p=0.758, d=0.506), and FSS scores (p=0.867, d=0.555) of BCTSQ and PSFS scores (p=0.737, d=0.496) after six weeks of treatment (Table 2).

The reduction in median CSA of the median nerve in the NGE group was statistically significant at week 6, and the effect size of this difference was large (p=0.006, d=1.101) (Table 2). However, within-group changes in the CSA of the median nerve in control

(p=0.096, d=0.917) and NTGE groups (p=0.140, d=0.743) were not statistically significant at week 6 (Table 2). The effect sizes of these differences for the groups were large and medium, respectively.

After six weeks of treatment, a statistically significant improvement was observed in SSS scores in the control (p=0.012, d=1.630) and NGE groups (p=0.005, d=1.611), with large effect sizes. The reduction in median SSS score in the NTGE group was not statistically significant, with a large effect size (p=0.097, d=0.851) (Table 2). There was a statistically significant difference in the FSS scores in all groups in the within-group comparisons (control: p=0.012, d=1.630; NGE group: p=0.012, d=1.352; NTGE group: p=0.021, d=1.303) (Table 2). All the effect sizes of these differences were large.

At week 6, within-group changes in the PSFS scores were statistically significant for the NGE (p=0.008, d=1.128) and NTGE groups (p=0.028, d=1.620) (Table 2). The effect sizes of these differences were large. However, the improvement in the median PSFS score in the control group was not statistically significant, with a large effect size (p=0.069, d=1.022) (Table 2).

All patients completed the orthosis log. There was no significant difference between groups in wearing time from baseline to post-intervention, with a medium effect size (p=0.757, d=0.506). Patients in the exercise groups also completed the exercise log. Exercise adherence was similar between NGE and NTGE groups at week 6, with a large effect size (p=0.604, d=3.001) (Table 3).

Patients in all groups reported no adverse events during or after the treatment. Additionally, the patients stated they did not use any medication during the treatment period.

Discussion

After six weeks of treatment, we found that orthosis and exercises improved symptoms and function in patients with CTS. There were no significant differences between all groups. However, education, orthosis, and NGE significantly reduced the CSA of the median nerve at week 6.

The plausible target of the treatment in patients with mild-to-moderate CTS is to prevent the transition of the median nerve to the fibrotic stage by reducing edema (28). It was shown that either orthosis or NTGE exercises reduced signal intensity of the median in patients with CTS (28). Ultrasound, which is widely used in diagnosing CTS, is another tool used to investigate the effectiveness of treatments. Twelve-week volar-supported neutral wrist splint treatment decreased the CSA of the median nerve at the pisiform level (29,30). In our study, the CSA of the median nerve at the pisiform level decreased by 3.7% after six weeks of volar-supported neutral wrist orthosis treatment. This magnitude of change in the intraneural edema did not exceed minimal detectable change (MDC) thresholds (3.8-6.2%) (31).

Hand therapists often prescribe a combination of exercise and orthosis (9). Yıldırım et al. (32) stated that the combination of the orthosis with the NTGE did not significantly affect the CSA of the median nerve. This result is consistent with our findings.

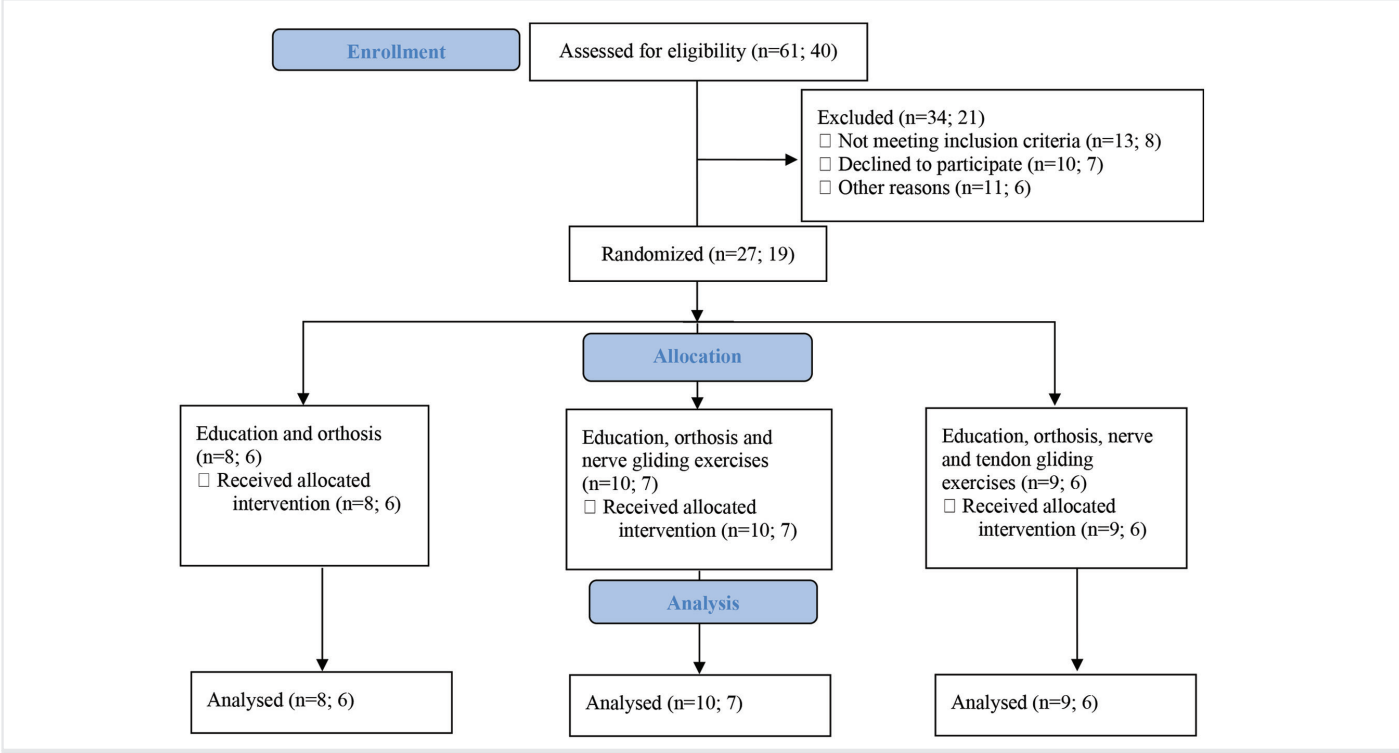

Figure 1. Flow chart of the study

n: Number of hands; the number of patients

The way the patients do the tendon gliding exercises may have led to this result. Strong fist movement performed during tendon gliding exercises causes migration of lumbrical muscles into the tunnel. This movement could have aggravated nerve compression and neutralized the effects of NGE (27). We taught them that if their symptoms worsened during these movements, they should reduce their range of motion. The patients did the exercises independently, so whether they followed the instructions during the sessions was unclear.

Polat et al. (12) reported that a combination of orthosis and NGE did not affect intraneural edema after six weeks of treatment. They didn't report which nerve gliding exercise was used in the study. However, the combination of NGE with orthosis reduced the median CSA of the median nerve by 13.6% in our study. This magnitude of change in the intraneural edema exceeded (MDC) thresholds (31). This may be due to our study's different NGE.

A significant improvement of 1.41 for SSS and 1.50 for FSS was achieved in the orthosis group in our study. This improvement was better than Gatheridge et al. (33), whose treatment protocol included a 4-week neutral wrist orthosis. The differences in orthotic designs, wearing regimes, treatment, and follow-up times used in the studies make it difficult to conclude. However, our results supported that the custom-made neutral wrist splint, worn only at night for six weeks, as suggested in the guideline (34), reduced the symptoms and improved the patients' function.

After six weeks of treatment, a significant improvement of 1.36 for SSS and 1.24 for FSS was achieved in the NGE group. Orthosis and NGE could facilitate venous return and reduce intraneural edema. This contributes to symptom resolution and improvement in function in patients with mild-to-moderate CTS.

Compared to the baseline, a significant improvement of 1.37 for FSS was achieved in the NTGE group. This improvement is better than the improvements in the studies of Figueiredo et al. (13) and Schmid et al. (28). An improvement of 1.09 for SSS in the NTGE group was also achieved, lower than the finding of the study by Figueiredo et al. (13). However, this was not a statistically significant improvement. Although the number of repetitions, treatment duration, and application method of the NTGE used in these studies differed, we had similar results to previous studies. It can be concluded that a combination of orthosis and NTGE could decrease the symptoms and improve the functions of mild-to-moderate CTS patients. However, we don't know whether these differences exceed the MCD of SSS and FSS. The MCD of SSS and FSS is only calculated for surgical treatment (35). Moreover, it is impossible to interpret these findings in terms of MCD.

The patients' PSFS scores improved in all groups, and the activities they rated were similar to those on the FSS of BCTSQ. However, there were some activities not included in BCTSQ, like "using hand and power tools as an occupational activity", "sewing stitches as a free time activity", and "using makeup tools as a personal care", where they faced difficulties. Hence, to evaluate the functional status of CTS patients in more detail, we recommend using PSFS along with BCTSQ.

Monitoring a patient's adherence to treatment is crucial, particularly in studies that involve exercise and orthosis. In our research, we had patients fill out logs for both orthosis and exercise to monitor and enhance their motivation and adherence. We found that patients complied well with the treatment, and this should be closely monitored in future studies involving exercise and orthosis.

Table 1. Baseline characteristics of patients

	Orthosis group (Group 1) (n=8; patients =6)	Orthosis, and nerve gliding exercises group (Group 2) (n=10; patients =7)	Orthosis, nerve, and tendon gliding exercises group (Group 3) (n=9; patients =6)	p-value
Age (y), mean (SD)	40.83 (13.61)	44.00 (9.87)	43.00 (6.48)	0.828
Body mass index, mean (SD)	29.56 (4.85)	26.28 (2.51)	28.30 (5.61)	0.513
Gender, n (%)				
Female	6 (100)	4 (57.1)	6 (100)	
Male	-	3 (42.9)	-	
Affected side, n (%)				
Dominant	4 (66.70)	4 (57.10)	2 (33.30)	
Non-dominant	-	-	1 (16.70)	
Bilateral	2 (33.30)	3 (42.90)	3 (50.00)	
Electrodiagnostic test grade				
Mild	3	4	4	
Moderate	5	6	5	
CTS-6 score (points)	17.94 (3.74)	17.55 (1.96)	19.38 (5.17)	0.813
Duration of symptoms (m)	44.25 (29.90)	24.25 (21.83)	24.55 (21.67)	0.119

y: Years, SD: Standard deviation, BMI: Body mass index, m: Months

Non-surgical treatments are recommended before considering surgery for patients with mild-to-moderate CTS. This study's findings can help healthcare professionals formulate treatment plans and reduce intraneural edema in the median nerve in patients with mild-to-moderate CTS. Education, night splinting, and exercise could improve mild-to-moderate CTS patients' functionality and intraneural edema in the median nerve.

The study's major strengths are the objective measurement of intraneural edema, the randomized design, and the use of valid

Table 2. Comparison of median values of outcome measures for all groups and between-group and within-group differences

Outcome measures	Group	Baseline Median (25th percentile-75th percentile)	Week 6 Median (25th percentile-75th percentile)	Between-group difference p-value	Within-group difference p-value
Median nerve cross-sectional area (mm^2)	Group 1	13.50 (10.25-16.50)	13.00 (9.25-14.75)	Baseline 0.352[a]	0.096[b]
	Group 2	11.50 (10.00-14.00)	10.00 (9.00-10.25)		0.006[b]*
	Group 3	10.00 (10.00-13.00)	10.00 (9.00-10.50)	Week 6 0.238[a]	0.140[b]
Symptom severity scale (points)	Group 1	2.99 (2.90-3.18)	1.58 (1.20-1.63)	Baseline 0.867[a]	0.012[b]*
	Group 2	2.85 (2.40-3.63)	1.49 (1.00-2.20)		0.005[b]*
	Group 3	2.54 (1.90-4.31)	1.45 (1.45-3.17)	Week 6 0.758[a]	0.097[b]
Functional status scale (points)	Group 1	3.25 (2.65-3.50)	1.75 (1.37-2.43)	Baseline 0.508[a]	0.012[b]*
	Group 2	2.81 (1.42-3.15)	1.57 (1.28-2.40)		0.012[b]*
	Group 3	2.87 (1.62-3.84)	1.50 (1.25-2.78)	Week 6 0.867[a]	0.021[b]*
Patient specific functional scale (points)	Group 1	3.80 (1.60-5.15)	6.00 (5.00-7.95)	Baseline 0.052[a]	0.069[b]
	Group 2	4.50 (2.47-5.00)	6.80 (5.60-8.42)		0.028[b]*
	Group 3	4.80 (2.75-5.10)	8.10 (7.05-8.80)	Week 6 0.737[a]	0.008[b]*

mm^2: Square millimetres, [a]: P-values determined using the Kruskal-Wallis test. [b]: P-values determined using the Wilcoxon test. *: p<0.05

Table 3. Comparison of orthosis wearing times and exercise sessions between groups

Outcome measures	Group	Mean (SD)	Median (25th percentile-75th percentile)	p-value
Orthosis wearing time (hours)	Group 1	7.26 (1.14)	6.86 (6.40-8.49)	0.757[c]
	Group 2	6.63 (1.38)	6.83 (5.58-7.78)	
	Group 3	6.87 (0.83)	7.00 (5.94-7.50)	
Number of exercise sessions in a day	Group 2	4.58 (0.35)	4.59 (4.30-5.00)	0.604[d]
	Group 3	4.22 (0.84)	4.86 (3.28-4.94)	

SD: Standard deviation, [c]: P-value determined using Kruskal-Wallis test. [d]: P-value determined using Mann-Whitney U test

and reliable measurements. Additionally, our data can be used to calculate the sample size for confirmatory studies.

Study Limitations

The major limitation of this study was the need for a follow-up evaluation. We did not perform follow-up assessments. Therefore, it is still being determined whether the effects of these proposed treatments last longer. The long-term effects of orthosis and exercise on intraneural edema and function need further investigation. Although the sample size in each group was small, most of the calculated effect sizes were medium-to-large. So, we could argue that the current sample size was sufficient to test the research hypothesis. We did not perform the classification of patients according to the severity of CTS. Further studies can also assess the effectiveness of orthosis, nerve, and tendon gliding exercises on median nerve morphology and function of CTS patients with varying degrees of CTS severity. Finally, exercise and orthosis adherence were monitored by self-reporting, which could overestimate the actual adherence rate.

Conclusion

Custom-made nocturnal wrist orthosis and a 6-week home-based nerve gliding exercise program are safe and feasible in patients with mild-to-moderate CTS. Participating in and completing the proposed CTS treatment program substantially benefits patients' intraneural edema, symptoms, and functions.

Ethics

Ethics Committee Approval: The clinical research Ethics Committee of Kütahya Dumlupınar University approved the study (decision no: 2017-4/2, date: 22.03.2017).

Informed Consent: Participants signed written informed consent before their inclusion.

Authorship Contributions

Surgical and Medical Practices: H.A., M.K., S.C.K., H.H.G., Concept: H.A., M.I.A., Design: H.A., M.I.A., Data Collection or Processing: H.A., M.K., S.C.K., H.H.G., Analysis or Interpretation: H.A., M.I.A., Literature Search: H.A., Writing: H.A., M.I.A., M.K., S.C.K., H.H.G.

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Original Article

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Chemical Contents and Bioactivities of Green Algae *Ulva rigida* C.Agardh Red Algae *Grateloupia turuturu* Yamada Extracts

Yeşil Alg *Ulva rigida* C.Agardh ve Kırmızı Alg *Grateloupia turuturu Yamada* Ekstrelerinin Kimyasal İçerikleri ve Biyoaktiviteleri

Begüm ZENGİN[1], Gülbahar Özge ALİM TORAMAN[2], Rabia Sare YANIKOĞLU[3], Fatma GÖÇ[2], Harika Öykü DİNÇ[4], Emine Şükran OKUDAN[5], Gülaçtı TOPÇU[2,6], Halil ŞENOL[7]

[1]Bezmialem Vakıf University Institue of Health Science, Department of Pharmacognosy, İstanbul, Türkiye
[2]Bezmialem Vakıf University Faculty of Pharmacy, Department of Pharmacognosy, İstanbul, Türkiye
[3]Bezmialem Vakıf University Faculty of Pharmacy, Department of Biochemistry, İstanbul, Türkiye
[4]Bezmialem Vakıf University Faculty of Pharmacy, Department of Microbiology, İstanbul, Türkiye
[5]Akdeniz University Faculty of Aquaculture, Department of Basic Sciences Fisheries, Antalya, Türkiye
[6]Bezmialem Vakıf University Pharmaceutical Application and Research Center, İstanbul, Türkiye
[7]Bezmialem Vakıf University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Türkiye

ABSTRACT

Objective: Edible seaweeds, valued for their rich content of bioactive compounds, have gained recognition in complementary medicine beyond their traditional role in the food and pharmaceutical sectors. Notable examples include *Ulva rigida* C.Agardh and *Grateloupia turuturu* Yamada. These seaweeds offer a promising avenue for exploring their bioactive potential in medical and dietary contexts.

Methods: Hexane extracts were prepared to elucidate the fatty acid composition of seaweeds via Gas Chromatography-Mass Spectrometry analyses. Subsequently, investigations were conducted to assess the cytotoxicity on human breast cancer cell lines (MCF-7 and MDA-MB-231), as well as examine the antimicrobial and anti-cholinesterase activities of extracts obtained using hexane, dichloromethane:methanol (CH_2Cl_2:MeOH, 1:1), methanol (MeOH), and water solvents.

Results: The most abundant substances for the *U. rigida* and *G. turuturu* species were hexadecanoic acid, octadecenoic acid, and erucic acid compounds. The CH_2Cl_2:MeOH (1:1) extract of *G.*

ÖZ

Amaç: Zengin biyoaktif bileşik içeriği nedeniyle değer verilen yenilebilir deniz yosunları, gıda ve ilaç sektöründeki geleneksel rollerinin ötesinde tamamlayıcı tıpta da tanınmaktadır. Dikkate değer örnekler arasında *Ulva rigida* C.Agardh ve *Grateloupia turuturu* Yamada bulunmaktadır. Bu deniz yosunları, tıbbi ve diyetsel bağlamlarda biyoaktif potansiyellerini keşfetmek için umut verici bir yol sunmaktadır.

Yöntemler: Deniz yosunlarının yağ asidi kompozisyonunu Gaz Kromatografisi-Kütle Spektrometrisi analizleri yoluyla aydınlatmak için hekzan ekstreleri hazırlandı. Daha sonra, insan meme kanseri hücre dizileri (MCF-7 ve MDA-MB-231) üzerindeki sitotoksisiteyi değerlendirmek ve ayrıca hekzan, diklorometan:metanol (CH_2Cl_2:MeOH, 1:1), metanol (MeOH) ve su çözücüleri kullanılarak elde edilen ekstrelerin antimikrobiyal ve antikolinesteraz aktivitelerini incelemek için araştırmalar yapıldı.

Bulgular: *U. rigida* ve *G. turuturu* türleri için en bol bulunan maddeler hekzadekanoik asit, oktadekenoik asit ve erusik asit

Address for Correspondence: Halil Şenol, Bezmialem Vakıf University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, İstanbul, Türkiye
E-mail: halilsenol25@gmail.com **ORCID ID:** orcid.org/0000-0002-8333-035X

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ABSTRACT

turuturu was found as the highest toxicity on MCF-7 and MDA-MB-231 breast cancer cells (IC_{50}: 28.7 µg/mL). *G. turuturu* extracts showed inhibition on *E. coli* and *C. albicans*. The hexane extracts of *U. rigida* and *G. turuturu* inhibited AChE enzyme in both algae species.

Conclusion: The results highlight seaweeds' bioactive potential for therapeutic and dietary products. Further research can explore medical and nutritional applications.

Keywords: *Ulva rigida*, *Grateloupia turuturu*, chemical composition, bioactivity studies

ÖZ

bileşikleridir. *G. Turuturu*'nun CH_2Cl_2:MeOH (1:1) ekstresinin, MCF-7 ve MDA-MB-231 meme kanseri hücreleri üzerinde en yüksek toksisiteye sahip olduğu bulundu (IC_{50}: 28,7 µg/mL). *G. turuturu* ekstreleri *E. coli* ve *C. albicans* üzerinde inhibisyon gösterdi. Her iki alg türünün hekzan ekstreleri AChE enzimini inhibe etti.

Sonuç: Deniz yosunlarının tedavi edici ve diyetsel ürünler için biyoaktif potansiyelini vurgulamaktadır. Daha fazla araştırma tıbbi ve beslenme uygulamalarını keşfedebilir.

Anahtar Sözcükler: *Ulva rigida*, *Grateloupia turuturu*, kimyasal içerik, biyoaktivite çalışmaları

Introduction

Edible seaweeds are recognized for their significant content of bioactive antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids. While traditionally utilized as gelling and concentrating agents in the food and pharmaceutical sectors, contemporary scientific inquiries have disclosed their auspicious prospects in the realm of complementary medicine (1,2). Two noteworthy examples are *Ulva rigida*, C.Agardh, a green macroalgae commonly referred to as "Sea lettuce", and *Grateloupia turuturu*, Yamada, a red macroalgae known colloquially as "Devil's Tongue Grass", "Jinuari" (Korean), and "Ratanho" (Portuguese), originating from Asia.

Marine macroalgae are known to produce a diverse array of volatile organic compounds, including hydrocarbons, terpenes, phenols, alcohols, aldehydes, ketones, esters, fatty acids, and halogen or sulfur-containing compounds. Macroalgae serve as valuable sources of antimicrobial compounds, omega-3 fatty acids, antioxidants, and other bioactive constituents, thus garnering increasing interest for integration into functional foods and nutraceutical products. Notably, polyphenols, flavonoids, and polysaccharides have been identified for their antioxidant and antimicrobial activities within brown, red, and green algae (3).

The use of macroalgae has an extensive history, with indigenous South Americans employing macroalgae for sustenance and medicinal purposes as far back as 12,000 years ago. The earliest recorded use of algae in medicinal contexts can be traced to Shen Nung's "Materia Medica" from 2700 BCE, although the systematic scientific investigation of algae-based products primarily emerged in the last century (4).

Despite approximately ten thousand identified algae species, only around 5% of them find use in human or animal diets. Nevertheless, over a hundred seaweed types are utilized worldwide, particularly in Asian nations where they serve as sea vegetables (5,6). Seaweed consumption is associated with various health benefits, including immunity enhancement, cholesterol reduction, blood sugar level regulation, antioxidant activity, memory enhancement, blood pressure control, fatigue alleviation, weight management, growth and development support, anemia prevention, liver protection, acne reduction, skin moisture improvement, intestinal flora regulation, digestion promotion, fecal excretion facilitation, and gastric mucosal injury prevention (7).

In previous studies conducted on *U. rigida* and *G. turuturu* species, antioxidant, antimicrobial, anti-inflammatory and anticancer activities of these algae were detected and secondary metabolites such as terpenic compounds, polysaccharides and fatty acids were revealed (8-17).

The primary objective of this study was to investigate the cholinesterase inhibitory activities, antimicrobial activities, and cytotoxic effects of *U. rigida* and *G. turuturu* species on MCF-7 and MDA-MB231 human breast cancer cells.

Methods

Algae Collection and Extraction

Ulva rigida and *Grateloupia turuturu* (Figure 1) were harvested from the coastal regions bordering the Mediterranean and the Aegean Sea. The algae specimens (E105 and E137) were deposited at the Faculty of Aquatic Sciences and Fisheries, Akdeniz University, Antalya, Türkiye, and their identification was verified by Dr. Emine Şükran Okudan.

Figure 1. *Ulva rigida* (left) and *Grateloupia turuturu* (right)

The dried and pulverized algae *Ulva rigida* (10 g) and *Grateloupia turuturu* (10 g) were extracted with Hexane, MeOH, water (200 mL × 5 days) respectively, and *Ulva rigida* (280 g) and *Grateloupia turuturu* (370 g) were percolated with dichloromethane:methanol (CH_2Cl_2:MeOH 1:1, 200 mL × 5 days) at room temperature. The water extract underwent lyophilization and drying, whereas the remaining extracts were concentrated using a rotary evaporator under low pressure and at 45 °C.

Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Thermo Scientific TSQ GC-MS/MS. TG-5MS 0.25 mm silica column was used. The GC oven temperature was kept at 80 °C and programmed to increase 300 °C. Using scan mode, a mass range from 50 to 950 m/z. The identification of components was conducted using commercial libraries such as Wiley and Nist, along with referencing literature data. Relative percentage amounts of the separated compounds were determined from Total Ion Chromatograms.

Cytotoxic Activity

Human breast cancer cell lines (MCF-7 and MDA-MB-231) and a healthy skin fibroblast cell line (CCD-1079Sk) were obtained from the American Tissue Culture Collection. The cells were cultured in T75 cell culture flasks in a controlled humidified incubator with a 5% carbon dioxide (CO_2) atmosphere at 37 °C, utilizing DMEM/F12 medium supplemented with 10% fetal bovine serum and 100 units per milliliter (U/mL) of penicillin/streptomycin. Upon reaching an appropriate confluency of 80%, the cells underwent PBS washing and were detached through trypsin/EDTA treatment. Prior to treatments, $5x10^3$ cells were seeded into 96-well plates for 24 and 48 hours. The plant extracts were introduced into the cell cultures at final concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.60 µg/mL. After 24 and 48 hours of incubation, MTT solution (5 mg/mL in PBS) was added to each well and further incubated at 37 °C with 5% CO_2 for 4 hours in the dark. Subsequently, the cell culture media was aspirated, and 100 µL of DMSO was added to each well to dissolve the formed formazan crystals. Absorbance values were measured at 540 nm using an Elisa microplate reader. The experiments were conducted in triplicate, and the results were presented as the mean ± standard deviation (SD).

Determination of Selectivity Index

Selectivity index (SI) is an equation calculated to evaluate the cytotoxic potential in cancer cells relative to toxicity in normal cells; high SI value indicates high potency and low cell toxicity. An SI value higher than 3 serves as a marker for the selection of extracts (18,19).

Anticholinesterase Activity Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity were measured using a spectrophotometric method described by Ellman (20). This procedure involved mixing 10 µL of the sample solution, 20 µL of AChE or BChE solution, and 130 µL of 0.1 M sodium phosphate buffer (pH 8.0). The mixture was incubated for 10 minutes at 25 ºC. Then, 20 µL of DTNB was added to the combination. Acetylthiocholine iodide or butyrylthiocholine iodide (20 µL) was added to start the reaction, which resulted in the enzymatic hydrolysis of the aforementioned iodides and the release of thiocholine. At a wavelength of 412 nm, the yellow-colored 5-thio-2-nitrobenzoic acid anion that resulted from the reaction of thiocholine with DTNB was detected using spectrophotometry. Three parallel studies were conducted for each sample and the following equation was used to compute the results:

% Inhibition = (A control - A sample) / A control x 100

Antimicrobial Activity Assay

Material

Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), Gram (-) bacteria *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028) yeast were used. Vancomycin and ciprofloxacin were used as antibiotics and amphotericin B was used as antifungals for the control (21).

Resazurin Microplate Test

The Resazurin microplate method was employed to assess the antibacterial and antifungal properties as well as the minimum inhibitory concentrations (MIC) of the compounds. Two replicates were conducted for the activity determination. The tested compounds were prepared as stock solutions at a concentration of 1000 µg/mL, which were then sterilized through a 0.22 µm diameter membrane filter. In each well of a 96-well microplate, 50 µL of Mueller Hinton Broth medium was initially added. The first well received diluted samples at 1000 µg/mL, while the control antibiotic and antifungal compound were added to the first well and further diluted in a series. One column of the plate was designated for DMSO as the negative control, and another column was assigned for 50 µL of standard bacteria and yeast as the positive control, also diluted in a series. A McFarland suspension with a turbidity value of 0.5 was prepared from microorganism colonies and diluted at a 1:100 ratio. Subsequently, 10 µL of the final suspension was added to the plate wells. The plates were covered with parafilm and subjected to incubation at 37 °C for 24 hours for bacteria and 48 hours for yeast. After incubation, 10 µL of a solution containing 33.75 mg of resazurin dissolved in 5 mL of distilled water and 10 µL of 20% Tween 80 were added to all wells. Visual evaluation of the results was performed after allowing the plates to incubate for an additional 2 to 4 hours. The MIC value was determined as the lowest concentration preventing a color change from purple to pink.

Statistical Analysis

IC_{50} values were obtained with the equation y=50 and statistical analyses were calculated with Graphpad Prism 10 (San Diego, USA). In the equation, log-transformations of the concentration values of the extracts were plotted on the X-axis and fluorescence absorbance values were plotted on the Y-axis to obtain a sigmodial curve. IC_{50} values had ± 95% confidence interval.

The results were represented as mean ± SD, $^{*}p<0.05$ (p-values were obtained paired t-test).

Results

The non-polar hexane extract of the edible seaweed *U. rigida* was subjected to GC-MS analysis to examine its fatty acid composition and other compounds. The analysis results revealed that among the fatty acids, hexadecanoic acid and octadecenoic acid were identified as major compounds (Table 1). Similarly, in the hexane extract of *G. turuturu*, fatty acids such as hexadecanoic acid was observed as major constituent (Table 2).

As shown in Table 3, *U.rigida* methanol extract (1) and *U. rigida* CH_2Cl_2:MeOH (1:1) extract (2) were the most toxic against MCF-7 and MDA-MB-231 cell lines. The most active extract from the *U. rigida* plant was extract 1, with IC_{50} values of 11.1 g/mL and 20.3 g/mL after 24 and 48 hours of exposure, respectively. In all cell lines, the most cytotoxic effect of the extracts obtained from *G. turuturu* plant was obtained by extract (4) on MCF-7 line. *U. rigida* water extract (5) and *G. turuturu* MeOH extract (3) had the least cytotoxic effect against a healthy cell line CCD-1079Sk. When the viability data of the extracts at 2 time slots in each cell line were compared with the pared t-test, significant differences were obtained in extracts 1, 2 and 3 (Figure 2). Since the plants from which the extracts were obtained could be consumed as food, high IC_{50} values were an expected result. Extracts 5 and 3 had the least cytotoxic effect on the healthy cell line CCD-1079Sk. Similarly, these extracts had the maximum IC_{50} values obtained after 48 hours of exposure in two cancer cell lines. In the graphs in (Figure 3), the percent viability data of the extracts according to time and concentration increased depending on the decrease in concentration. It was revealed that the percentage of time-dependent viability in healthy cell line and two cancer cell lines was increased. These findings indicated that the extracts showed their effects within twenty-four hours.

When the SI results were evaluated, it was determined that extracts 3, and 5 had the S>3 value, indicating that they had a specific effect on cancer cells; the S<3 value corresponds to the general toxicity of the other extracts. In 48 hours, the SI values of all extracts decreased relative to the 24 hour data. The SI values for extract 3, which had a significant effect on two cancer lines based on 24 hour treatment data, were 9.9 and 4.7, as shown in Table 4. The IC_{50} and SI values of extract 3 were consistent, and extract 3 demonstrated high selectivity against two cancer cell lines.

Table 1. Compounds detected in hexane extract of *Ulva rigida* by GC-MS

RT (min)	Name of the compound	Molecular formula	%
3.90	Benzaldehyde	C_7H_6O	0.65
4.08	Sabinene	$C_{10}H_{16}$	1.23
5.39	α-Terpinene	$C_{10}H_{16}$	0.16
6.06	Benzoic acid, methyl ester	$C_8H_8O_2$	0.43
6.33	Benzaldehyde dimethyl acetal	$C_9H_{12}O_2$	2.43
7.69	Terpinen-4-ol	$C_{10}H_{18}O$	0.54
9.61	Nonanal dimethyl acetal	$C_{11}H_{24}O_2$	0.4
11.85	Geranyl acetate	$C_{12}H_{20}O_2$	6.3
14.71	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	0.44
15.18	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	0.39
18.48	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	1.43
20.24	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	0.51
21.58	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	0.53
21.92	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	34.16
23.52	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	0.6
24.59	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	0.8
24.68	6-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2.19
24.76	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2.46
25.06	Octadecenoic acid, methyl ester	$C_{19}H_{38}O_2$	24.21
27.94	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	0.75
30.29	Erucic acid	$C_{22}H_{42}O_2$	2.93
30.60	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	1.12
30.89	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	3.98
		Total	88.64

GC-MS: Gas chromatography - Mass spectrometry

Table 2. Compounds detected in hexane extract of *Grateloupia turuturu* by GC-MS

RT (min)	Name of the compound	Molecular formula	%
4.09	Sabinene	$C_{10}H_{16}$	1.73
6.34	Benzaldehyde dimethyl acetal	$C_9H_{12}O_2$	4.73
11.86	Geranyl acetate	$C_{12}H_{20}O_2$	3.15
18.48	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	4.93
20.24	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	3.08
21.58	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	2.84
21.66	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	0.78
21.92	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	37.95
23.53	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	1.64
24.68	6-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.28
24.77	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.28
25.06	Octadecenoic acid, methyl ester	$C_{19}H_{38}O_2$	4.43
30.29	Erucic acid	$C_{22}H_{42}O_2$	1.53
30.88	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	5.86
		Total	81.21

GC-MS: Gas chromatography - Mass spectrometry

Table 3. IC_{50} values of the cytotoxicity of synthesized molecules at MCF-7, MDA-MB-231 and CCD-1079Sk cell line

IC_{50} value (µg/mL)

Extracts	Codes	CCD-1079Sk		MCF-7		MDA-MB-231	
		24h	48h	24h	48h	24h	48h
U. rigida MeOH	1	32.7±0.05	42.6±0.11	11.1±0.07	20.3±0.05	13.5±0.06	58.3±0.02
U. rigida CH_2Cl_2:MeOH (1:1)	2	27.1±0.06	36.5±0.09	12.8±0.12	14.1±0.1	10.1±0.15	31.2±3.81
G. turuturu MeOH	3	107.0±0.04	140.5±0.09	10.8±0.35	135.3±0.06	22.8±0.07	118.7±0.08
G. turuturu CH_2Cl_2:MeOH (1:1)	4	36.7±0.09	15.7±0.11	24.5±0.11	28.8±0.09	89.0±0.06	24.2±0.13
U. rigida water	5	86.8±0.06	25.8±0.09	384.7±0.05	166.6±0.02	10.7±0.77	221.5±0.1
G. turuturu water	6	15.1±0.19	9.1±0.18	79.2±0.05	69.7±0.06	54.1±0.13	17.3±0.15

According to the data obtained in this study, although antibacterial and antifungal activity was higher in methanol extracts for *Ulva rigida*, lower inhibition was observed for *S. aureus*. The MIC values for the inhibition of the methanol extract of *Ulva rigida* on *E. coli* and *C. albicans* were 125 µg/mL for both. There was also inhibition for hexane and CH_2Cl_2:MeOH (1:1) extracts of *Ulva rigida*. All of the *Grateloupia turuturu* extracts showed inhibition on *E. coli* at a concentration of 125 µg/mL, but lower on *S. aureus*. The highest antifungal activity was found in the methanol extract of *Ulva rigida* and *G. turuturu* at a concentration of 125 µg/mL (Table 5).

Hexane, CH_2Cl_2:MeOH (1:1), and MeOH extracts were investigated for the detection of AChE and BChE enzymes inhibitor activity. Due to their inability to penetrate the blood-brain barrier and their notably low AChE activities, water extracts were not included in this study of cholinesterase inhibitor activity (21). According to the results of cholinesterase enzyme inhibition experiments, 63-84% inhibition of AChE inhibition was observed in the range of 50-200 µg/mL on hexane extracts for both species. Inhibition values of control Galantamine under the same conditions were in the range of 75-84%. The results may be promising for future studies when compared with galantamine. BChE inhibition results were moderate to good only for the methanol extract of *Grateloupia turturu* (Table 6).

Discussion

The GC-MS is highly sensitive, allowing for the detection of trace amounts of compounds present in herbal extracts. This is important for identifying minor constituents that may contribute to the overall therapeutic effects or serve as markers for quality control. In prior chemical investigations, the chemical constituents of these algae were predominantly identified through GC-MS analysis of essential oils obtained via hydrodistillation. Notably, there is a dearth of literature on GC-MS analysis of hexane extracts. To address this gap, the chemical composition of hexane extracts, prepared using the maceration technique from both algae species, was determined through GC-MS analysis (12,17,18). Both hexadecanoic acid and octadecanoic acid act as energy sources for the body, as they are metabolized to produce energy needed for physical activities. Furthermore, these fatty acids are commonly incorporated into cosmetic product formulations, serving as moisturizing agents in skincare products

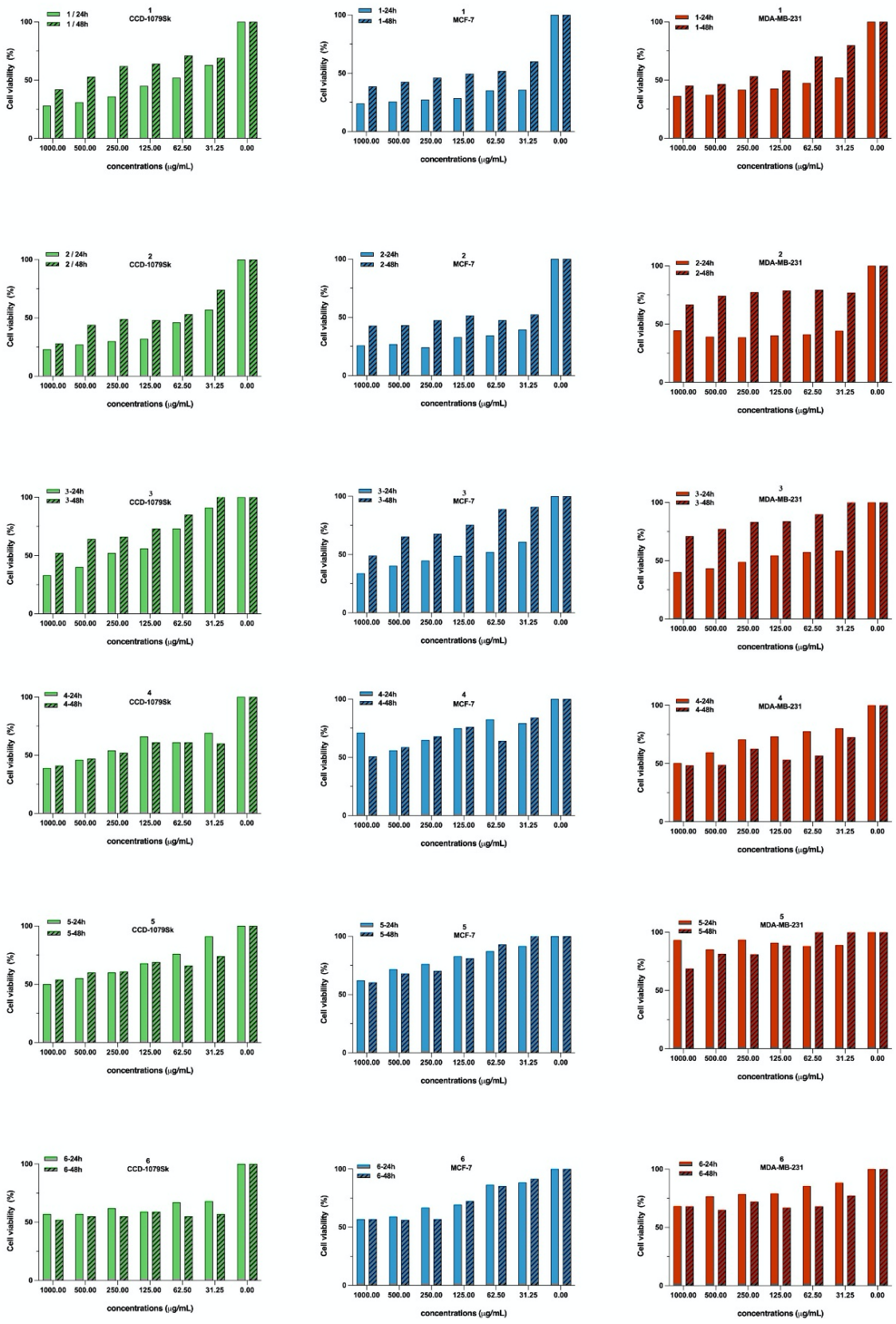

Figure 2. *In vitro* cytotoxicity of CCD-1079Sk, MCF-7 and MDA-MB-231 cells treated with extracts from *U. rigida* and *G. turuturu*. The cell viability was assessed by a MTT assay

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

and as foaming agents in soaps (8). The fact that *U. rigida* and *G. turturu* algae are rich in fatty acid composition and the major presence of hexadecanoic acid and octadecanoic acid in their composition gives importance to these algae.

The MCF-7 and MDA-MB-231 represent two distinct subtypes of breast cancer, each with unique characteristics. MCF-7 cells are estrogen receptor-positive (ER+) and typically respond to hormonal therapies, while MDA-MB-231 cells are triple-negative (ER-, PR-, HER2-) and tend to be more aggressive. Studying cytotoxic effects on both cell lines helps address the heterogeneity of breast cancer, allowing for a more comprehensive understanding of potential treatment options. Studying cytotoxic effects on breast cancer cell lines (MCF-7, MDA-MB-231) and a normal cell line (CCD-1079Sk) is important to observe any differential responses between cancerous and non-cancerous cells. This information is crucial for identifying compounds/extracts that selectively target cancer cells while sparing healthy ones.

U. rigida methanol extract (1) and *U. rigida* CH_2Cl_2:MeOH extract (2) were most toxic to cancer cells, with extract (1) showing significant cytotoxicity after 24 and 48 hours. Extracts (5) and (3) from *U. rigida* water and *G. turuturu MeOH* extracts, respectively, had minimal impact on healthy cells and the highest IC_{50} values in cancer cells after 48 hours. Statistical analysis revealed concentration-dependent effects, with significant differences in viability for extracts 1, 2, and 3 over time. Extract (3) demonstrated high selectivity against cancer cells (SI>3), maintaining consistency in efficacy at 48 hours. These findings suggest extract 3 as a promising candidate for further investigation in cancer treatment, highlighting the potential of natural compounds in therapeutic applications.

Table 4. Table of selectivity index for the two cell types

Extracts	Codes	Selectivity index			
		MCF-7		MDA-MB-231	
		24h	48h	24h	48h
U. rigida MeOH	1	2.9	2.1	2.4	0.7
U. rigida CH_2Cl_2:MeOH (1:1)	2	2.1	2.6	2.7	1.2
G. turuturu MeOH	3	9.9	1.0	4.7	1.2
G. turuturu CH_2Cl_2:MeOH (1:1)	4	1.5	0.5	0.4	0.6
U. rigida water	5	0.2	0.2	8.1	0.1
G. turuturu water	6	0.2	0.1	0.3	0.5

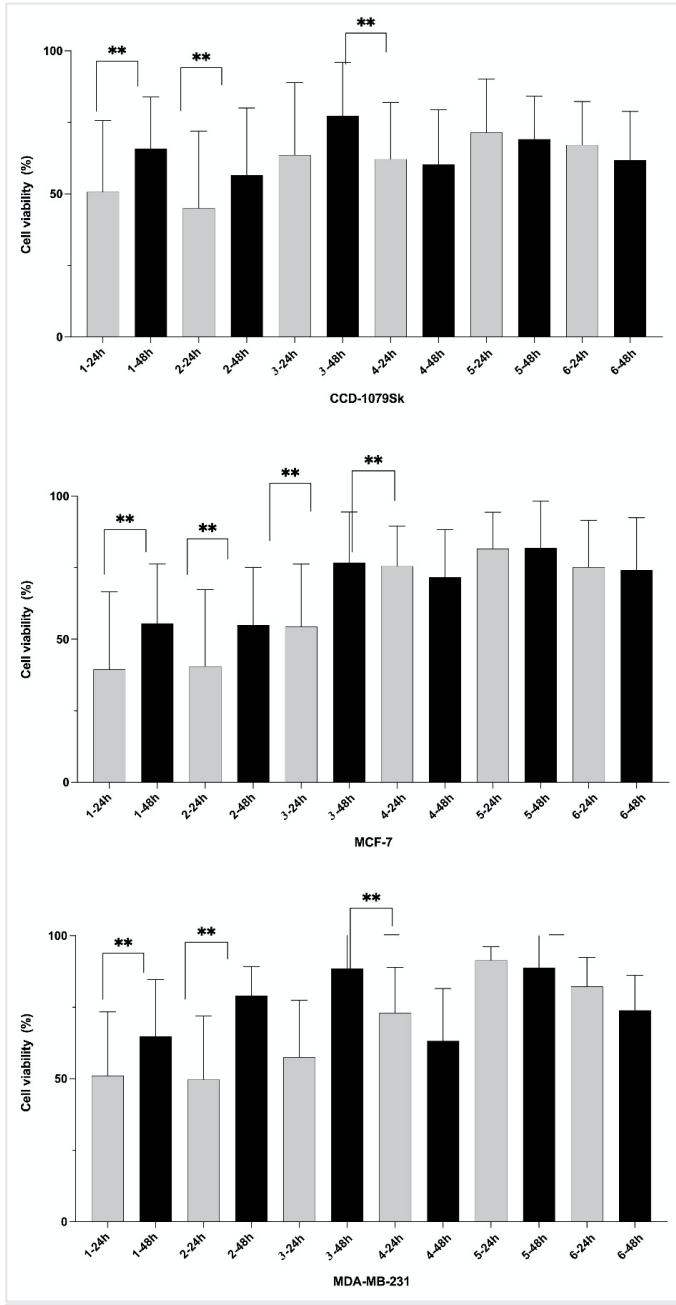

Figure 3. Cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Viability data obtained at two time points of extracts in each cell line. *p<0.05 (p-values were obtained paired t-test)

Table 5. Antibacterial and antifungal activities of *Ulva rigida* and *Grateloupia turuturu* extracts

	Ulva rigida MIC value (µg/µL)				***Grateloupia turuturu*** MIC value (µg/µL)			
	Hexane	CH_2Cl_2:MeOH (1:1)	MeOH	Water	Hexane	CH_2Cl_2:MeOH (1:1)	MeOH	Water
E. coli	125	250	125	NA	125	125	125	NA
S. aureus	250	250	250	NA	250	250	250	NA
C. albicans	250	125	125	NA	250	250	125	NA

Table 6. Acetylcholinesterase and butyrylcholinesterase inhibiton activities of *Grateloupia turuturu* and *Ulva rigida* extracts

Extracts	Inhibiton (%) Acetylcholinesterase			Inhibiton (%) Butyrylcholinesterase		
	200 µg/mL	100 µg/mL	50 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL
G. turuturu Hexane	80	77	69	29	21	19
U. rigida Hexane	84	68	63	34	28	27
G. turuturu CH_2Cl_2:MeOH (1:1)	67	56	52	15	5	NA
U. rigida CH_2Cl_2:MeOH (1:1)	55	45	40	24	12	8
G. turuturu MeOH	NA	NA	NA	NA	NA	NA
U. rigida MeOH	NA	NA	NA	NA	NA	NA
Galantamine	84	80	75	79	78	68

The search for balanced foods and nutraceuticals is fueled by the growing evidence that dietary patterns can either increase or decrease the risk of developing certain cancers. Research has supported the protective effects of seaweeds against various neoplastic illnesses, including colorectal, breast, and pancreatic cancer (22). No previous research has explored the cytotoxic activities of either the *Grateloupia* species or the *Ulva* species on breast cancer. This study marks the first investigation into the cytotoxicity of both species on breast cancer cells.

In studies on the antimicrobial activity of algae, different results have been reported depending on various factors (23). According to a study investigating the effectiveness of extraction methods, it was found that methanol extraction provided higher antimicrobial activity than hexane and ethyl acetate. However, another study reported that chloroform was better than methanol and benzene (24,25). In general, it has been observed that Gram-positive bacterial strains are more sensitive to seaweed extracts than Gram-negative ones (26). This may be due to the presence of a more accessible web-like peptidoglycan layer in Gram-positive bacteria for the extracts to penetrate. Conversely, Gram-negative bacteria have a resistant barrier consisting of a thin lipopolysaccharide outer membrane that can restrict contact with the extract (27).

Methanol extracts of *Ulva rigida* exhibited higher antibacterial and antifungal activity, with notable inhibition against *E. coli* and *C. albicans* at a concentration of 125 µg/mL. However, *S. aureus* showed lower inhibition, indicating selectivity in the antibacterial effects. Interestingly, hexane and CH_2Cl_2:MeOH (1:1) extracts of *Ulva rigida* also demonstrated inhibitory effects, emphasizing the diverse bioactive compounds present in different extracts. *Grateloupia turuturu* extracts, on the other hand, exhibited inhibition on *E. coli* at 125 µg/mL, while demonstrating lower activity against *S. aureus*. Notably, the methanol extracts of both *Ulva rigida* and *Grateloupia turuturu* displayed the highest antifungal activity at a concentration of 125 µg/mL. This suggests the potential of these extracts in inhibiting fungal growth, particularly against *C. albicans.*

The observed variations in antibacterial and antifungal activities among different extracts and microbial strains highlight the complexity of the bioactive compounds present in these seaweeds. The consistent antifungal activity at a specific concentration indicates a potential therapeutic value against fungal infections. Further research is warranted to identify and isolate the specific bioactive compounds responsible for these activities and explore their potential applications in the development of antimicrobial agents. Additionally, understanding the selectivity of these extracts against different microbial strains is crucial for their targeted use in combating specific infections.

AChE and BChE play essential roles in regulating cholinergic neurotransmission by breaking down acetylcholine, ensuring proper neuronal signaling. Their balanced activity is critical for cognitive function, and alterations in AChE and BChE levels have implications in neurodegenerative diseases, emphasizing their importance in maintaining neurological health and cognitive integrity.

The investigation into AChE and BChE inhibitor activities of hexane, CH_2Cl_2-MeOH (1:1), and MeOH extracts revealed notable findings. Water extracts were excluded due to their limited ability to penetrate the blood-brain barrier and low AChE activities.

In the cholinesterase inhibition experiments, the hexane extracts of both species demonstrated substantial inhibition, ranging from 63% to 84% at concentrations between 50 and 200 µg/mL. The inhibition values for the control galantamine, under comparable conditions, fell within the range of 75% to 84%. These results suggest that the hexane extracts exhibit promising AChE inhibitory activity, comparable to the positive control galantamine. This raises the prospect of further investigations into the potential therapeutic use of these extracts in conditions associated with cholinesterase dysfunction. However, BChE inhibition results were only moderately to good for the methanol extract of *Grateloupia turuturu*. While not as pronounced as the AChE inhibition, these findings still indicate potential for the methanol extract in modulating cholinesterase activities.

The observed inhibitory effects, particularly with the hexane extracts, provide a foundation for future studies exploring the specific compounds responsible for cholinesterase inhibition and their potential neuroprotective effects. Understanding the molecular mechanisms underlying these inhibitory activities can contribute to the development of novel therapeutic agents for

conditions involving cholinergic dysfunction, such as Alzheimer's disease. Further investigations should focus on isolating and characterizing the bioactive compounds responsible for these inhibitory effects to advance the potential application of these extracts in neurodegenerative disorders.

Study Limitations

The isolation study for the master's thesis could not be conducted due to insufficient project support.

Conclusion

In this investigation, distinct from previous studies, the chemical composition of hexane extracts from *U. rigida* and *G. turuturu* was elucidated using GC-MS analysis, identifying hexadecanoic acid and octadecanoic acid as predominant metabolites. The study assessed the cytotoxic effects on previously unexplored MCF-7 and MDA-MB-231 breast cancer cell lines and examined their potential implications for Alzheimer's disease through the analysis of cholinesterase inhibitory activities. Additionally, antimicrobial activity was investigated. These results highlight the bioactive potential of the studied seaweed species, suggesting their prospective application in the development of therapeutic and dietary products. Further research could explore their applications in diverse medical and nutritional contexts, leveraging their diverse bioactive profiles. The varied compounds identified in these extracts present opportunities for future investigations and innovations in the realm of complementary medicine and beyond.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: G.Ö.A.T., G.T., H.Ş., Design: F.G., G.T., H.Ş., Data Collection or Processing: B.Z., G.Ö.A.T., R.S.Y., E.Ş.O., G.T., H.Ş., Analysis or Interpretation: B.Z., G.Ö.A.T., R.S.Y., F.G., H.Ö.D., G.T., H.Ş., Literature Search: B.Z., G.Ö.A.T., R.S.Y., F.G., G.T., H.Ş., Writing: B.Z., G.Ö.A.T., R.S.Y., F.G., G.T., H.Ş.

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

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Original Article

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Comparison of Free Gingival Graft and Modified Apical Repositioned Flap Techniques to Create Attached Gingiva: Long-Term (2 Years) Retrospective Study

Yapışık Diş Eti Oluşturmak için Uygulanan Serbest Diş Eti Grefti ve Modifiye Apikale Repozisyone Flep Tekniklerinin Karşılaştırılması: Uzun Dönem (2 Yıl) Retrospektif Çalışma

Melis YILMAZ[1], Emrah TÜRKMEN[1], Nur BALCI[1], Hilal TOYGAR[1], Ali ÇEKİCİ[2]

[1]İstanbul Medipol University Faculty of Dentistry, Department of Periodontology, İstanbul, Türkiye
[2]İstanbul University Faculty of Dentistry, Department of Periodontology, İstanbul, Türkiye

ABSTRACT

Objective: An ideal anatomical component for maintaining gingival health is the attached gingiva. Increasing the width of the attached gingiva can be achieved using the predictable surgical methods of the modified apically repositioned flap (MARF) and the free gingival graft (FGG).

Methods: Fifteen (female) systemically and periodontally healthy patients were enrolled for this study. The treatment of a total of 21 teeth with recession in the lower jaw, absence of bone dehiscence, and attached gingiva ranging from a minimum of 0.5 mm to a maximum of 1.5 mm was conducted through FGG and modified apical positioned flap techniques. These procedures were randomly selected. Pocket depth on probing, gingival recession (GR), clinical attachment loss, bleeding index on probing, attached gingival width (AGW), keratinized tissue width and plaque index values were recorded before the surgical procedure and repeated at the 3rd month, 1st and 2nd years.

Results: The changes in GR levels at baseline, 3 months, first and second year in both the FGG and MARF groups were statistically significant (p=0.001; p<0.05). The changes observed in AGW levels at baseline, 3 months, first and second year in both MARF and FGG groups were statistically significant (p=0.001; p<0.05).

ÖZ

Amaç: Diş eti sağlığını korumak için ideal bir anatomik bileşen yapışık diş etidir. Yapışık diş eti bölgesinin artırılması, modifiye apikale repozisyone flep (MARF) ve serbest diş eti grefti (SDG) gibi öngörülebilir cerrahi yöntemler kullanılarak elde edilebilir.

Yöntemler: Bu çalışmaya sistemik ve periodontal açıdan sağlıklı 15 (kadın) hasta dahil edildi. Alt çenede diş eti çekilmesi bulunan, kemik dehisensi olmayan, yapışık diş eti minimum 0,5 mm ile maksimum 1,5 mm arasında değişen toplam 21 dişin tedavisi SDG ve MARF teknikleri ile gerçekleştirildi. Bu prosedürler rastgele seçilmiştir. Sondalamada cep derinliği, diş eti çekilmesi, klinik ataşman kaybı, sondalamada kanama indeksi, yapışık diş eti genişliği (YDG), keratinize doku genişliği ve plak indeksi değerleri cerrahi işlem öncesinde kaydedilerek 3. ay, 1. ve 2. yılda tekrarlandı.

Bulgular: Hem SDG hem de MARF gruplarında başlangıçta, 3. ayda, birinci ve ikinci yılda diş eti çekilme seviyelerindeki değişiklikler istatistiksel olarak anlamlıydı (p=0,001; p<0,05). Hem MARF hem de SDG gruplarında başlangıçta, 3 ayda, birinci ve ikinci yılda YDG düzeylerinde gözlenen değişiklikler istatistiksel olarak anlamlıydı (p=0,001; p<0,05).

Address for Correspondence: Melis Yılmaz, İstanbul Medipol University Faculty of Dentistry, Department of Periodontology, İstanbul, Türkiye
E-mail: yilmazmelis9@gmail.com **ORCID ID:** orcid.org/0000-0003-3435-1856

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ABSTRACT

Conclusion: Both techniques have been shown to result in a statistically significant increase in the width of keratinized tissue and the amount of attached gingiva in the long term.

Keywords: Free gingival graft, apically repositioned flap, attached gingiva

Clinical Trial Registration: The study was registered at ClinicalTrials.gov (NCT06253910).

ÖZ

Sonuç: Her iki tekniğin de uzun vadede keratinize doku genişliğinde ve yapışık diş eti miktarında istatistiksel olarak anlamlı bir artışa neden olduğu gösterilmiştir.

Anahtar Sözcükler: Serbest diş eti grefti, apikale repozisyone flep, yapışık diş eti

Klinik Araştırma Kaydı: Bu çalışma, "ClinicalTrials.gov" adresine kaydedilmiştir (NCT06253910).

Introduction

The attached gingiva serves to defend the periodontium from external harm, contributes to the stabilization of the gingival margin and prevents gingival recession (GR). It forms a strong barrier against physiological and friction forces with the thick collagen fibers attached to the bone (1). The dimensions of the attached gingiva differ based on the specific area within the oral cavity. In the consensus report published in 2015, it was reported that the width of the attached gingiva around the teeth should be at least 1 mm to maintain periodontal health (2). Adequate presence of attached gingiva facilitates enhanced efficacy in oral hygiene practices, thereby reducing the risk of periodontal inflammation (3).

The most widely accepted and implemented technique for addressing the deficiency of attached keratinized tissue is the free gingival graft (FGG). The FGG offers distinct advantages such as ample donor tissue availability and the capacity to address multiple teeth simultaneously (4). However, drawbacks associated with this technique encompass postoperative discomfort, unpredictability in color matching of tissues, and the necessity for an additional surgical procedure to obtain donor tissue (4). Carnio and Miller (5) described the modified apically repositioned flap (MARF) technique in 1999 as a method to increase the attached gingiva on multiple adjacent teeth. This surgical intervention employs a single horizontal incision at the designated site. The recognized advantages of employing the MARF technique include its surgical simplicity, ease of application, elimination of the requirement for a palatal donor site, reduced surgical duration, and a heightened predictability in achieving a harmonious color match of the tissues (5). Taking into consideration the advantages of the MARF technique, the authors suggest that it needs to be more widely implemented.

The aim of this study is to apply the frequently preferred FGG technique and the alternative MARF technique to areas with insufficient attached gingiva in order to establish attached gingiva. Furthermore, the objective is to assess changes in attached gingiva over the long-term period (baseline, 3rd month, 1st and 2nd year) resulting from the application of these techniques.

Methods

The present study was approved by the İstanbul Medipol Universitesi Non-Invasive Clinical Research Ethics Committee (decision no: 79, date: 18.01.2024) for the use and access of human subjects in research and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Fifteen (female) systemically and periodontally healthy patients were enrolled for this study. The general exclusion criteria were as follows: use of antibiotics and/or anti-inflammatory, non-steroidal anti-inflammatory drugs, steroids, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, and hormonal contraceptives within 3 months preceding the study, smokers, surgical periodontal treatment (previous 12 mo.), having less than 15 natural teeth excluding third molar, diabetes, having systemic diseases such as rheumatoid arthritis and cardiovascular disorders. All participants gave oral informed consent.

The treatment of the total of 21 teeth with recession in the lower jaw, absence of bone dehiscence, and attached gingiva ranging from a minimum of 0.5 mm to a maximum of 1.5 mm, was conducted through FGG (FGG group, n=10; mean age of 39.5±5.85) and modified apical positioned flap (MARF group, n=11; mean age of 49.4±9.14) techniques. These procedures were randomly selected (coin toss) and applied by a single researcher.

Surgical methods were performed on a maximum of 2 teeth in the treated areas. At least 2 months before surgical procedures, patients received non-surgical periodontal treatment and oral hygiene practices were demonstrated. After applying surgical treatment to all patients, a simple numerical rating scale was used to assess postoperative comfort and pain approximately 10 days later. Patients were asked to provide a score between 0 (no pain) and 10 (unbearable pain) for the 10 days following the procedure.

Clinical Periodontal Parameters

To determine the periodontal status of the teeth to be treated, pocket depth on probing (PPD) (mm), GR (mm), clinical attachment loss (CAL) (mm), bleeding index on probing (BOP) (%), attached gingival width (AGW) (mm), keratinized tissue width (KTW) (mm) and plaque index (PI) values were recorded by 2 calibrated researchers. AGW was calculated by subtracting the PPD value from the KTW measurement value. Clinical periodontal parameters were recorded before surgical treatment (baseline) and repeated at third months, first and second years after treatment was completed.

Free Gingival Graft Technique

For the FGG surgery, a half-thickness flap was raised and expanded from approximately 0.5 mm coronal to the mucogingival border,

with a horizontal incision in the attached gingiva and two vertical incisions at the mesial and distal ends of the horizontal incision (6). Subsequently, donor tissue (10 mm x 5 mm) was harvested from the palatal region through a rectangular incision of 1-1.5 mm thickness (7). The wound bed that formed on the palate was promptly treated using local hemostatic measures. The tissue obtained from the palate was secured to the recipient site using 5-0 silk sutures, and a periodontal dressing was placed in the wound area. Immediately after the surgery, patients were instructed to avoid hot/acidic foods and beverages.

Modified Apically Repositioned Flap Technique

The MARF technique was implemented according to the previously established protocol by Carnio et al. (6). Following the administration of local anesthesia to the surgical area, a horizontal bevel incision was made from the mucogingival junction towards the attached gingiva, maintaining a distance of 0.5 mm with a no. 15 Bard-Parker scalpel. To avoid vertical relaxing incisions, a horizontal incision was extended mesiodistally to the buccal aspect of adjacent teeth parallel to the mucogingival attachment. The prepared half-thickness flap was secured apically to the periosteum using 5-0 silk sutures. To prevent dead space between the flap and the periosteal bed, gentle finger pressure was applied, and a periodontal dressing was placed in the wound area.

Statistical Analysis

The IBM SPSS Statistics 22 software was used for statistical analyses. The normal distribution of parameters was assessed using the Shapiro-Wilk test. For intergroup comparisons of parameters, the Mann-Whitney U test was utilized. Meanwhile, intragroup comparisons of parameters were conducted using the Friedman test and post hoc Wilcoxon signed-rank test. Significance was evaluated at the $p<0.05$ level.

Results

The study was conducted with a total of 21 cases, ranging in age from 31 to 70. The mean age was 44.45±9.03 years.

Evaluation of periodontal parameters within and between groups is shown in Table 1 and Figure 1. Briefly, there was no statistically significant difference between the treatment groups in terms of PI, PPD, BOP, CAL, GR at baseline, 3 months, first and second years ($p>0.05$). The changes in GR levels at baseline, 3 months, first and second years in both the FGG and MARF groups were statistically significant ($p=0.001$; $p<0.05$). The 3rd month, 1st year and 2nd year AGW level of the FGG group was statistically significantly higher than the MARF group (respectively: $p=0.006$; $p=0.015$; $p=0.007$; $p<0.05$). The changes observed in AGW levels at baseline, 3 months, first and second years in both MARF and FGG groups were statistically significant ($p=0.001$; $p<0.05$). The 3rd month and 2nd year KTW levels of the FGG group were statistically significantly higher than the MARF group (respectively: $p=0.006$; $p=0.004$; $p<0.05$). The changes in KTW levels at baseline, 3 months, 12 months, and 24 months in both the FGG and MARF groups were statistically significant (Table 1).

Table 1. Evaluation of periodontal parameters within and between groups

	FGG	MARF	
	Mean ± SD (median)	Mean ± SD (median)	[1]p-value
PI Baseline	0.68±0.65 (0.5)	1.03±0.7 (1.1)	0.192
3rd month	1±0.55 (1)	1.35±0.78 (1.6)	0.192
1st year	1.08±0.51 (1)	1.35±0.77 (1.5)	0.208
2nd year	0.78±0.72 (0.5)	1.13±0.6 (1.3)	0.220
[2]p	0.174	0.340	
PPD Baseline	1.3±0.4 (1.1)	1.23±0.32 (1.1)	0.806
3rd month	1.28±0.4 (1.1)	1.15±0.32 (1)	0.324
1st year	1.23±0.3 (1)	1.17±0.26 (1)	0.306
2nd year	1.26±0.4 (1.1)	1.15±0.3 (1)	0.320
[2]p	0.102	0.381	
BOP Baseline	12.5±17.68 (0)	7.5±16.87 (0)	0.399
3rd month	15±17.48 (12.5)	15±24.15 (0)	0.734
1st year	1.28±0.4 (1.1)	1.25±0.41 (1)	0.677
2nd year	1.35±0.52 (1)	1.4±0.43 (1.3)	0.425
[2]p	0.903	0.089	
GR Baseline	1.25±0.65 (1.1)	2.05±1.3 (1.5)	0.118
3rd month	1.20±0.66 (1)	1.83±0.95 (1.5)	0.102
1st year	1.08±0.61 (1)	1.70±0.95 (1.5)	0.099
2nd year	1.0±0.59 (1)	1.55±0.9 (1.5)	0.104
[2]p	0.001*	0.001*	
CAL Baseline	2.55±0.65 (2.5)	3.28±1.4 (2.6)	0.322
3rd month	2.48±0.66 (2.4)	2.98±0.82 (2.8)	0.148
1st year	2.35±0.63 (2.4)	2.95±0.79 (2.9)	0.072
2nd year	2.35±0.56 (2.3)	2.95±0.88 (2.9)	0.102
[2]p	0.141	0.314	
AGW Baseline	0.5±0.47 (0.5)	0.6±0.46 (0.8)	0.625
3rd month	4.3±1.16 (4)	2.8±0.79 (3)	0.006*
1st year	4.3±1.25 (5.5)	2.9±0.99 (4)	0.015*
2nd year	4.4±1.65 (5)	2.4±1.17 (3)	0.007*
[2]p	0.001*	0.001*	
KTW Baseline	1.7±0.63 (1.8)	1.6±0.46 (1.8)	0.840
3rd month	5.4±1.35 (5)	3.8±0.79 (4)	0.006*
1st year	5.38±1.72 (6)	3.76±1.15 (5)	0.005*
2nd year	5.27±1.57 (6)	3.06±1.17 (5)	0.004*
[2]p	0.001*	0.001*	

[1]Mann-Whitney U test, [2]Friedman test, *p<0.05
PPD: Pocket depth on probing, GR: Gingival recession, CAL: Clinical attachment loss, BOP: Bleeding index on probing, AGW: Attached gingival width, KTW: Keratinized tissue width, PI: Plaque index, FGG: Free gingival graft, MARF: Modified apically repositioned flap, KTW: Keratinized tissue width

The comparison of changes in the PI, PPD, BOP, GR, CAL, AGW, and KTW parameters of the treatment groups at baseline, 3rd month, 1st year, and 2nd year is presented in Table 2. Briefly, there was a statistically significant difference between the treatment groups in terms of the changes in BOP levels in the 24th month compared to the 12th month (p=0.045; p<0.05). The increase in AGW levels at 3 months, first and second years compared to baseline was statistically significantly higher in the FGG group than in the MARF group (respectively; p=0.004, p=0.012, p=0.004; p<0.05). The increase in KTW levels at 3 months, first and second years compared to baseline was statistically significantly higher in the FGG group than in the MARF group (p=0.006, p=0.005, p=0.004; p<0.05).

Patients reported a higher comfort score within the first 10 days after the FGG procedure compared to the MARF group, and this difference was statistically significant (p=0.011; p<0.05) (Table 3). The changes in representative cases for the FGG and MARF groups at baseline, 3rd month, 1st year, and 2nd year are showed in Figures 2 and 3.

Discussion

The main aim of this study is to evaluate the long-term changes in the formation of attached gingiva and keratinized tissue using the FGG and MARF methods. The width of attached gingiva and the amount of keratinized tissue increased significantly more with the FGG method compared to MARF, both techniques demonstrated a significant increase compared to the baseline.

Insufficient or lacking width of attached gingiva is a significant factor that increases susceptibility to periodontal disease and contributes to GR (1). For this purpose, various surgical techniques are being explored, and the FGG method is often preferred due to its successful outcomes (8-10). However, it has disadvantages such as the need for a donor site, the possibility of postoperative bleeding at the donor site, and color incompatibility at the recipient site (6). In order to eliminate the disadvantages of this technique, the MARF method was described in 1999 (5). With this technique, the need for a second surgical site and the problem of color incompatibility are eliminated.

To the best of our knowledge, there is only one study in the literature comparing MARF and FGG techniques in the long term (1 year) (6). Authors reported that, after 1 year, the FGG technique resulted in a greater amount of keratinized tissue and attached gingiva compared to the MARF technique. However, both methods resulted in a significant increase in AGW and KTW within their respective groups (6). In our results, similar to this study, the amounts of AGW and KTW at all time intervals (3rd month, 1st year, and 2nd year) were greater in the FGG technique compared to MARF. However, the increases in AGW and KTW amounts in both groups were statistically significant. The reason for this difference can be explained by the fact that in the FGG method, the recipient site is prepared more extensively compared to the MARF technique. In the FGG technique, the recipient site is prepared to be wider than the palatal tissue, considering postoperative shrinkage. In the MARF technique, it has been suggested that preparing the recipient site with a width of 4 mm in the apico-coronal direction is sufficient for the formation of an adequate width of attached gingiva after the procedure. Furthermore, Carnio et al. (6) reported that there was no difference between and within the groups in terms of PPD and GR after surgical procedures. In our study, in addition to PPD and GR, we also evaluated PI, BOP, and CAL levels. Similar to the previous study, while there was no statistically significant difference between groups and within groups in PD levels, GR levels showed a significant decrease within both groups (Table 1). The fact that clinical periodontal parameters (PI, BOP, PPD, GR,

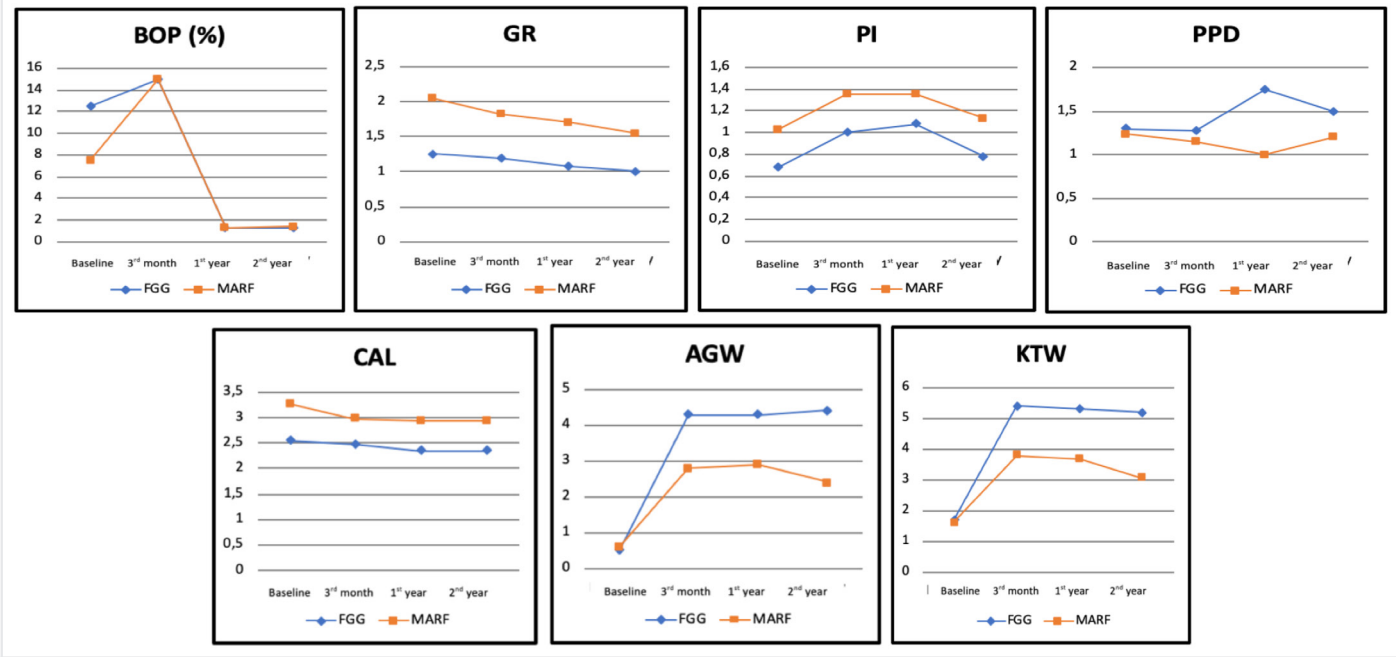

Figure 1. Evaluation of periodontal parameters within and between groups

Table 2. The comparison of changes in the PI, PPD, BOP, GR, CAL, AGW, and KTW parameters of the treatment groups at baseline, 3 months, 1 year, and 2 years

	FGG	MARF	
	Mean ± SD (median)	Mean ±SD (median)	p-value
PI 0-3	0.33±0.46 (0.1)	0.33±0.58 (0.4)	0.939
PI 0-12	0.4±0.49 (0.3)	0.33±0.66 (0.4)	0.819
PI 0-24	0.1±0.52 (0)	0.1±0.39 (0)	1.000
PI 3-12	0.08±0.17 (0)	0±0.12 (0)	0.304
PI 3-24	-0.23±0.64 (-0.1)	-0.23±0.65 (-0.4)	0.819
PI 12-24	-0.3±0.67 (-0.3)	-0.23±0.7 (-0.4)	0.939
PPD 0-3	-0.03±0.28 (0)	-0.08±0.17 (0)	0.233
PPD 0-12	-0.03±0.28 (0)	0.03±0.38 (0)	0.544
PPD 0-24	0.05±0.39 (0)	0.18±0.43 (0)	0.442
PPD 3-12	0±0 (0)	0.1±0.32 (0)	0.317
PPD 3-24	0.08±0.47 (0)	0.25±0.42 (0.1)	0.258
PPD 12-24	0.08±0.47 (0)	0.15±0.24 (0.1)	0.331
BOP 0-3	2.5±18.45 (0)	7.5±12.08 (0)	0.543
BOP 0-12	5±19.72 (0)	2.5±21.89 (0)	0.868
BOP 0-24	12.5±21.25 (12.5)	0±23.57 (0)	0.273
BOP 3-12	2.5±7.91 (0)	-5±25.82 (0)	0.584
BOP 3-24	10±12.91 (0)	-7.5±26.48 (0)	0.060
BOP 12-24	7.5±12.08 (0)	-2.5±7.91 (0)	0.045*
GR 0-3	-0.05±0.16 (0)	-0.23±0.43 (0)	0.486
GR 0-12	-0.18±0.21 (-0.1)	-0.35±0.41 (-0.3)	0.444
GR 0-24	-0.25±0.2 (-0.3)	-0.5±0.5 (-0.4)	0.311
GR 3-12	-0.13±0.18 (0)	-0.13±0.18 (0)	1.000
GR 3-24	-0.2±0.2 (-0.3)	-0.28±0.32 (-0.3)	0.748
GR 12-24	-0.08±0.17 (0)	-0.15±0.24 (0)	0.516
CAL 0-3	-0.08±0.43 (0)	-0.3±0.69 (0)	0.549
CAL 0-12	-0.2±0.4 (-0.1)	-0.33±0.77 (-0.1)	0.875
CAL 0-24	-0.2±0.47 (-0.3)	-0.33±0.76 (-0.3)	0.908
CAL 3-12	-0.13±0.18 (0)	-0.03±0.32 (0)	0.518
CAL 3-24	-0.13±0.57 (-0.1)	-0.03±0.42 (-0.1)	0.641
CAL 12-24	0±0.54 (0)	0±0.17 (0)	0.679
AGW 0-3	3.8±1.06 (3.8)	2.2±0.89 (2.5)	0.004*
AGW 0-12	4.1±1.21 (5.3)	3.1±1.03 (3.5)	0.012*
AGW 0-24	4.2±1.47 (4.8)	2.8±1.16 (3)	0.004*
AGW 3-12	1±0.47 (1)	1.1±0.57 (1)	0.654
AGW 3-24	1.1±0.88 (1)	0.6±0.97 (0,5)	0.249
AGW 12-24	0.1±0.88 (0)	-0.5±0.53 (-0,5)	0.104
KTW 0-3	3.4±1.09 (3.3)	2.2±0.89 (2.5)	0.006*
KTW 0-12	4.3±1.6 (4.8)	3.2±1.24 (3.8)	0.072
KTW 0-24	4.7±1.31 (4.8)	3±1.22 (3)	0.005*
KTW 3-12	1.1±0.74 (1)	1.2±0.79 (1)	0.744
KTW 3-24	1.3±0.95 (1)	0.8±1.03 (1)	0.324
KTW 12-24	0.2±0.92 (0.5)	-0.4±0.52 (0)	0.107

Mann-Whitney U test, *p<0.05
PPD: Pocket depth on probing, GR: Gingival recession, CAL: Clinical attachment loss, BOP: Bleeding index on probing, AGW: Attached gingival width, KTW: Keratinized tissue width, PI: Plaque index

Table 3. Discomfort score within the first 10 days after surgical procedures

	Discomfort score
	Mean ± SD (median)
FGG	7.0±0.82 (7)
MARF	5.2±1.55 (5)
p-value	0.011*

Mann-Whitney U test, FGG: Free gingival graft, MARF: Modified apically repositioned flap, SD: Standard deviation, *p<0.05

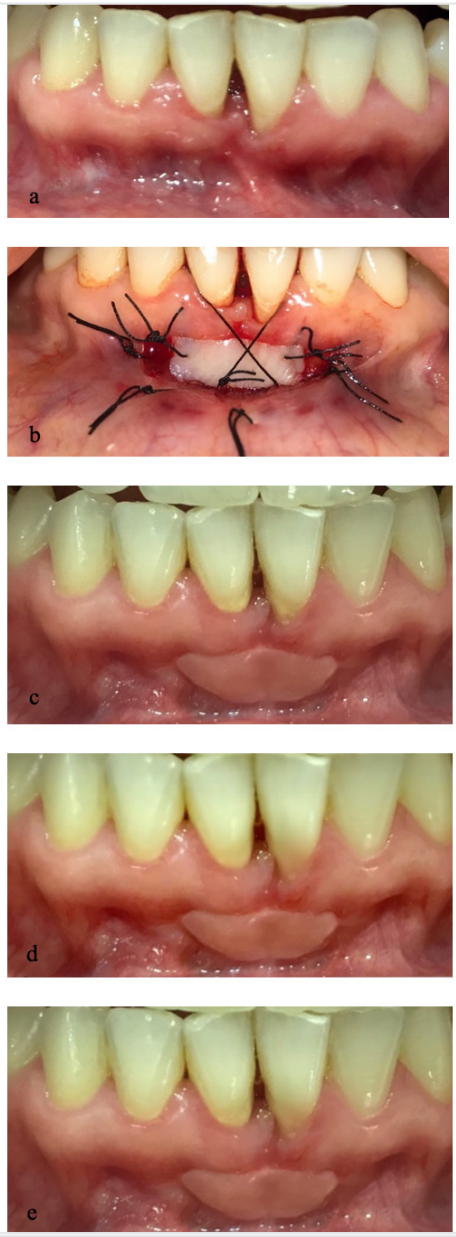

Figure 2. The changes in representative cases for the FGG: at baseline (a), after FGG procedure (b), at 3rd month (c), at 1st year (d), at 2nd year (e)

FGG: Free gingival graft

CAL) did not differ between the two groups at all time intervals showed that the MARF technique supported periodontal health at least as much as the FGG technique (Tables 1, 2).

Carnio et al. (11) reported an average increase of 3.6 mm in keratinized tissue and 2.21 mm in attached gingiva after following 21 teeth treated with the MARF technique for 1 to 11 years. In another study by Carnio et al. (12), where they evaluated the MARF technique over a period of 4 to 16 years, an average gain of 2.06 mm in keratinized tissue and an increase of 2.15 mm in attached gingiva width were reported. Moreover, both studies reported no significant differences in GR and PPD levels after the procedures (11,12). In a study with a 13-year case follow-up, it was reported that there was an average increase of 2.5 mm in attached gingiva and a 3 mm increase in keratinized gingival width compared to the baseline (13). In our study, an average increase of 2.2 mm in attached gingiva width was observed at the end of the third month compared to the baseline, 2.3 mm at the end of the first year and 1.8 mm at the end of the second year. The width of keratinized tissue increased by an average of 2.2 mm at the end of the third month compared to the baseline, 2.1 mm at the end of the first year, and 1.46 mm at the end of the second year. The results of our study are compatible with other clinical studies and case reports aiming to increase the amount of attached gingiva with the MARF technique (6,11-14).

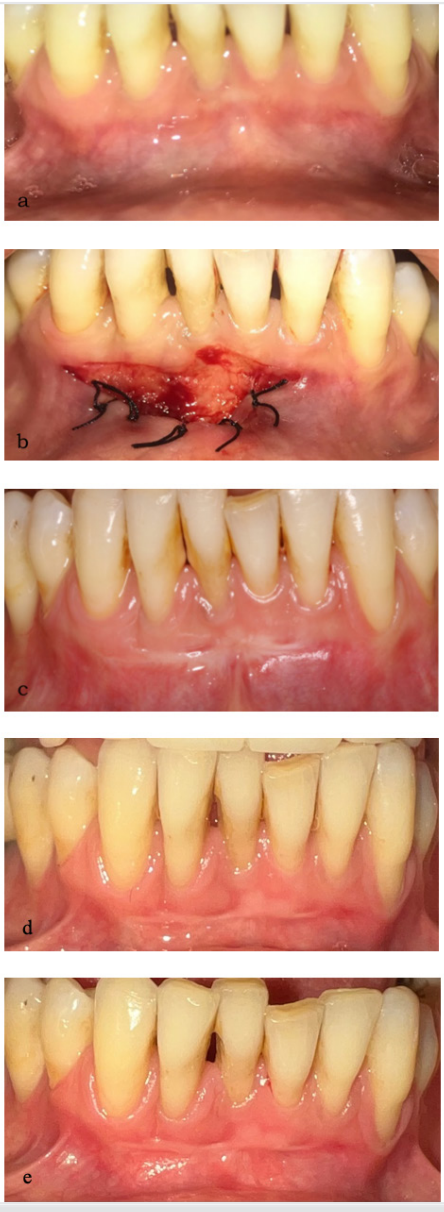

Figure 3. The changes in representative cases for the MARF: at baseline (a), after FGG procedure (b), at 3rd month (c), at 1st year (d), at 2nd year (e)

FGG: Free gingival graft, MARF: Modified apically repositioned flap

In our study, post-op comfort was also evaluated and scored by the patients with a simple numerical scale (0-10). As a result, patients stated that MARF technique was more comfortable and caused less postoperative pain (Table 3). Parallel to our study, a study evaluating post-procedure comfort as more or less found the MARF method to be more comfortable.[6] These results suggest that the MARF method will be a more preferred method by patients in the future.

Study Limitations

The main limitation of this clinical study comparing MARF and FGG methods in the long term is the small sample size. Additionally, the measurement of keratinized and AGW using only a visual method can be identified as another limitation. In future studies, it is considered essential to increase the sample size, record the procedure duration and the correlation between duration and patient comfort should not be overlooked.

Conclusion

Both techniques have been shown to result in a statistically significant increase in the width of keratinized tissue and the amount of attached gingiva in the long term. However, MARF technique has many advantages, including not requiring a second surgical site, being technically simpler, providing more postoperative comfort for patients and achieving better tissue color match. Although it is not applicable for root coverage like FGG, the advantages of the MARF technique lead to the consideration of it as an alternative to FGG.

Ethics

Ethics Committee Approval: The present study was approved by the İstanbul Medipol University Non-Invasive Clinical Research Ethics Committee (decision no: 79, date: 18.01.2024) for the use and access of human subjects in research and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Informed Consent: All participants gave oral informed consent.

Authorship Contributions

Surgical and Medical Practices: M.Y., N.B., H.T., Concept: E.T., A.Ç., Design: M.Y., E.T., Data Collection or Processing: H.T.,

Analysis or Interpretation: N.B., Literature Search: M.Y., E.T., A.Ç., Writing: M.Y.

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Original Article

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Investigation of the Effect of Sodium Hypochlorite, EDTA, Propolis, Boric Acid and Citric Acid Irrigation Solutions on Push-out Bond Strengths of Root Canal Sealers

Sodyum Hipoklorit, EDTA, Propolis, Borik Asit ve Sitrik Asit Yıkama Solüsyonlarının Kök Kanal Patlarındaki Bağlanma Dayanımları Üzerine Etkisinin İncelenmesi

Damla İLKGELEN, Emre İRİBOZ

Marmara University Faculty of Dentistry, Department of Endodontics, İstanbul, Türkiye

ABSTRACT

Objective: The aim of this study is to investigate the effect of different root canal irrigation solutions, including propolis and boric acid (BA), on the bonding strengths of epoxy-resin-based AH Plus and bioceramic-based Bioserra root canal sealers on root dentin.

Methods: One hundred extracted teeth with single root-single canal were shaped and divided into 2 groups according to filling material (AH Plus and Bioserra) and each group was divided into 5 subgroups according to irrigation solution (5.25% NaOCl, 17% EDTA, 10% citric acid, 10% BA, 10% propolis). The teeth of which canals were filled were incubated at 37 °C for one week, then embedded in acrylic resin. Horizontal sections with thickness of 2 mm were taken from the middle level of the root of each tooth and pressing force was applied in vertical direction until a break occurred in the connection, POBS (push-out bond strength) values were calculated. Statistical analysis was performed using Kruskal-Wallis H and Mann-Whitney U tests. Finally samples were examined with binocular microscope.

Results: Regardless of the sealer used, mean POBS values of 5.25% NaOCl solution groups were found significantly higher than others ($p<0.05$). Also regardless of the irrigation solution used, mean POBS values of AH Plus groups were significantly higher than Bioserra groups ($p<0.05$). Among the experimental groups that

ÖZ

Amaç: Bu çalışmanın amacı, propolis ve borik asit (BA) dahil olmak üzere farklı kanal yıkama solüsyonlarının epoksi-rezin bazlı AH Plus ve biyoseramik bazlı Bioserra kanal patlarının kök dentini üzerindeki bağlanma dayanımlarına etkisini araştırmaktır.

Yöntemler: Tek kök-tek kanallı 100 adet çekilmiş insan dişi döner aletle şekillendirildikten sonra kanal dolum malzemesine (AH Plus ve Bioserra) göre 2 gruba ayrılmış ve her grup yıkama solüsyonuna (%5,25 NaOCl, %17 EDTA, %10 sitrik asit, %10 BA, %10 propolis) göre 10 örnek içeren 5 alt gruba ayrılmıştır. Kanalları doldurulan dişler bir hafta 37 °C'de etüvlendikten sonra akrilik reçineye gömülmüştür. Her bir dişin kökünün orta hizasından 2 mm kalınlığında horizontal kesitler alınmış ve bağlantıda kopma oluşana kadar dikey yönde basma kuvveti uygulanmıştır, bağlanma dayanım değerleri hesaplanmıştır. Ayrıca numuneler binoküler mikroskopla incelenmiştir. Değerlerin istatistiksel analizleri Kruskal-Wallis H ve Mann-Whitney U testleri kullanılarak yapılmıştır.

Bulgular: Kullanılan kanal patından bağımsız olarak, %5,25 NaOCl yıkama solüsyonu gruplarının ortalama bağlanma dayanım değerleri diğerlerine göre anlamlı derecede yüksek bulunmuştur ($p<0,05$). Ayrıca kullanılan yıkama solüsyonundan bağımsız olarak, AH Plus kullanılan grupların bağlanma dayanımlarının ortalaması, Bioserra kullanılan gruplardan anlamlı derecede yüksektir ($p<0,05$).

Address for Correspondence: Damla İlkgelen, Marmara University Faculty of Dentistry, Department of Endodontics, İstanbul, Türkiye
E-mail: ilkgelen@hotmail.com **ORCID ID:** orcid.org/0000-0003-1619-173X

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ABSTRACT

used Bioserra, 5.25% NaOCl irrigation group had significantly higher POBS value than other solution groups. Meanwhile, there was no difference between POBS values of irrigation solution groups when AH Plus was used. In addition, in 17% EDTA and 10% citric acid groups, the mean POBS values of the samples using AH Plus were found significantly higher according to Bioserra samples ($p<0.05$). When mode of failures were examined; cohesive failure was mostly observed in NaOCl-AH Plus group, adhesive failure was mostly in BA-AH Plus group, mixed failure was mostly in Propolis-Bioserra group.

Conclusion: The bonding strength is significantly affected by the sealer used and the sealer/irrigation solution interaction. The bonding strength of AH Plus is superior to Bioserra. If 17% EDTA or 10% citric acid is to be used as irrigation solution, then AH Plus should be preferred instead of Bioserra sealer.

Keywords: Push-out bond strength test, propolis, boric acid, root canal sealer, root canal irrigation

ÖZ

Bioserra'yı kanal dolum patı olarak kullanan deney grupları arasında %5,25 NaOCl ile irrigasyon yapılan grup, diğer yıkama solüsyon gruplarına göre anlamlı derecede daha yüksek bağlanma dayanımı değerine sahiptir. Bu arada kök kanal patı olarak AH Plus kullanıldığında yıkama solüsyon gruplarının bağlanma dayanım değerleri arasında fark görülmemiştir. Ayrıca %17 EDTA ve %10 sitrik asit yıkama solüsyonu gruplarında, kanal patı olarak AH Plus kullanılan numunelerin bağlanma dayanımlarının ortalaması, Bioserra kullanılan numunelerin ortalamasına göre anlamlı olarak daha yüksek bulunmuştur ($p<0,05$). Bağlantı başarısızlıkları incelendiğinde; koheziv kopma en sık NaOCl-AH Plus grubunda, adeziv kopma en sık BA-AH Plus grubunda, karışık kopma en sık Propolis-Bioserra grubunda görülmüştür.

Sonuç: Bağlanma dayanım gücü; kullanılan kök kanal patından ve kanal patı/yıkama solüsyonu etkileşiminden önemli ölçüde etkilenmektedir. AH Plus'ın bağlanma dayanımı, Bioserra'dan üstündür. İrrigasyon solüsyonu olarak %17'lik EDTA veya %10'luk sitrik asit kullanılacak ise ardından dolumda Bioserra yerine AH Plus patı tercih edilmelidir.

Anahtar Sözcükler: Basma dayanım testi, propolis, borik asit, kök kanal patı, kök kanal irrigasyonu

Introduction

The aim of a successful root canal treatment is to clean and shape the root canals and then fill them in a three-dimensional hermetic way (1). Gutta percha, which is a root canal filling material, should be used together with a sealer because it does not have the ability to bind to the root canal walls alone (2). The task of the sealer is to ensure the adhesion of the gutta percha to the root canal walls and also to fill the gaps between the master apical file and the other gutta percha points during the lateral condensation technique (3). An effective irrigation can ensure that even the lateral canals, isthmus, apical deltas that cannot be reached by the gutta percha are filled with root canal sealer.

There are some agents such as Sodium hypochlorite (NaOCl), EDTA, citric acid, which have been used for a long time as an irrigation solution in endodontic treatments and have proven many benefits. NaOCl exhibits strong antibacterial properties, dissolves organic substances and removes necrotic tissue. Some clinicians work by filling the pulp chamber with NaOCl during the entire root canal preparation process to maximize the lubrication, antibiofilm and proteolytic effects (4). EDTA is a chelating agent used to dissolve inorganic components. It acts on calcium ions in the content of hydroxyapatite, the main inorganic compound of dentin, and accelerates the removal of smear layer by demineralizing its' inorganic components (5). Qian et al. (6) concluded in a study that EDTA and citric acid led to negative features such as intertubular and peritubular dentin erosion. Although there are studies like these in the literature, these two solutions are the most commonly used irrigation solutions for complete removal of the smear layer; it is recommended to use citric acid at a concentration of 10% and EDTA at a concentration of 17% (7). The use of boric acid (BA), which has antifungal, antiseptic, strong antibacterial properties and has proven biocompatibility with tissue, as an endodontic irrigation agent is also on the agenda and represents an ideal irrigant (8,9). A limited number of studies have been conducted on the use of BA in dentistry (10). Apart from these, when we examine the literature, we see that propolis, which is a natural and easy-to-obtain material that is already used in different areas of daily life and has many different biological properties, is also used in dentistry and researches on it are increasing (11).

AH Plus (Dentsply Sirona, Germany), one of the root canal sealers to be used, is an epoxy-resin based root canal filling material whose superior physical properties have been demonstrated many times (12). The other root canal sealer Bioserra (Meta Biomed Co. Ltd., Korea) is one of the calcium-silicate-based materials of which use has increased especially in recent years due to its' superior biocompatibility and sealing properties.

The interaction of all these irrigation solutions with the root canal filling materials is inevitable. Irrigation solutions should not negatively affect the bonding properties of the root canal sealer to the root dentin. For this purpose, it is important to know the effect of irrigation solutions on the push-out bond strengths of root canal sealers. "Push-out bond strength", also called dislodgement resistance, is considered as the determining factor in evaluating the connection of a root canal sealer to the root canal dentin and core material (13). Instead of shear and tensile tests, push-out test has been advocated as a more appropriate test to evaluate the bonding resistance of intra-canal filling materials (14).

In our research, we aim to make useful contributions to the endodontic literature by keeping the diversity range of the

materials wide. In this study, it was aimed to investigate the effects of some root canal irrigaiton solutions, including propolis and BA, on the bond strength of epoxy resin-based AH Plus and bioceramic-based Bioserra sealers on root dentin.

Methods

In our study, 100 human teeth with single root-single canal extracted for caries, orthodontic or periodontal reasons and did not have any cracks/fractures on the root surface and also did not have any anomalies in the root canal morphologies were used. Because of human tissues were utilized for this *in vitro* study, the ethics committee approval of this study was obtained from the Clinical Research Ethics Committee of the Faculty of Dentistry of Marmara University under decision number 2022/91, dated September 29, 2022. All procedures of the study were carried out by the same operator. Organic and inorganic tissue residues on the root surfaces of the teeth that complied with the criteria and were included in the study were cleaned with the help of periodontal curettes and cavitron. Then, all the teeth were kept in 10% NaOCl for 1 and a half hours and disinfected; at the end of this time, the teeth were washed under running water and kept in 10% formalin solution until the experiment started. The crowns of the teeth were removed with a diamond fissure burr. The working length was determined with a 15 K-file to be 1 mm behind the root canal length and the root canals were shaped with a Protaper Next X3 (Dentsply Maillefer, Ballaigues, Switzerland) rotary file. One hundred teeth with shaped root canals were randomly distributed to 10 experimental groups containing 10 samples each. A total of 5 mL of irrigation solution was applied to each tooth in the experimental groups with an endodontic irrigation needle for 4 minutes. Then, the root canals were dried with paper-points and root canals were filled. According to this:

- Group 1A: irrigation with 5.25% NaOCl, cold lateral condensation with AH Plus sealer (n=10).

- Group 1B: irrigation with 5.25% NaOCl, single cone technique with Bioserra sealer (n=10).

- Group 2A: irrigation with 17% EDTA, cold lateral condensation with AH Plus sealer (n=10).

- Group 2B: irrigation with 17% EDTA, single cone technique with Bioserra sealer (n=10).

- Group 3A: irrigation with 10% citric acid, cold lateral condensation with AH Plus sealer (n=10).

- Group 3B: irrigation with 10% citric acid, single cone technique with Bioserra sealer (n=10).

- Group 4A: irrigation with 10% BA, cold lateral condensation with AH Plus sealer (n=10).

- Group 4B: irrigation with 10% BA, single cone technique with Bioserra sealer (n=10).

- Group 5A: irrigation with 10% propolis, cold lateral condensation with AH Plus sealer (n=10).

- Group 5B: irrigation with 10% propolis, single cone technique with Bioserra sealer (n=10).

After the root canal fillings completed, the orifices of the root canals were filled with temporary filling material. The specimens were kept at 37 °C temperature in the incubator for 1 week for the sealer to set completely. Then teeth were embedded in cold acrylic resin blocks vertically. Horizontal sections with thickness of 2 mm were taken from the middle level of the each tooth's root under water cooling with a low-speed-saw (IsoMet 1000, Buehler, Illinois, USA).

Specimens were placed on a metal plate with a hole in the middle. A universal testing machine (Shimadzu Corporation, Kyoto, Japan) was used in the push-out bond strength (POBS) test to provide a break in the connection (debonding) of the samples. In order to apply this test to root canals of different diameters, metal pins with diameters of 0.5, 0.7 and 1 mm were designed. The diameters were measured and checked with a digital caliper.

Vertical load was applied in apico-coronal direction with the universal testing machine until the bond failure occured. The maximum force measured at the moment of bond failure was recorded in Newtons (N) for each sample in the Trapezium X software (Shimadzu Corporation, Kyoto, Japan) on the computer connected to the universal testing machine. After it, the bond strength value in Megapascal of each sample was calculated.

The bond strength value was calculated according to the following formula:

$$\text{Push} - \text{out Bond Strength} = \frac{F}{2\pi(r_1 + r_2)h}$$

F: the maximum force measured at the moment of bond failure (N); π: The number of Pi = 3,14; r_1: the apical radius of the root canal section (mm); r_2: the coronal radius of the root canal section (mm); h: thickness of sample =2 mm.

After the POBS test, the root canals of the specimens were examined and photographed with a binocular microscope (Leica, Danaher Corporation, Germany) at 4x magnification and the types of failures in each sample were detected and recorded. The mode of failures were classified into three groups as adhesive failure (no material left on the canal wall), mixed failure (partial material left on the canal wall) and cohesive failure (material left along the entire canal wall) (Figure 1).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 23.0 software (IBM Corporation, Armonk, New York, USA) and the conformity of the data to normal distribution was checked by Kolmogorov-Smirnov test. At the irrigation solution groups without normal distribution, the Kruskal-Wallis test was used to compare the mean of the groups and in the root canal sealer groups without normal distribution, the Mann-Whitney U test was used to compare the mean. When the p-value was below 0.05, it was accepted that there was statistically significant difference.

Results

All specimens had measurable adhesion to the root dentin and no premature failure occurred. The mean ± standard deviation, median, minimum and maximum push-out bond strength values of all experimental groups are shown in Table 1. It was accepted that there was a statistically significant difference if the results were p<0.05. In the 17% EDTA and 10% citric acid irrigation solution groups; the samples using AH Plus as root canal sealer produced significantly higher POBS values than the samples using Bioserra (p<0.05). There was no significant difference in the push-out bond strength values of other root canal irrigation solution groups in terms of any sealer.

There was no statistically significant difference between the mean POBS values compared to the irrigation solution groups using AH Plus root canal sealer (p>0.05). Besides that; in the groups using Bioserra root canal sealer, when it was compared according to the irrigation solutions, the group irrigated with 5.25% NaOCl revealed significantly the highest dislodgement resistance (p<0.05) (Table 2).

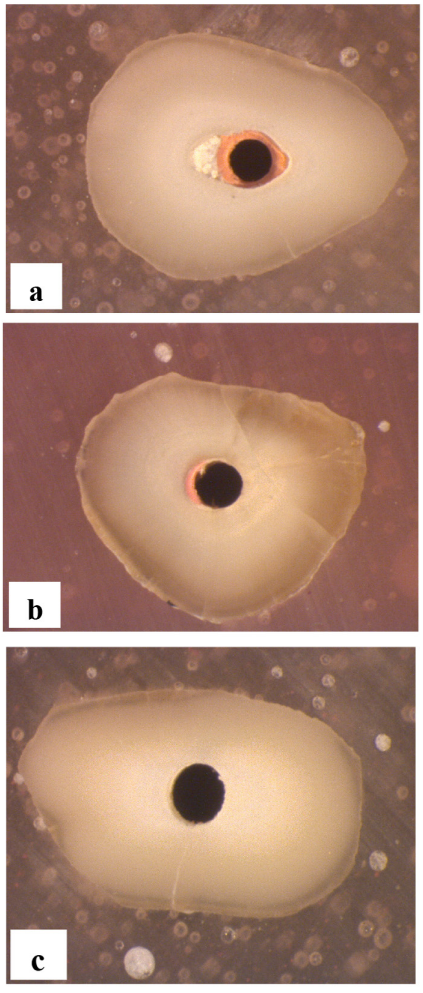

Figure 1. Images obtained by binocular microscope at 4x magnification for observation of the mode of failure types in samples; a: Cohesive failure, b: Mixed failure, c: Adhesive failur

When the groups were compared according to just the irrigation solutions regardless of the root canal sealer used, the mean push-out bond strength values were found to be statistically significantly higher in the groups where 5.25% NaOCl was used as irrigation solution compared to other solution groups (p<0.05) (Table 3). Also, when it was compared according to the root canal sealer regardless of the irrigation solution, AH Plus groups produced significantly higher POBS values than those of the groups using Bioserra sealer (p<0.05) (Table 4).

According to the samples examined by binocular microscope, the results of the rates of mode of failures in the experimental groups are shown in Figure 2. Cohesive failure type was most often observed in NaOCl-AH Plus group, adhesive failure was most often observed in BA-AH Plus group and in the Propolis-Bioserra group, it was most often observed mixed failure type (Figure 2).

Table 1. Mean ± standard deviation, median, minimum and maximum push-out bond strength values of all experimental groups in MPa

Groups	Mean ± SD (MPa)	Median (minimum-maximum)
5.25% NaOCl		
1A (AH Plus)	10.4±5.5	9.7 (5.0-21.9)
1B (Bioserra)	10.5±4.9	10.7 (4.7-20.6)
17% EDTA		
2A (AH Plus)	7.2±2.6*	6.6 (4.2-12.6)
2B (Bioserra)	3.9±2.6	3.5 (1.6-10.8)
10% citric acid		
3A (AH Plus)	7.9±3.6*	8.9 (1.9-12.2)
3B (Bioserra)	4.5±2.6	4.2 (1.6-10.8)
10% boric acid		
4A (AH Plus)	7.3±3.6	6.7 (3.0-12.9)
4B (Bioserra)	6.9±3.5	6.9 (2.1-2.8)
10% propolis		
5A (AH Plus)	6.8±1.9	6.9 (3.6-9.4)
5B (Bioserra)	5.3±3.3	3.9 (2.4-12.6)

SD: Standard deviation, MPa: Megapascal, *p<0.05

Table 2. The mean POBS values of the experimental groups filled with Bioserra according to the irrigation solutions

Irrigation solution-Bioserra	Mean ± SD (MPa)
NaOCl (n=10)	10.5±4.9*
EDTA (n=10)	3.9±2.6
Citric acid (n=10)	4.5±2.6
Boric acid (n=10)	6.9±3.5
Propolis (n=10)	5.3±3.3
Total (n=50)	6.2±4.1

SD: Standard deviation, POBS: Push-out bond strength, *p<0.05

Table 3. The mean POBS values according to the root canal irrigation solution, regardless of the sealer

Irrigation solution	Mean ± SD (MPa)
NaOCl (n=20)	10.4±5.1*
EDTA (n=20)	5.6±3.1
Citric acid (n=20)	6.2±3.6
Boric acid (n=20)	7.1±3.4
Propolis (n=20)	6.1±2.7
Total (n=100)	7.1±4.0

SD: Standard deviation, POBS: Push-out bond strength, NaOCl: Sodium hypochlorite, EDTA: Ethylenediamine tetraacetic acid, *p<0.05

Table 4. The mean POBS values according to the root canal sealer, regardless of the irrigation solution

Root canal sealer	Mean ± SD (MPa)
AH Plus (n=50)	7.9±3.7*
Bioserra (n=50)	6.2±4.1
Total (n=100)	7.1±4.0

SD: Standard deviation, POBS: Push-out bond strength, MPa: Megapascal *p<0.05

Discussion

In an ideal endodontic treatment, it is very important to fill the root canals three-dimensionally and hermetically to prevent reinfection after they are completely cleared and cleaned of pathogens, vital or necrotic tissues (15). In a review, root canals prepared with both current nickel-titanium rotary tool systems and traditional stainless steel hand files were examined as two separate groups and it was found that about half of the root canal walls in both groups were left unprepared (16). Due to this condition, microorganisms in the canal system may not be completely cleaned and a growth medium for bacterias may form on them. In order to completely clean the root canals, irrigation protocol is very important and various irrigation solutions are used to support the mechanical preparation and to open the dentin tubules on root surface. However, it has also been shown that the use of antimicrobial irrigation solutions significantly reduces bacteria, but cannot completely eliminate them and achieve a sterile root canal system (17).

A study has been conducted showing that interfacial stress distributions and POBS value measurements are mostly unaffected when the ratio of the tip diameter to the diameter of the sample is less than 0.85 and the ratio of the section thickness of the sample to the diameter of the sample is greater than 0.6 (18). We have prepared sample groups and experimental materials by paying attention to these criteria in our own study.

We have done research on which root canal obturation technique we should choose for root canal filling of sample groups and decided that we should use cold obturation techniques and stay away from warm gutta percha obturation techniques when using bioceramic-based sealer Bioserra. Because studies have shown

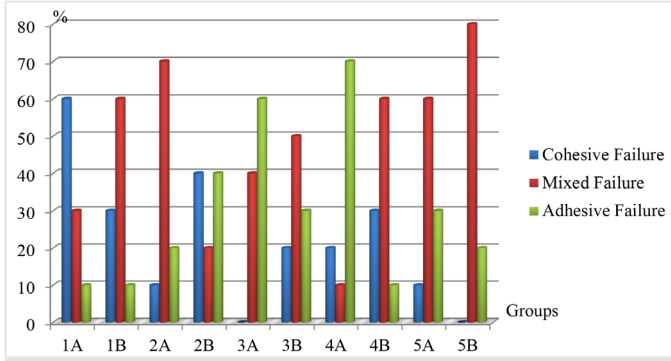

Figure 2. Distribution of mode of failure types in all experimental groups (in %)

that heat can alter the physical properties of bioceramic-based sealers, especially their viscosity and hardening time (19,20). This may affect the quality of the root canal filling.

Donnermeyer et al. (21) found that AH Plus revealed significantly higher POBS values than other root canal sealers used, and also found that EDTA irrigation solution positively affected POBS values of AH Plus root canal sealer. These results are parallel to the results we have indicated in our study. In addition, it was found that EDTA had a negative effect on the POBS values of the bioceramic-based root canal sealer used in the study (21). Similar to this; in our study, when we used EDTA solution and bioceramic-based sealer Bioserra together, we found the lowest POBS values.

They conducted another similar study in which they compared the bond strength resistance of 3 different calcium-silicate-based sealer with that of epoxy resin-based AH Plus, and as a result, AH Plus sealer had higher POBS values than all other calcium-silicate-based root canal sealer groups (22). Our study also found that the mean POBS values of the groups using AH Plus root canal sealer were significantly higher than the groups using calcium-silicate-based Bioserra sealer, regardless of the irrigation solution used. These findings correlate with their results. This result also confirms the findings of Donnermeyer et al. (23) in another study conducted in 2018 that the retreatment of the root canal fillings using calcium-silicate-based sealer led to more effective and efficient clinical results than the fillings using epoxy-resin-based sealer. On the contrary, in a study, the effect of warm gutta-percha obturation techniques on the bond strength of bioceramic-based sealer was investigated and, contrary to our results, they found significantly higher POBS values in calcium-silicate-based bioceramic root canal sealer than epoxy-resin-based AH Plus (24). In this research, Dewi et al. (24) defined a control group in which AH Plus, which had a lot of research on it and almost all of its' properties were known today, was applied by warm gutta-percha obturation techniques, and compared the bond strength values of the relevant sealers. The reason for this result, which contradicts our study, may be that heat application accelerates the chemical reaction of epoxy-resin-based sealer AH Plus and therefore the sealer cannot flow into complex anatomies such as the dentin tubules of the root canal system and cannot fill the gaps adequately, ultimately leading to lower bonding strength (25).

In a study conducted with a calcium-silicate-containing sealer known as "BioRoot RCS", the POBS values of this sealer were compared with those of AH Plus. As a result, the bonding strength of BioRoot RCS was found to be higher than AH Plus (26). Also in a study conducted by Ballal et al. (27); among the samples obturated with AH Plus and gutta-percha, the 5.25% NaOCl irrigation group had the lowest POBS values. The results of these two aforementioned studies contradicted the results of our study, in which we observed higher POBS values in the AH Plus sealer compared to the bioceramic-based one. The reasons for these differences may be factors such as different volumes of NaOCl solution used, the taper angle created as a result of the mechanical preparation of the root canal, and whether or not core material is used together with the root canal sealer during the endodontic obturation procedure.

When determining root canal irrigation solution groups, we also included citric acid, which is one of the demineralization agents, in our study. When we conducted a literature research about what percentage of citric acid we would use; we saw study results showing that 10% citric acid was a sufficient and effective material for removing the smear layer on the dentin tubules and surface (28), and a 10% concentration of citric acid could provide demineralization more effectively than a 1% concentration (29). For these reasons, we determined the concentration of citric acid to be used as irrigation solution in our study as 10%.

In our study, when evaluated regardless of the root canal sealer, the mean POBS values of the group which was irrigated with NaOCl solution were found to be significantly higher compared to other solution groups. At one study, the POBS values of bioceramic-based root canal sealers after irrigation with 2.5-3% NaOCl and 17% EDTA were compared in an experiment (30). As a result of this study, it was concluded that the NaOCl group, which is an alkaline solution, showed higher POBS values compared to the acidic EDTA solution group. One reason for this may be the composition of bioceramic-based root canal sealers containing calcium silicate, which are sensitive to low pH and the mechanism of hardening in an acidic environment can be adversely affected (30).

After chemomechanical preparation in the root canal system, the residual EDTA remaining in the root canal continues to chelate calcium ions released during hydration of bioceramic-based root canal sealers and affects the precipitation process, so reduces the adhesion of the root canal sealer to the root dentin walls (31). According to the findings we obtained from our study, in the study groups using 17% EDTA irrigation solution, average of POBS values was found to be significantly higher when AH Plus was used as root canal sealer compared to using bioceramic-based Bioserra sealer. This result can be explained by the result found by Lee et al. (31) in their study.

During the use of NaOCl in root canal treatment, many mishaps and accidents may occur, such as overflow from the apical foramen due to large apical foramen structure, root resorption and incorrect placement of the needle and as a result, periapical irritation and inflammation; injection instead of anesthesia by accident; occurence of allergic reaction in the patient to the solution; splashing into the patient's or dentist's eye, bleaching of the clothes when dripping on the patient and physician (32-34). For these reasons, the search for a different solution that can replace NaOCl with the same properties and with less risk of complications should be one of the main research topics of today's scientists. But it is an easy fact to predict that NaOCl has been a cornerstone among root canal irrigation solutions throughout the history of endodontics and will be for quite a while longer. If we compare the numerous advantages of NaOCl proven in studies conducted from the past to the present with its' disadvantages, it is preferable to continue using NaOCl in root canal treatment by taking the necessary precautions (for example; the use of endodontic irrigation needle tips in irrigation procedure, the use of protective glasses in the patient and the physician, the use of protective clothes by the physician) (35). Already in our study, NaOCl supports this preference by proving its' superiority once again with the superior POBS values in terms of bonding strength with root canal sealers in different groups. The application of the gel form of NaOCl instead of the solution form is an alternative that can reduce the risk of overflow from the apical of the root and thus reduce the occurrence of postoperative pain (36-38). But at this point, it is also a matter of debate whether the gel form of NaOCl, which has a more solid consistency than the solution, can penetrate into the dentin tubules at a sufficient depth during root canal irrigation (39).

Herbal ingredients have advantages such as having minimal side effects compared to routine irrigation materials in endodontics in general, being better tolerated by patients and being able to be renewed naturally (40). Propolis, which is rich in flavonoids and its' ethanolic extract has different biological properties such as antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, local anesthetic, is applied as an intracanal medicament and can be considered as a preferred material in the irrigation solution group, as Castaldo and Capasso (11) also suggested in their study on propolis. When we examined the 10% propolis solution as a root canal irrigation solution option, in the average POBS values that we found in our study, there was no significant difference that would provide an advantage between the other groups, and there was also no aspect that negatively affected the bonding of root dentin with the sealer. Accordingly, 10% propolis can also be considered as a material that can be used in root canal irrigation procedures in endodontics, just like other more commonly used irrigation solutions in our study.

Study Limitations

This study was prepared with *in vitro* design. *In vivo* conditions such as sudden intraoral temperature changes could not be provided, and the effects of these conditions on the properties of root canal sealers were not included. However, this study is first in which the effect of propolis on POBS was compared with this combination of irrigation solutions and sealer groups.

Conclusion

The bonding strength of AH Plus root canal sealer is superior to bioceramic-based Bioserra sealer. The POBS values of the NaOCl irrigation solution groups, regardless of the root canal sealer used; and the AH Plus root canal sealer groups, regardless of the irrigation solution, are high compared to other groups. If 17% EDTA or 10% citric acid is to be used as the irrigation solution during the root canal treatment procedures, then choosing AH Plus sealer for root canal filling will be a factor that increases the bonding strength values compared to choosing Bioserra sealer. When we examined the 10% propolis solution as an option for root canal irrigation procedure, there was no significant difference in the mean POBS values that would provide an advantage between the other groups, nor was there a difference that negatively affected the binding to the root canal dentin. Nevertheless, more researches need to be done on propolis as an irrigation solution option in endodontics, especially in *in vivo* conditions.

Ethics

Ethics Committee Approval: The ethics committee approval of this study was obtained from the Clinical Research Ethics Committee of the Faculty of Dentistry of Marmara University under decision number 2022/91, dated September 29, 2022.

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: D.İ., E.İ., Design: D.İ., E.İ., Data Collection or Processing: D.İ., Analysis or Interpretation: D.İ., Literature Search: D.İ., Writing: D.İ., E.İ.

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Original Article

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Comparison of the Results of Conventional and Laparoscopic Methods of Lymph Node Dissection Performed in Endometrial Cancer Surgery

Endometriyum Kanser Cerrahisinde Yapılan Lenf Nodu Diseksiyonunun Konvansiyonel ve Laparoskopik Yöntem Sonuçlarının Karşılaştırılması

Cihan COMBA[1], Gökhan DEMİRAYAK[2], Sema KARAKAŞ[3], Şakir Volkan ERDOĞAN[4], Ömer DEMİR[5], Güneş Özlem YILDIZ[6], Ayşe Büşra ÖNDER[3], Aliye ERDOĞAN[7], Selim AFŞAR[8], İsa Aykut ÖZDEMİR[9]

[1]İstanbul Aydın University VM Medical Park Hospital, Clinic of Gynecologic Oncology, İstanbul, Türkiye
[2]Memorial Şişli Hospital, Clinic of Gynecologic Oncology, İstanbul, Türkiye
[3]University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Gynecologic Oncology, İstanbul, Türkiye
[4]Bayrampaşa Kolan Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Türkiye
[5]Karadeniz Technical University Faculty of Medicine, Department of Obstetrics and Gynecology, Trabzon, Türkiye
[6]University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Anesthesiology, İstanbul, Türkiye
[7] University of Health Sciences Türkiye, Bağcılar Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Türkiye
[8]Balıkesir City Hospital, Clinic of Gynecologic Oncology, Balıkesir, Türkiye
[9]Medipol University Hospital, Clinic of Gynecologic Oncology, İstanbul, Türkiye

ABSTRACT

Objective: The aim of this study was to determine a current approach to en bloc para-aortic lymphadenectomy and to compare the results of this new approach performed by way of laparotomy and endoscopically.

Methods: This study was conducted on 191 patients with endometrial cancer (EC) who had undergone para-aortic lymph node (PaLN) dissection with the current method (en. bloc paraaortic lymphadenectomy; protect inferior mesenteric artery and superior hypogastric plexus) between January 2015 and September 2019. A description of the paraaortic lymphadenectomy technique was made in this study. Harvested lymph node counts, operational information, pathological features, postoperative complications, recurrence were presented.

Results: A total of 191 EC patients were analyzed in two separate groups with regard to surgical approach. Open and minimally

ÖZ

Amaç: Çalışmada; en blok paraaortik lenfadenektomiye güncel bir yaklaşım belirlemek ve laparotomi ile endoskopik olarak yapılan bu yeni yaklaşımın sonuçlarının karşılaştırılması amaçlanmıştır.

Yöntemler: Çalışma, Ocak 2015 ile Eylül 2019 tarihleri arasında güncel yöntemle (en blok paraaortik lenfadenektomi; alt mezenterik arteri ve süperior hipogastrik pleksusu koruyarak) para-aortik lenf nodu (PaLN) diseksiyonu yapılan 191 endometriyum kanserli (EC) hasta üzerinde gerçekleştirildi. Makalede uygulanan teknik anlatıldı. Alınan lenf nodu sayıları, operasyon bilgileri, patolojik özellikler, postoperatif komplikasyonlar ve nüks oranları belirtildi.

Bulgular: Toplam 191 EC tanılı hasta cerrahi yaklaşım açısından iki ayrı grupta incelendi. Laparotomi grubu ve minimal invaziv cerrahi grubu sırasıyla 141 ve 50 hastadan oluşuyordu. Gruplar arasında yaş, vücut kitle indeksi, menopoz durumu, anestezi süresi,

Address for Correspondence: Cihan Comba, İstanbul Aydın University VM Medical Park Hospital, Clinic of Gynecologic Oncology, İstanbul, Türkiye
E-mail: cihan.comba@yahoo.com.tr **ORCID ID:** orcid.org/0000-0002-3161-2689

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ABSTRACT

invasive surgery was composed of 141 and 50 patients, respectively. There was no difference between the groups in respect of age, body mass index, menopausal status, anesthesia time, chylous ascites, the presence of recurrence. We found in open surgery group that pre-operative CA125 level, amount of intraoperative bleeding, erythrocyte suspension transfusion, number of PaLNs, metastatic PaLN counts, and early postoperative complications were significantly higher than the other group.

Conclusion: The current method has some advantages such as protecting normal body structures, resulting in the same lymph node counts with published articles by other authors previously, and having lower recurrence rates. Two approaches of the current technique are feasible, easy to perform and effective.

Keywords: Paraaortic lymphadenectomy, method, endometrial cancer, minimally invasive surgery

ÖZ

şilöz asit, nüks varlığı açısından fark yoktu. Laparotomi grubunda ameliyat öncesi CA125 düzeyi, intraoperatif kanama miktarı, eritrosit süspansiyon transfüzyonu, PaLNs sayısı, metastatik PaLNs sayısı ve erken postoperatif komplikasyonların diğer gruba göre anlamlı derecede yüksek olduğu tespit edildi.

Sonuç: Mevcut yöntemin, normal vücut yapılarını koruması, lenf nodu sayılarının daha önce başka yazarlar tarafından yayınlanan makalelerle benzer olması ve nüks oranlarının daha düşük olması gibi bazı avantajları vardır. Mevcut tekniğin iki yaklaşımı da uygulanabilir, gerçekleştirilmesi kolay ve etkilidir.

Anahtar Sözcükler: Paraaortik lenfadenektomi, metod, endometriyum kanseri, minimal invaziv cerrahi

Introduction

Para-aortic lymph nodes (PaLNs) involvement in tumor cells is important in the stages of gynecologic malignancies including cervical, endometrial, ovarian, and vulvar cancers. Endometrial cancer (EC) is the most common gynecological cancer in the world. Although the sentinel lymph node concept has become widespread in EC surgery in recent times, PaLN dissection is an important part of the surgical procedure (1-3).

The superior hypogastric plexus (SHP) is a preaortic nervous plexus formed by two laterals which originate mainly from the lowest lumbar splanchnic nerves and include a sympathetic component; one median root around the level of aortic bifurcation (4-6). The median root, or a continuation of the abdominal aortic plexus from the inferior mesenteric plexus (4,7), includes both sympathetic and parasympathetic components (8). The SHP is divided into paired hypogastric nerves either at the same level or below the sacral promontory (4,5,7).

The inferior mesenteric artery (IMA) supplies blood to the left side of the colon through the bifurcate left colic artery (LCA) and the sigmoid artery. The frequencies of the bifurcation patterns of the IMA were determined by Murono et al. (9) in 2015 using three-dimensional computed tomography angiography. The anatomy of the artery and the sparing of it are very important for surgeons and patients who suffer from gynecological cancers or cancers in the left colon segments (9).

The aim of this study was to determine a current approach to en bloc para-aortic lymphadenectomy which would protect the IMA and hypogastric nervous plexus and to compare the results of this new approach performed by way of laparotomy and endoscopically.

Methods

This study was conducted on patients with EC who had undergone PaLN dissection with current methods between January 2015 and September 2019 with the approval of the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Board (approval Number: 2019/434, date: 30.09.2019). Bakırköy Dr. Sadi Konuk Training and Research Hospital is a center accredited by the European Society of Gynecological Oncology.

Patients who had been diagnosed as having EC and who had undergone a para-aortic lymphadenectomy were included in this study due to the modified Mayo criteria (10).

The study participants were divided into two groups as laparotomy patients (minimally invasive surgery conversion to laparotomy), and those who had undergone minimally invasive surgery (laparoscopy or robotics) according to surgeon's decision. In the groups the same surgical techniques were performed by the same surgical teams.

Informed consent was obtained from patients before the procedure. A statistical program (G*Power version 3 [Heinrich Heine University, Düsseldorf, Germany]) was used to estimate the sample size via a 1-tailed hypothesis using an independent sample t-test with an α error of 0.05 and a power of 0.90. For a moderate effect size (d=0.50), a total sample size of 176 was calculated to be required. In the present study, there were 50 women in the minimally invasive group and 141 women in the laparotomy group. Post-hoc analysis for moderate effect size indicated that the power of the test was increased to 0.95. The inclusion criteria for the study were patients who suffered EC and who underwent comprehensive surgical staging with PaLN dissection.

The study exclusion criteria were patients who suffered from another gynecological cancer, synchronous gynecological (ovarian, cervical) or non-gynecological tumors (breast or gastrointestinal tumors), patients who underwent sentinel lymph node dissection without a para-aortic lymphadenectomy, or in whom a pelvic lymphadenectomy only was performed, and those who had undergone a fertility sparing procedure.

Statistical Analysis

SPSS 20 program designed for Windows was used for statistical analysis. All continuous variables were expressed as mean and standard deviation values and categorical variables were expressed as number (n) and percentage of the total group (%). All statistical tests were performed by comparing two groups. Mann-Whitney U test was used for comparative analysis of independent variables and chi-square test was used for categorical variables. A value of $p<0.05$ was considered statistically significant.

Description of the Surgical Technique

After pelvic lymph node dissection, PaLN dissection begins with an incision to the right pelvic side parietal peritoneum up to the ileocecal region. The right ureter is separated laterally up to the same level. The peritoneum is incised from the medial of the right infundibulopelvic ligament to the inferior mesenteric vein in the open procedure. However, in the minimally invasive procedure, the incision is finished up to 4 cm further from the vessel to tent the peritoneum and to restrain the bowels. The foamy tissues are dissected though the lateral and medial sides up to the ileopsoas muscle and are visible on the right side of the vena cava. The right ureter and right infundibulopelvic ligament are dissected from the medial side up to the level of the right ovarian vein's entrance to the vena cava and the right ovarian vein is then ligated and cut. In general the left renal vein which crosses over the aorta and flows into the vena cava can be visible by dissecting porous tissue and lymphatic veins around the vena cava. Thus the right side is prepared for the lymphadenectomy. Afterwards the incision is enlarged to the pelvic floor from the lateral side of the sigmoid colon to the peritoneum and the ureter is lateralized by way of left ureter dissection. The lymph nodes are dissected through this level with a dissection above the right common iliac artery to isolate the IMA which is located approximately 4 or 5 centimeters above the aorta bifurcation and rises up slightly to the left side. By preserving the superior hypogastric nerves, a passage is carved out between the iliac veins and nerves, ensuring that these nerves remain on the anterior side. The left ureter and left ovarian vein are dissected to the level of the IMA. From that point the lymph nodes are located on the lateral side of the right common iliac artery and the superior and right lateral side of the vena cava are then carefully dissected. It is important to ensure that the associated veins which are at the level of the iliac artery dissection point and between the vena cava and the lymphatic vessel are intact. Until the level of the IMA para aortocaval lymph nodes is dissected, as the first dissection on the side of the aorta in order to reach the level of the left renal vein, the cranial part is left undissected. Starting from the lateral side of the left common iliac artery, on the medial side the common iliac and the aorta and on the lateral side the psoas muscle are dissected. By preserving the chain, the PaLNs are dissected through the track of the vertebrae to the level of the IMA. Afterwards, in the minimal invasive procedure, the camera is replaced in the suprapubic trocar and the operation is performed from the pelvis to the thorax. First the para aorto-caval lymph nodes, which are left without dissection of its cranial parts, are dissected to the level of the left renal vein and the cranial parts are occulted by using endoclips or suture materials. The PaLNs which are left as a chain to the level of the IMA are dissected a further 1-2 cms and are retracted by crossing over the IMA in order to maintain easier traction on the right side. The superior PaLNs between the aorta and the IMA are harvested as a chain up to the left renal vein cranially and between the aorta and the left ovarian vein. The cranial part is occulted by endoclip or sutures as are the aorto-caval lymph nodes. Afterwards to control hemorrhaging, BLOODCARE powder is applied to the cranial side of the lymph node dissection area. A dissection of presacral lymph nodes is performed with precision starting from the medial side of the right common iliac artery to the left common iliac vein and the middle sacral artery and veins. Thus the PaLN dissection is completed.

Results

A total of 191 patients in accordance with the inclusion criteria of the study were identified in the specified date range and the results were analyzed retrospectively.

These patients were divided into two groups for examination. While the first group was the PaLN dissection using the laparotomy method, the second group was the group undergoing PaLN dissection using minimally invasive surgery (laparoscopy/robotic surgery). The bridge technique described above was used in both groups. The demographic characteristics of the entire population and the groups are shown in Table 1.

Table 1. Demographic characteristics of the all population and individual groups

	All population n=191	Group 1 Open surgery n=141	Group 2 Minimal invasive surgery n=50	p-value
Age	61±8.9 (30-82)	62.8±8.4	58.6±9.7	0.247
BMI	35.5±7.7 (20-68)	36.6±7.4 (20-59)	33.3±7.9 (23-68)	0.578
Menopausal Status	24-premenopause (12.6 %) 167-postmenopause (87.4 %)	14- premenopause (9.9 %) 127- postmenopause (90.1 %)	10- premenopause (20 %) 40- postmenopause (80 %)	0.059
Pre-operative CA125	38.3±90.4 (3-757)	45.8±105.8 (4-757)	19.8±18.3 (3-111)	**0.012**
Pre-operative CA19-9	17.9±38.7 (1-293)	21.8±45.8 (1-293)	9.5±8.7 (3-40)	**0.009**

BMI: Body mass index

Table 2. Operation-specific features of the all population and individual groups

	All population n=191	Group 1 Open surgery n=141	Group 2 Minimal invasive surgery n=50	p-value
Type of operation	141- open surgery 50- minimal invasive surgery (laparoscopic / robotic)	137- total abdominal hysterectomy + pelvic + paraaortic lymphadenectomy 4- re-staging surgery (open)	33- laparoscopic hysterectomy + pelvic + paraaortic lymphadenectomy 10- robotic hysterectomy + pelvic + paraaortic lymphadenectomy 5- type B hysterectomy + pelvic + paraaortic lymphadenectomy 2- re-staging surgery (laparoscopic)	
Omentectomy	141 (73.8%) - Present 50 (26.2%) - None	121 (85.8%) - Present 20 (14.2%) - None	20 (40%) - Present 30 (60%) - None	**0.000**
Operation time (min)	278±71.6 (100-500)	262.9±68.4 (100-470)	320.8±63.1 (180-500)	**0.000**
Anesthesia time (min)	29.4±9.9 (15-60)	28.5±8.8 (15-50)	32.2±12.3 (15-60)	**0.022**
Amount of Intraoperative Bleeding (mL)	320.1±265.9 (50-2000)	372.3±282.7 (50-2000)	172.6±125.3 (50-500)	**0.000**
Pre-operative hemoglobin	12.3±1 (8.3-14.9)	12.3±0.9 (8.3-14.9)	12.3±1.2 (9.2-14.4)	0.877
Post-operative hemoglobin	11.4±0.9 (8.8-14.4)	11.3±0.9 (8.8-14.4)	11.6±1.1 (9-13.2)	0.079
Erythrocyte suspension transfusion	48 (25.1%) - Present 143 (74.9%) - None	44 (31.2%) - Present 97 (68.8%) - None	4 (8 %) - Present 46 (92 %) - None	**0.001**
Fresh frozen plasma transfusion	47 (24.6%) - Present 144 (75.4%) - None	43 (30.5%) - Present 98 (69.5%) - None	4 (8%) - Present 46 (92%) - None	**0.001**
Intraoperative complication	6 (3.1%) - Present 185 (96.9%) None	5 (3.5 %) - Present 136 (96.5%) - None	1 (2%) - Present 49 (98%) -None	0.504
Early postoperative complication	27 (14.1%) - Present 164 (85.9%) - None	24 (17%) - Present 117 (83%) - None	3 (6%) - Present 47 (94%) - None	**0.039**
Type of early postoperative complication	24 - open surgery (24/141) - 17 % 3 - minimal invasive surgery (3/50) - 6%	9 - wound infection 3 - dehiscence 3 - subileus 2 - atelectasis 2 - embolism + wound infection 2 - atelectasis+ wound infection 2 - acute kidney failure 1- obturator nerve injury	2 - wound infection 1 - intraabdominal abscess	
Chylous ascites	17 (8.9%) - Present 174 (91.1%) - None	15 (10.6%) - Present 126 (89.4%) - None	2 (4%) - Present 48 (96%) - None	0.127
Treatment of chylous ascites		5 (33.4%) - Diet 10 (66.6%) - Diet + Total parenteral nutrition	2 (100%) - Diet	

The operations performed in both groups and their features are shown in Table 2. The duration of anesthesia and the duration of the operation were statistically significantly longer in the group that underwent the minimally invasive surgery (p=0.000; p=0.022, respectively). In addition, the amount of intraoperative bleeding was statistically significantly lower in the group undergoing minimally invasive surgery compared to the group undergoing open surgery (p=0.000). The need for erythrocyte suspensions and fresh frozen plasma transfusions was found to be less common in the laparoscopy group (p=0.001, 0.001, respectively). While there was no significant difference between the two groups in terms of intraoperative complication rates, the early postoperative complication rate was statistically significantly higher in the laparotomy group (p=0.039).

Among the groups, the number of pelvic and PaLNs and metastatic lymph node numbers collected were statistically significantly higher in favor of open surgery. While the presence of lymphovascular area invasions was more common in the open surgery group (p=0.045), there was no difference between the groups in terms of intraabdominal fluid cytology positivity and uterine lower segment involvement (p=0.973; p=0.316). There was no statistically significant difference between the groups in terms of recurrence rates (p=0.072). Pathological outcomes of the cases and their comparisons are summarized in Table 3.

The preoperative and postoperative histological diagnoses, grades and stages of the cases examined are as shown in Figure 1 and Figure 2 (histological grade information of 182 patients preoperatively was obtained). Having regard to the distribution by stages, the first three rankings in both groups were Stage 1a, Stage 1b and Stage 3c2, respectively.

Discussion

The results of the study which compared two methods for the same operation indicated that the same survival rate, complication-free period, and the recurrence. However, there were some differences including operation times and extent of peroperative blood loss. The both techniques were more practical, successful, effective, and straightforward when compared to conventional techniques.

Systemic para aortic lymphadenectomy is defined as the entire dissection and removal of fat and nodal tissues around the aorta, inferior vena cava, and renal vessels. The dissection level includes the left renal vein cranially and the midpoint of the common iliac vessels caudally (11).

Herd et al. (12) developed a technique based on 100 cases with patients who had undergone a transperitoneal or extraperitoneal approach for PaLN dissection. Dargent et al. (13) described extraperitoneal laparoscopic total PaLN dissection as a two sided approach up to the renal vein in 2000. Lymph node dissection was commenced below the left renal vein. This dissection was performed bluntly, scissors and monopolar and bipolar devices were not commonly used and the lymph nodes were harvested, which placed the left side of the aorta into two separate chains, one of which was the left and ventral surface part, the other was the dorsal part of the aorta (13). However, we demonstrated en

Table 3. Comparative analysis of pathological data of cases

	All population n=191	Group 1 Open surgery n=141	Group 2 Minimal invasive surgery n=50	p-value
Tumor size	3.7±1.8 (0,5-12)	3.9±1.9 (0.5-12)	3.4±1.3 (1-8)	0.062
Number of pelvic lymph nodes	20.5±7.7 (10-70)	21.4±7.9 (10-70)	18±6.1 (10-42)	**0.002**
Number of paraaortic lymph nodes	18±7.7 (4-46)	19.3±7.8 (7-46)	14.3±6.1 (4-29)	**0.000**
Total number of lymph nodes	38.6±12.8 (16-93)	40.8±12.9 (21-93)	32.3±9.8 (16-67)	**0.000**
Metastatic pelvic lymph node count	0.6±1.5 (0-10)	0.7±1.7 (0-10)	0.2±0.6 (0-3)	**0.005**
Metastatic paraaortic lymph node count	1±3.1 (0-20)	1.2±3.5 (0-20)	0.5±1.6 (0-8)	**0.044**
Total number of metastatic lymph nodes	1.6±4.5 (0-29)	1.9±5 (0-29)	0.7±2.2 (0-10)	**0.018**
Intraabdominal fluid cytology	7 (3.7%) - positive 184 (96.3%) - negative	6 (4.3%) - positive 135 (95.7%) - negative	1 (2%) - positive 49 (98%) - negative	0.973
Lymphovascular space invasion	45 (23.6%) - present 146 (76.4%) - none	38 (27%) - present 103 (73%) - none	7 (14%) - present 43 (86%) - none	**0.045**
Uterine lower segment involvement	41 (21.5%) - present 150 (78.5%) - none	32 (22.7%) - present 109 (77.3%) - none	9 (18 %) - present 41 (82 %) - none	0.316
The presence of recurrence	14 (7.3%) - present 177 (92.7%) - none	13 (9.2%) - present 128 (90.8%) - none	1 (2 %) - present 49 (98 %) - none	0.072
Recurrence area		7 - vaginal cuff 3 - pelvis 1 - abdomen 2 - multiple	1 - vaginal cuff	

bloc removal of the left PaLNs dissection from under the superior hypogastric nerves and upper IMA with contralateral traction in the current study. In the English literature, the effective numbers of PaLN removals have not yet been described. Some researchers have established the removal of more than ten PaLNs in their studies (14-16). In the current study, in total the number of lymph nodes removed was 18, 14 in the open and the minimally invasive surgery groups, respectively. The technique allowed the harvesting of a large number of PaLNs because an en bloc lymphadenectomy was performed based on ease of dissection

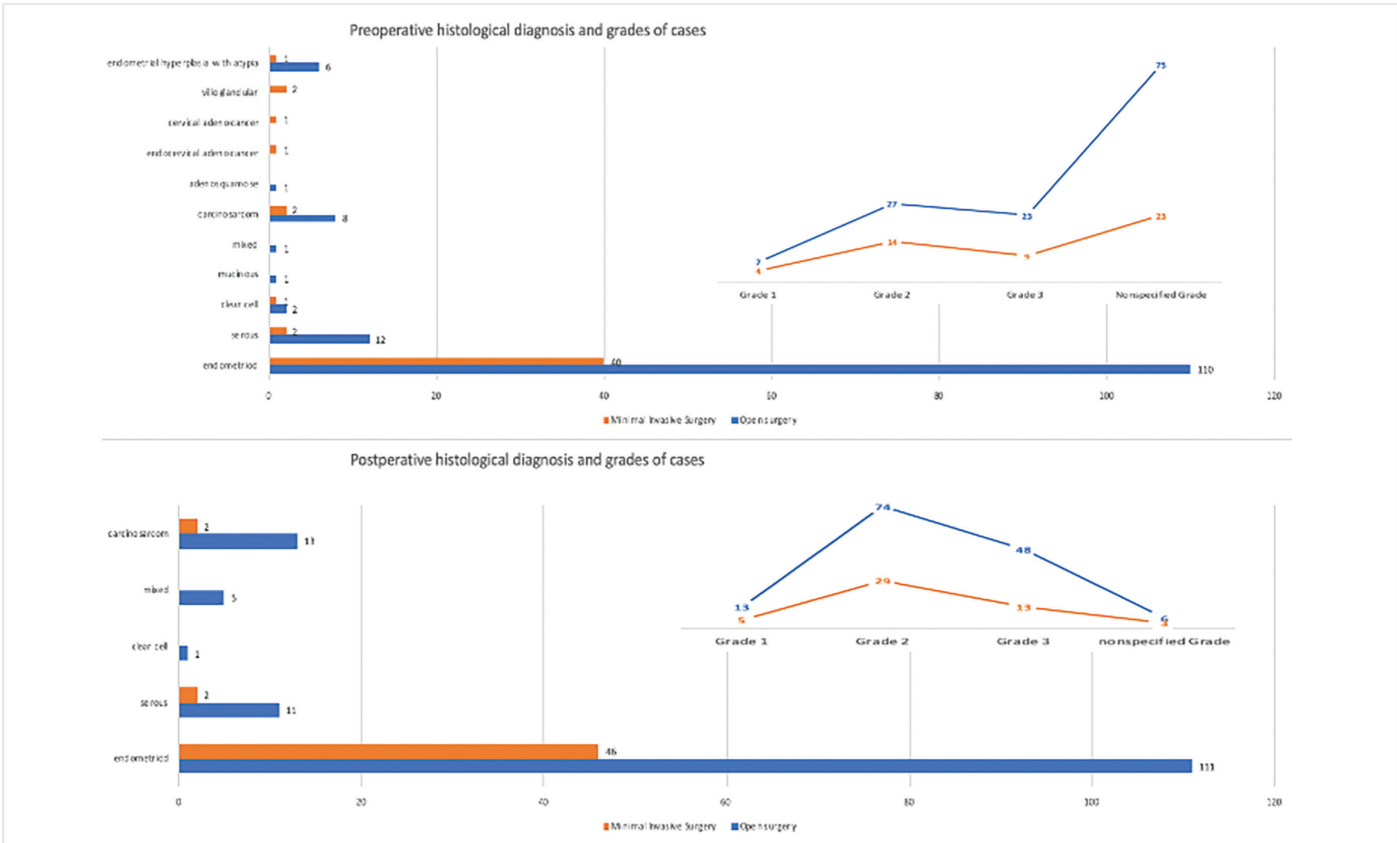

Figure 1. Pre-post operative histological diagnosis and grades of cases

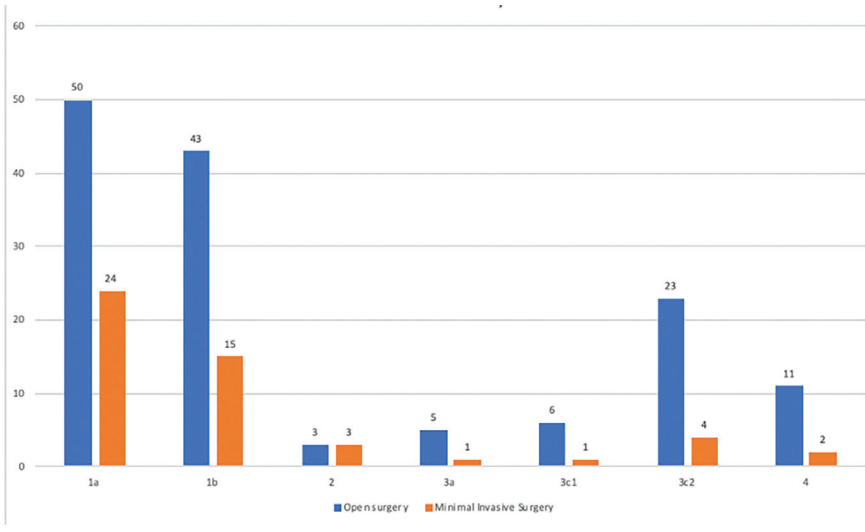

Figure 2. Distribution of cases by stage

with contralateral traction of the lymphatic chain, allowing enhanced vision. Some studies reached the same PaLN counts as our study by way of extraperitoneal PaLN dissection (17).

The current concept of lymph node assessment follows a risk-based algorithm. It suggests a systematic LND in patients with EC and uterine high risk factors. In these patients, the incidence of lymph node involvement is high. Furthermore, in these patients, lymph node metastasis of EC is found to be associated with lymphovascular space involvement (LVSI), non-endometrioid histology, and stage IB (16). In our study, the non-endometrioid histology of EC was higher at 56 (29.32%), LVSI 45 (23.6%), and at higher stages than stage 1A 117 (61.25%). Nevertheless, the recurrence rate was significantly lower, it occurred in only 14 patients in total. Approximately 15% of patients with stage I and II EC will suffer a recurrence, this rate in advanced stages and all stages EC is higher than 15% (18,19).

IMA supplies blood to the left part of the colon and divides the sigmoidal (SA) and LCA. The division can occur in four patterns: 1) the LCA arises independently from the SA; 2) the LCA and SA arise from the IMA at the same point; 3) the LCA and SA have a common trunk; 4) there is a deficit of the LCA (20). The level of the IMA at its aortic origin can cause some complications such as bowel anastomosis leakage in patients with colorectal cancer (21). These issues indicate that protection of the IMA during PaLN dissection is very critical to prevent blockage of the blood supply to the left colon segments. Using the current technique, we were able to protect them and to harvest lymph nodes which were placed above the IMA with contra traction of them.

Study Limitations

The hypogastric nerve plays a role in terms of bowel, internal genital organs, and bladder functions. Damage to the fibers may lead to male impotence additionally problems of micturition and defecation were unable to be identified in the female population (4,8). As we were able to protect these structures, we avoided postoperative complications related to these organs and we were able to discharge our patients sooner.

Conclusion

Our particular approach has been evolving since 2015. The authors believe that en bloc lymphadenectomies have increased survival rates and have reduced the recurrence of EC. The protection of the IMA and hypogastric nerve fibers can help to avoid certain postoperative complications.

Ethics

Ethics Committee Approval: This study was conducted on patients with EC who had undergone PaLN dissection with current methods between January 2015 and September 2019 with the approval of the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Board (approval Number: 2019/434, date: 30.09.2019).

Informed Consent: Informed consent was obtained from patients before the procedure.

Authorship Contributions

Surgical and Medical Practices: C.C., G.D., S.K., S.A., İ.A.Ö., Concept: C.C., G.D., Ş.V.E., A.B.Ö., İ.A.Ö., Design: C.C., Ş.V.E., A.B.Ö., İ.A.Ö., Data Collection or Processing: C.C., S.K., Ş.V.E., G.Ö.Y., A.B.Ö., İ.A.Ö., Analysis or Interpretation: C.C., G.D., S.K., Ş.V.E., Ö.D., G.Ö.Y., S.A., İ.A.Ö., Literature Search: C.C., Writing: C.C., Ö.D., A.E., S.A.

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

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Original Article

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Who Predicts Mainly Anxiety and Depression Severity in The Community Sample: Metacognitions or Intolerance of Uncertainty?

Toplum Örnekleminde Anksiyete ve Depresyonu Kim Yordar: Üstbilişler mi? Belirsizliğe Tahammülsüzlük mü?

Kübra SEZER KATAR[1], Ayşe Gökçen GÜNDOĞMUŞ[2]

[1]University of Health Sciences Türkiye, Ankara Training and Research Hospital, Department of Psychiatry, Ankara, Türkiye
[2]Ministry of Health, Etlik City Hospital, Department of Psychiatry, Türkiye

ABSTRACT

Objective: Our study aims to find the predictors of anxiety and depression in a community sample.

Methods: Beck depression inventory (BDI), Beck anxiety inventory (BAI), Intolerance of Uncertainty Scale (IUS), and Metacognition Questionnaire-30 (MCQ-30) were rated by 172 individuals who were without ongoing psychiatric disorders and treatment.

Results: There were significant relationships between BAI and cognitive confidence and the total score of MCQ (r=0.175 and r=0.157, respectively; p<0.05). BDI had significant relationships with all subscales and total scores of MCQ-30 and IUS (r=0.234-0.520, p<0.05). There was a significant relationship between the total score of MCQ-30 and IUS (r=0.707, p<0.05). MCQ-30 and IUS did not predict anxiety symptoms. However, "need to control thoughts" (β=0.49, p<0.05) and "cognitive self-consciousness" (β=-0.27, p<0.05) predicted levels of depression and explained 34% of the variance in depression.

Conclusion: In conclusion, our study showed depression had a more close relationship with metacognitions than anxiety did. Despite the limitations, our findings highlight the possible relationship between two important cognitive models and anxiety and depression.

Keywords: Anxiety, depression, intolerance of uncertainty, metacognition

ÖZ

Amaç: Çalışmamız bir toplum örnekleminde anksiyete ve depresyon yordayıcılarını bulmayı amaçlamaktadır.

Yöntemler: Halihazırda psikiyatrik bir tanısı ve devam eden bir tedavisi olmayan 172 bireyde Beck depresyon envanteri (BDE), Beck anksiyete envanteri (BAE), Belirsizliğe Tahammülsüzlük Ölçeği (BTÖ) ve Üstbiliş Ölçeği-30 (ÜÖ-30) puanlanmıştır.

Bulgular: BAE ile ÜÖ-30 toplam puanı ve bilişsel güven alt boyutu arasında anlamlı ilişkiler saptanmıştır (sırasıyla r=0,175 ve r=0,157, p<0,05). BDE ile ÜÖ-30 ve BTÖ ölçeğinin tüm alt boyutları ve toplam puanları arasında anlamlı ilişkiler saptanmıştır (r=0,234-0,520, p<0,05). Ayrıca ÜÖ-30 ve BTÖ ölçeklerinin toplam puanları arasında anlamlı bir ilişki saptanmıştır (r=0,707, p<0,05). ÜÖ-30 ve BTÖ anksiyete skorunu predikte etmezken düşünceleri ÜÖ-30'un kontrol ihtiyacı (β=0,49, p<0,05) ve bilişsel farkındalık (β=-0,27, p<0,05) alt boyutları depresyon skorunu predikte etmiş ve %34'ünü açıklamıştır.

Sonuç: Sonuç olarak, çalışmamız depresyon skorunun anskiyete skoruna göre üstbilişler ile daha yakın bir ilişkide olduğunu göstermiştir. Kısıtlılıklarına rağmen bulgularımız anksiyete, depresyon ve iki önemli bilişsel model arasındaki ilişkiye ışık tutmaktadır.

Anahtar Sözcükler: Anksiyete, depresyon, belirsizliğe tahammülsüzlük, üstbiliş

Address for Correspondence: Kübra Sezer Katar, University of Health Sciences Türkiye, Ankara Training and Research Hospital, Department of Psychiatry, Ankara, Türkiye
E-mail: kubrasezerkatar@gmail.com **ORCID ID:** orcid.org/0000-0001-7184-7960

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Introduction

Depression is a common and severe psychiatric disorder related to morbidity, mortality, and decreased quality of life and functioning (1). Specifically, in Western countries, around 16-20% of people face depression in their lifetimes (2). Anxiety is also a very common psychiatric problem. Literature shows that the current prevalence of all anxiety disorders ranges between 0.9% and 28.3% (3). Depression and anxiety disorders are common comorbid psychiatric disorders (4). The Netherlands Study of Depression and Anxiety (NESDA) study showed that the lifetime comorbidity rate of anxiety disorder was 75% in individuals with depression (5). Although comorbidity rates of anxiety and depressive symptoms in community samples were relatively lower, studies proved that comorbidity rates aligned with NESDA rates (6). Identifying the key factors that may affect depression and anxiety symptoms is important; if clinicians could find these factors, they could find the factors playing role in the onset, maintenance, and potential treatment target of depression (7).

The metacognitive model explains psychological disorders such as depression and anxiety disorders with the self-regulatory executive function model (8). According to the metacognitive model of emotional disorders, "rumination, worry, fixated attention, and unhelpful self-regulatory strategies or coping behaviors" called cognitive attentional syndrome (CAS) is the core of the model (8). Mostly, this type of thinking style is temporary. However, CAS could impact people by forcing them into perseverative, toxic, and persistent thinking styles. CAS results from incorrect metacognitive beliefs (MBs) and is controlled by these positive and negative MBs. Positive MBs about rumination and unhelpful coping and negative MBs such as uncontrollability and harmfulness of this process lead to depression and anxiety (9). Empirical studies have demonstrated that MBs play an important role in depression (9). Several studies examined the relationship between MBs and depression in healthy participants and found further evidence for the metacognitive model of depression (10,11). The metacognitive model is also one of the most esteemed cognitive models of anxiety disorders, and literature has proved that significantly negative MBs play an important role in worry, generalized anxiety disorder, panic disorder (PD), and social anxiety disorder (12,13).

The other most prominent cognitive factor underlying depression and anxiety disorders is intolerance of uncertainty (IoU). IoU could be defined as a vast and possibly transdiagnostic construct characterized by emotional, cognitive, and behavioral reactions to uncertainty in life (14). First, IoU was initially developed from worry, a distinctive feature of generalized anxiety disorder (14). However, studies suggest that IoU could also be related to depression (15,16). There are contrary results in the literature about the relationship between IoU, depression, and anxiety. Some studies put forward that IoU is associated with anxiety more than depression (15,17), and others put forward a unique relationship between IoU and depression (16).

Some researchers recognized maladaptive beliefs as a modulator of IoU (18). IoU might be related to metacognitions (19). Studies in clinical and non-clinical samples showed that IoU and MBs had a significant positive relationship (20,21). Furthermore, Einstein's IoU model underlines the "need for predictability" beliefs at the center of IoU (18). "Need for predictability" beliefs could cause undesired feelings and coping behaviors such as rumination, worry, and avoidant behaviors (22). Although Einstein's model and previous data reported a more critical relationship between anxiety and MBs than IoU, there is still a need to investigate these important cognitive factors (MBs, IoU) and their relationship between anxiety and depression in different samples.

The current study aims to investigate the effects of metacognitions and IoU on depressive symptoms and anxiety symptoms in individuals without ongoing psychiatric disorders and treatment processes. Based on the literature, our hypotheses are: (i) There is a significant positive correlation between MBs and depression and anxiety severity. (ii) There is a significant positive correlation between IoU and depression and anxiety severity. (iii) MBs could predict depression and anxiety severity better than IoU.

Methods

Sample

We conducted an a priori power analysis using G*Power version 3.1.9.7 for sample estimation. The minimum sample size was n=68 with a significance criterion of alpha=0.05 and beta=0.20 for detecting a medium effect. According to the study's aim, we collected data from 177 individuals. Five individuals were excluded from the study due to missing values.

One hundred seventy-two nonclinical participants aged between 18-65 and literate were included in the study. The participants were informed about the study, and written informed consent was obtained. Participants who were not diagnosed as having any psychiatric disease during the psychiatric interview were included in the study. Individuals with previous psychiatric treatment history were included in the study if their treatment had been concluded. Exclusion criteria included ongoing psychiatric disorder, organic mental diseases, such as delirium, mental disability, medical illnesses with significant cognitive sequelae, epilepsy, dementia, and active alcohol or substance use disorders. The participants were not paid for their contribution to the study. The Ethics Committee of Ankara Training and Research Hospital approved the procedures and the purposes of the study (decision number: E-23-1197, date: 08.03.2023).

Sociodemographic Data Form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Intolerance of Uncertainty Scale (IUS), and Metacognition Questionnaire-30 (MCQ-30) were filled out by individuals.

Measurements

The Sociodemographic Data Form, was used to collect personal information such as age, sex, level of education, and information about the history of applying to a psychiatric unit.

Beck Anxiety Inventory, was used to measure anxiety scores. This 21-item and 4-point Likert-type scale was developed by Beck et al. (23). The Cronbach α of BAI was 0.88 in the current study.

Beck Depression Inventory, was developed to measure depression levels (24). This test includes 21 items in six subscales answered using a 4-point Likert-type scale. The Cronbach α of BDI was 0.89 in the current study.

Intolerance of Uncertainty Scale, is a 12-item scale that measures IoU (25). IUS has two subscales (inhibitory and prospective). IUS is a 5-point Likert scale ranging from 1 (not at all characteristic of me) to 5 (entirely characteristic of me). Higher scores indicate higher IoU. IUS's Cronbach α was 0.95 in the current study.

Metacognition Questionnaire-30 (MCQ-30), was developed by Cartwright-Hatton and Wells (26). It is a 30-item 4-point Likert-type scale ranging from 1 (do not agree) to 4 (agree very much). Higher scores obtained from the scale indicate more dysfunctional MBs. MCQ-30 provides information on five subscales. These subscales are cognitive confidence, positive beliefs about worry, cognitive self-consciousness, negative beliefs about uncontrollability and danger of worry, and the need to control thoughts. The Cronbach α of MCQ-30 was 0.94 in the current study.

Statistical Analysis

The IBM SPSS Statistics for Windows v.25.0 was used to analyze the data (IBM Corp., Armonk, NY). Initially, we performed descriptive statistics to describe the study group. The Pearson correlation coefficient was used to examine the relationship between measurement tools. Finally, multiple linear regression analysis was performed to examine the predictors for depression and anxiety. In the models, BAI and BDI were entered as the dependent variables, and subscales of MCQ-30 and IUS were entered as the regressors. The significance level was considered as 0.05.

Before performing multiple linear regression analysis, the assumptions that should be met were tested. First, the ZPRED-ZRESID graph was examined to determine the normal distribution of the differences between the estimated values and the observed values, and it was determined that this graph was in the form of an ellipse, that is, the residual values showed a normal distribution. When the graphs were drawn to determine whether the relationship between the predictor and the predicted variable was linear, which is the second assumption, it was found that this assumption was met. For the third assumption, the multicollinearity problem, variance inflation factor (VIF), and tolerance values were examined, and it was observed that VIF values ranged from 4.26 to 1.60, and tolerance values ranged from 0.24 to 0.63, and it was determined that there was no multicollinearity problem among the predictive variables. Skewness and kurtosis values were examined to determine whether the predicted variable, which is the last assumption, had a normal distribution. It was seen that the predicted variables had a normal distribution. After testing all assumptions, multiple regression analysis was performed.

Results

The sociodemographic data of the sample are shown in Table 1. The mean age of the participants was 36.45±9.36 years, and 56.4% of the sample (n=97) was female. The mean education level was 14.80±6.21 years, and 69.8% of the participants (n=120) was employee/officer.

Descriptive statistics of the scales and the subscales are given in

Table 1. The sociodemographic data of the sample

Variables		f	%
Sex	Female	97	56.4
	Male	75	43.6
Marital status	Single	65	37.8
	Married	100	58.1
	Divorced	7	4.1
Occupational status	Unemployed	13	7.6
	Self-employment	21	12.2
	Employee/officer	120	69.8
	Student	10	5.8
	Retired	8	4.6
History of psychiatric treatment	Yes	28	16.3
	No	144	83.7
Total		58	100
	Minimum-maximum	$\bar{X}$	S
Age (years)	19-59	36.45	9.36
Level of education (years)	2-27	14.80	6.21

Table 2. Descriptive statistics of the scales and the subscales

	Min-Max	$\bar{X}$	SD
BAI	0.00-33.00	9.05	7.76
BDI	0.00-36.00	10.58	8.52
PBW	6.00-24.00	11.10	4.48
NBC and DW	6.00-24.00	11.26	4.70
CC	6.00-22.00	11.60	4.22
NCT	6.00-22.00	11.63	4.24
CSC	6.00-23.00	13.14	4.16
MCQ-30 (Total)	30.00-108.00	58.73	18.05
Prospective anxiety	7.00-35.00	19.41	7.05
Inhibitory anxiety	5.00-25.00	12.35	5.76
IUS (Total)	12.00-60.00	31.76	12.03

BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, PBW: Positive beliefs about worry, NBC and DW: Negative beliefs about uncontrollability and danger of worry, CC: Cognitive confidence, NCT: Need to control thoughts, CSC: Cognitive self-consciousness, MCQ-30: Metacognition Questionnaire-30, IUS: Intolerance of Uncertainty Scale, SD: Standard deviation, Min:Minimum, Max: Maximum

Table 2.

Correlational analyses of the variables were summarized in Table 3. There was no significant relationship between the BAI and the subscales of MCQ-30 except cognitive confidence and the total score of MCQ (r=0.175 and r=0.157, respectively). Also, there was no relationship between the BAI and the total score and subscales of IUS. The BDI had significant and positive relationships with all subscales and total scores of MCQ-30 and IUS (r=0.234-0.520, p<0.05). There was a significant positive relationship between the total score of MCQ-30 and IUS (r=0.707, p<0.05).

After the correlational analyses between the variables were examined, multiple linear regression analyses were conducted to predict anxiety and depression in the study sample. The results were given in Table 4 for anxiety and Table 5 for depression. In the first model, BAI was entered as the dependent variable, and subscales of MCQ-30 and IUS were entered as regressors. The first model showed that neither MCQ-30 nor IUS significantly predicted anxiety symptoms (p>0.05). Also, this model did not show good fitness, F(7-164)=0.97, p=0.455, and adjusted R^2=0.04 for the anxiety model.

In the second model, BDI was entered as the dependent variable, and subscales of MCQ-30 and IUS were entered as regressors. This model showed a good fitness with F(7-164)=11.85, p=0.00, and adjusted R^2=0.34. According to the model, a higher "need to control thoughts" (β=0.49, p<0.05) and lower "cognitive self-consciousness" (β=-0.27, p<0.05) predicted higher levels of depression. The need to control thoughts and cognitive self-consciousness together explained 34% of the variance in

Table 3. Correlations between variables

Variables	1	2	3	4	5	6	7	8	9	10
1. BAI	--									
2. BDI	0.233*	--								
3. PBW	0.147	0.234*	--							
4. NBC and DW	0.095	0.445*	0.646*	--						
5. CC	0.175*	0.258*	0.406*	0.397*	--					
6. NCT	0.110	0.520*	0.588*	0.838*	0.497*	--				
7. CSC	0.126	0.256*	0.702*	0.712*	0.578*	0.702*	--			
8. MCQ-30 (Total)	0.157*	0.415*	0.811*	0.874*	0.687*	0.877*	0.890*	--		
9. Prospective anxiety	0.105	0.373*	0.615*	0.614*	0.337*	0.576*	0.564*	0.656*	--	
10. Inhibitory anxiety	0.118	0.434*	0.586*	0.647*	0.363*	0.629*	0.544*	0.672*	0.760*	--
11. IUS (Total)	0.118	0.426*	0.641*	0.670*	0.372*	0.639*	0.591*	0.707*	0.950*	0.924*

*p<0.05; BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, PBW: Positive beliefs about worry, NBC and DW: Negative beliefs about uncontrollability and danger of worry, CC: Cognitive confidence, NCT: Need to control thoughts, CSC: Cognitive self-consciousness, MCQ-30: Metacognition Questionnaire-30, IUS: Intolerance of Uncertainty Scale

Table 4. Multiple linear regression with anxiety as the dependent, and subscales of MCQ-30 and subscales of IUS as predictors

Variables	b	Standard deviation b	β	t-test
Constant	4.60	2.23	--	2.07
PBW	0.20	0.21	0.11	0.95
NBC and DW	-0.07	0.26	-0.04	-0.27
CC	0.27	0.18	0.15	1.54
NCT	0.01	0.28	0.00	0.02
CSC	-0.06	0.25	-0.03	-0.24
Prospective anxiety	-0.01	0.14	-0.01	-0.06
Inhibitory anxiety	0.06	0.17	0.05	0.36
R=0.20	R^2=0.04			
$F_{7\text{-}164}$=0.97	p=0.455			

p<0.05; BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, PBW: Positive beliefs about worry, NBC and DW: Negative beliefs about uncontrollability and danger of worry, CC: Cognitive confidence, NCT: Need to control thoughts, CSC: Cognitive self-consciousness, MCQ-30: Metacognition Questionnaire-30, IUS: Intolerance of Uncertainty Scale

Table 5. Multiple linear regression with depression as the dependent, and subscales of MCQ-30 and subscales of IUS as predictors

Variables	b	Standard deviation b	β	t-test
Constant	-0.45	2.03	--	-0.22
PBW	-0.24	0.19	-0.13	-1.28
NBC and DW	0.18	0.24	0.10	0.75
CC	0.17	0.16	0.08	1.05
NCT	0.99	0.25	0.49	3.89*
CSC	-0.55	0.23	-0.27	-2.39*
Prospective anxiety	0.11	0.13	0.09	0.89
Inhibitory anxiety	0.26	0.16	0.18	1.68
R=0.58	R^2=0.34			
$F_{7\text{-}164}$=11.85	p=0.000			

*p<0.05; BAI: Beck Anxiety Inventory, BDI: Beck Depression Inventory, PBW: Positive beliefs about worry, NBC and DW: Negative beliefs about uncontrollability and danger of worry, CC: Cognitive confidence, NCT: Need to control thoughts, CSC: Cognitive self-consciousness, MCQ-30: Metacognition Questionnaire-30, IUS: Intolerance of Uncertainty Scale

depression.

Discussion

The primary aim of the current study was to investigate the MBs and IoU as potential predictors of anxiety and depression in a sample of community individuals. Also, we aimed to examine the relationship between MBs, IoU, and depression and anxiety. We found that while depression correlated with IoU and all MBs, anxiety only correlated with the "cognitive confidence" subscale of MCQ and total score of MCQ. Multiple linear regression analyses revealed that "cognitive self-consciousness" and "need to control thoughts" predicted depression severity. However, unexpectedly, there were no variables predicting anxiety severity.

In the present study, we observed significant correlations between variables. While depression correlated with all MB and IoU subscales, anxiety just correlated with cognitive confidence and total score of MCQ-30. The cognitive confidence subscale of MCQ-30 can be defined as "concerns with efficacy of ones' cognitive skills, distrust in memory." Nordahl et al. (27) found that trait anxiety had significant correlations with all subscales of MCQ. Although they obtained similar results between trait anxiety and MBs in their evaluations with an interval of 8 weeks, they could not find a relationship between cognitive confidence and anxiety in their first evaluation, but they did in their second evaluation (27). Some studies showed that "cognitive confidence" and negative beliefs about the "uncontrollability and danger of worry" were significantly associated with anxiety (28). Aydın et al. (13) studied individuals with PD and generalized anxiety disorder (GAD). They found a significant relationship between the "need to control thoughts" and GAD severity, while they did not find any relationships between MBs and PD severity (13).

On the other hand, another study showed a relationship between "self-consciousness" and PD severity (13). These findings suggest that the relationship between different anxiety disorders and MBs can vary, and evaluations made at different times can affect these results. Lastly, anxiety had a weak correlation with the MCQ-30 total score in our study. We found that our study's MCQ total and subscales' mean scores were lower than previous studies conducted in healthy controls (13). We could suggest that these results could explain the absence of correlations between anxiety and the subscales of MCQ-30, except for cognitive confidence and the weak relationship between anxiety and the total score of MCQ-30. Also, the total score of MCQ-30 was considered a more durable construct than its subscales, so our findings could be interpreted in line with the previous data (13). Consequently, we could suggest that the participants in our study had success avoiding negative MBs and anxiety.

Surprisingly, we found that the anxiety severity in healthy individuals did not correlate with IoU. Previous literature indicated a relationship between IoU and different anxiety disorders such as social anxiety disorder, generalized anxiety disorder, and PD, and the level of IoU is usually higher in clinical samples compared to control groups (19,29,30). Worry, a construct highly associated with anxiety, is related to IoU in the literature (29). In this study, we did not examine the relation of worry with IoU or focus on the difference between worry and anxiety. We conducted our study with a non-clinical sample. The absence of a correlation between anxiety and IoU in our sample could be related to the study design and sample above characteristics.

In the present study, all subscales of MCQ-30 and total score of MCQ significantly correlated with depression severity. Our findings are consistent with the previous literature suggesting that depression levels have significant correlations with positive and negative MBs in clinical and non-clinical samples (10,22,27). According to the metacognitive theory of depression, positive MBs such as the "need to control thoughts" about rumination could be considered as a solution to analyze the depressive thoughts to get over depression. However, this method's negative consequences could lead to negative MBs such as "uncontrollability and danger of worry" (8). Our findings agree with the theoretical background of the metacognitive model for the relations between positive and negative MBs and depression.

We found positive correlations between depression severity and inhibitory anxiety, prospective anxiety, and total score of IUS. Previous data suggest mixed results regarding the relationship between depression and IoU (17). Some studies suggest that depression is related to IoU (16,29), and some studies suggest that depression is less associated with IoU than anxiety (15,17); even some studies indicate that depression is not related to IoU (20). On the other hand, in studies that found a relationship between depression and IoU, when factors such as rumination and worry were searched for a mediator effect between these two concepts, it was determined that rumination had a mediator role between the two (29). In our study, depression was correlated with IoU moderately, consistent with the previous data (7). However, we needed to examine further analyses to understand the diverse nature of this relationship. Multiple regression analyses were performed to find predictors of depression severity in our study.

Unexpectedly, multiple linear regression analyses revealed that the anxiety model did not show good fitness. We found that neither IoU nor MBs predicted the anxiety severity in our sample. In many studies, previous data confirmed the association between anxiety and cognitive factors such as IoU and MBs (27,31). As mentioned in the introduction, IoU had some dimensions, and we examined "the desire for predictability" and the "tendency to become paralyzed in the face of uncertainty" as predictors in our study. Researchers have suggested that "the desire for predictability" could directly affect worry and that the "tendency to become paralyzed in the face of uncertainty" could be associated with avoidance behavior (32). Even though previous studies found a relationship between IoU and anxiety, our results could be related to different variables not investigated in the study, such as cultural differences.

Researchers showed that MBs were specific predictors of anxiety in nonclinical samples (27). MBs are accepted as a central factor in trait and state emotions and could be the main construct of anxiety (8). However, we used BAI to assess anxiety in our

sample, and BAI measures mostly the somatic component of anxiety. Essential components of anxiety as rumination and worry were not assessed in this study. This could be the reason for the results of the anxiety model.

Our regression model of depression showed that the "need to control thoughts" positively and "cognitive self-consciousness" negatively predicted depression severity. IoU subscales did not predict depression severity, and our third hypothesis was confirmed by multiple linear regression. Some studies found that negative beliefs about the uncontrollability and danger of worry and lack of cognitive confidence predicted depression but cognitive self-consciousness was not a predictor of depression (27). Yilmaz et al. (33) showed that negative MBs explained the additional variance in depression followed by positive MBs. Also, Huntley and Fisher (10) found that negative MBs about uncontrollability and harm contributed more to depression severity than positive MBs in a healthy sample. Contrary to our study, Solem et al. (34) showed that while cognitive confidence, positive beliefs about worry, and negative beliefs about worry were predicting depression, the need to control and cognitive self-consciousness were not predicting depression.

In the depression model, IoU total score and subscales did not predict depression. Prior studies argued that there was an uncertain relationship between IoU and depression (17). Many studies suggested that depression had a relationship with IoU via rumination or MBs (7,22). Some studies suggested that depression and IoU had a specific relationship after controlling other measures (16). Our results aligned with the previous literature suggesting that depression did not have a direct relationship with IoU. Further studies should focus on the mediational role of anxiety and conduct further analyses in different samples.

Study Limitations

Several limitations of the study should be mentioned. The first limitation was the cross-sectional design of our study. We examined our hypothesis in a nonclinical sample, and we could not generalize the findings to a clinical sample. We also excluded the individuals who had a psychiatric diagnosis with psychiatric interview; however, we did not use a structured clinical measurement. Another limitation of the study was that the participants fulfilled the measurement tools that could cause a bias. In addition, we did not examine the role of anxiety between depression and IoU with further analyses. Finally, although we examined the anxiety and depression severity, we did not control the contribution of rumination and worry in our study.

Conclusion

In conclusion, our study showed essential relations between the IoU, MBs, depression, and anxiety in a non-clinical sample. There was a significant positive relationship between MBs, IoU, and depression; however, anxiety correlated with cognitive confidence and total score of MCQ-30. Also, we found that cognitive self-consciousness and the need to control thoughts predicted the depression level, and IoU and MBs did not predict the anxiety level, while previous data addressed contradictory findings. Our findings showed that in our sample, depression had a more close relationship with MBs than anxiety did. Despite the limitations, our study expands the literature and enlightens the possible relationship between two critical cognitive models and anxiety and depression. Future research should focus on the role of rumination and worry in this relationship to extend our results and repeat our study in clinical samples for the generalizability of the findings.

Ethics

Ethics Committee Approval: The Ethics Committee of Ankara Training and Research Hospital approved the procedures and the purposes of the study (decision number: E-23-1197, date: 08.03.2023).

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

Authorship Contributions

Concept: K.S.K., A.G.G., Design: K.S.K., A.G.G., Data Collection or Processing: K.S.K., Analysis or Interpretation: K.S.K., A.G.G., Literature Search: K.S.K., A.G.G., Writing: K.S.K., A.G.G.

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Original Article

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Investigation of the Relationship Between Disc Degeneration and Cross-Sectional Area of Deep Extensor Muscles in Patients with Chronic Non-specific Neck Pain

Kronik Non-spesifik Boyun Ağrısı Olan Hastalarda Disk Dejenerasyonu ile Derin Ekstansör Kasların Kesit Alanı Arasındaki İlişkinin İncelenmesi

Ayla TEKİN[1], Gülüzar GÜL[1], Tuncay ÇOLAK[1], Özgür ÇAKIR[2]

[1]Kocaeli University Faculty of Medicine, Department of Anatomy, Kocaeli, Türkiye
[2]Kocaeli University Faculty of Medicine, Department of Radiology, Kocaeli, Türkiye

ABSTRACT

Objective: The objective of our research is to analyze the impact of the degree of C4-C5 disc degeneration on the relative cross-sectional area of the deep extensor muscles (R-CSA_{DEs}).

Methods: A total of 162 patients, 98 (60.5%) females and 64 (39.5%) males, who presented to our hospital between 2020 and 2022 with chronic non-specific neck pain, were included in our study. Retrospectively, the degrees of C4-C5 disc degeneration were determined through magnetic resonance imaging, and the R-CSA_{DEs} was measured.

Results: The average age of 162 patients was found to be 42.30±8.8, and the average relative CSA of the deep cervical extensor muscles (R-CSA_{DEs}) was found to be 1.96±0.45. C4-C5 disc degeneration was found in 41 (25.3%) patients at grade 0, in 99 (61.1%) patients at grade 1, and in 22 (13.6%) patients at grade 2. The mean R-CSA_{DEs} of male were higher than those of female, and this elevation was statistically significant ($p<0.05$). It was observed that both female ($p<0.001$) and male patients ($p<0.01$) had an increased age as the degeneration grade increased. There was a significant difference in R-CSA_{DEs} between the degrees of disc degeneration in female ($p<0.001$).

Conclusion: In patients with chronic neck pain, especially in female, there is a significant decrease in the R-CSA_{DEs} as the degree of disc degeneration increases.

Keywords: Intervertebral disc degeneration, MRI, neck muscles

ÖZ

Amaç: Çalışmamızın amacı C4-C5 disk dejenerasyonu derecesinin derin ekstansör kasların alanı üzerindeki etkisinin incelenmesidir.

Yöntemler: Araştırmamıza 2020-2022 yılları arasında kronik non-spesifik boyun ağrısı nedeni ile hastanemize başvuran 98'i (%60,5) kadın, 64'ü (%39,5) erkek olmak üzere toplam 162 hasta dahil edildi. Retrospektif olarak manyetik rezonans görüntülemeleri üzerinden C4-C5 disk dejenerasyonu dereceleri belirlenip derin ekstansör kasların alanları ölçüldü.

Bulgular: Yüz altmış iki hastanın yaş ortalaması 42,30±8,8 ve derin servikal ekstansör kasların göreceli kesit alanı (R-CSA_{DEs}) ortalaması 1,96±0,45 bulundu. C4-C5 disk dejenerasyonu 41 (%25,3) hastada derece 0,99 (%61,1) hastada derece 1,22 (%13,6) hastada derece 2 olarak bulundu. Erkeklerin R-CSA_{DEs} ortalamaları kadınlardan daha yüksekti ve bu yükseklik istatistiksel olarak anlamlıydı ($p<0,05$). Hem kadın ($p<0,001$) hem de erkek hastalarda ($p<0,01$) yaş arttıkça dejenerasyon derecesinin arttığı saptandı. Kadınlarda disk dejenerasyon dereceleri arasında R-CSA_{DEs} değerinde anlamlı fark bulundu ($p<0,001$).

Sonuç: Kronik boyun ağrılı hastalarda özellikle kadınlarda disk dejenerasyonunun derecesi arttıkça derin ekstansör kasların alanında anlamlı bir azalma gerçekleşir.

Anahtar Sözcükler: İntervertebral disk dejenerasyonu, MRG, boyun kasları

Address for Correspondence: Ayla Tekin, Kocaeli University Faculty of Medicine, Department of Anatomy, Kocaeli, Türkiye
E-mail: ayla.tekinorha@kocaeli.edu.tr **ORCID ID:** orcid.org/0000-0001-5793-7054

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Introduction

Neck pain is the most common musculoskeletal problem worldwide (1,2) and it can be classified based on its severity, type, etiology, and duration. Among different classification methods, duration is the best indicator and pain lasting beyond twelve weeks is considered chronic (3). In most cases of chronic neck pain, the pain is classified as non-specific because it cannot be attributed to a specific pathological condition (4).

Intervertebral discs are believed to be a major source of neck pain when they undergo degeneration due to their crucial role in the mobility and stabilization of the spine (5,6). Disc degeneration in the cervical region mostly occurs after middle age (7). As disc degeneration progresses, it can lead to herniation and spinal canal stenosis, resulting in costly interventions such as surgical treatment (8). The deep cervical extensor muscles, due to their direct connection of vertebrae to one another, hold a significance in the stabilization of the cervical spine and postural control, comparable to the importance of discs (9,10). Furthermore, it has been noted that there is a negative impact of neck pain on the cross-sectional area (CSA) of the deep cervical extensor muscles (11). Measurements of the CSA of the muscles are significant indicators of fundamental changes in muscle structure, particularly in terms of muscle strength and atrophy (12-14).

It is indeed crucial to diagnose and prevent the progression of disc degeneration and changes in muscle area in individuals with neck pain and those above middle age, to provide treatment without the need for surgical intervention. Therefore, our study aims to analyze the relationship among relative CSA of the deep extensor muscles (R-CSA_{DEs}) and disc degeneration, which we observed to be lacking in the literature, using cervical magnetic resonance imaging (MRI) (15), which are considered the gold standard for chronic neck pain patients.

Methods

Study Group

Between December 2020 and September 2022, a total of 1140 patients who presented to our clinic with complaints of chronic neck pain and underwent cervical MRI were retrospectively evaluated. Patients with masses in the cervical region, vertebral fracture or operation, spondylolysis or spondylolisthesis, congenital malformations, a history of cancer, symptoms related to sensory or motor impairments, and any disorders affecting the cervical region other than chronic neck pain were excluded from the study. Additionally, only three patients were found to have grade III disc degeneration and they were excluded from the research to prevent statistical bias. Finally, a total of 162 patients between the ages of 18 and 65 who met the inclusion criteria were investigated within the scope of the study. This research received approval from Kocaeli University Non-Interventional Clinical Research Ethics Committee (decision number: KÜ GOKAEK-2023/12.02 date: 13.07.2023). Consent was obtained from the patients participating in our study.

Evaluation of Disc Degeneration

T2-weighted MRI is widely used for the evaluation of disc degeneration (16). Cervical MRIs were obtained using a protocol designed for the evaluation of the cervical region from the hospital's Picture Archiving and Communications System radiographic system. MR images were acquired using a 1.5T MRI scanner (GE Healthcare), producing sagittal and axial views.

A comprehensive grading system and algorithm were developed based on previously used grading systems and literature reviews to determine cervical disc degeneration. In our study, we utilized this comprehensive grading system. Cervical disc degeneration was divided into four grades: Grade 0 indicating no degenerative changes, grade 1 (slight degeneration) representing low-density changes in the nucleus pulposus, grade 2 (moderate degeneration) involving annulus fibrosus degeneration along with disc bulging or herniation and finally grade 3 (severe degeneration) indicating more significant degeneration with a reduction in disc height by more than ¼ (17-18).

Calculating Cervical Muscle Area

Calculating muscle CSA based on MR scans, which is considered the top reference for muscle scanning, is an objective and non-invasive assessment method (19). The muscle CSA was measured on axial T2-weighted and T1-weighted scans at the level of the C4-C5 intervertebral disc. The imaging parameters included: Slice thickness=4 mm, field of view=160×160 mm, repetition time/echo time=4438/116 ms, and matrix=160×256, acquired from 16 slices. To minimize the influence of segmental levels, all muscles were examined at the same level (C4-C5 intervertebral disc level) (20). The reason for selecting the C4-C5 level was that the angle between the axial image passing through this level and the plane of the floor was minimal compared to other levels.

The CSAs of the bilateral multifidus cervicis and semispinalis cervicis muscles, which together form the deep cervical extensors, were measured by following the boundaries of the fascia and recorded in square millimeters (mm^2) by using the Sectra software program (Sectra workstation IDS7, Linköping, Sweden). Since the semispinalis cervicis and multifidus muscles could not be clearly distinguished, the measurements were combined (4). To control alterations in the muscle areas caused by changing body mass index, the relative muscle CSA/vertebral body area (VBA) ratios were preferred instead of exact muscle CSA values. The relative CSAs (R-CSAs), defined as the ratios of muscle CSA/VBA, were calculated by measuring the C5 VBAs on axial images (21). At the level of the C4-C5 intervertebral disc, the CSAs of the deep extensors on the right and left sides were individually summed and divided by the C5 VBAs to calculate the relative CSAs of the deep extensors (R-CSA_{DEs}) (Figure 1). All MRI evaluations, degeneration grades and R-CSA_{DEs} measurements were performed under the supervision of two experienced radiologists in the field.

Statistical Analysis

The statistical analysis was conducted utilizing IBM SPSS 20.0 (IBM Corp., Armonk) software package. The normal distribution assumption was assessed utilizing the Kolmogorov-Smirnov and Shapiro-Wilk tests. Numerical variables were presented as mean ± standard deviation or median (25^{th}-75^{th} percentile), while categorical variables were presented as frequency (percentage). Differences among groups were identified through independent samples t-test, Mann-Whitney U test, one-way analysis of variance (ANOVA), and Kruskal-Wallis test. Tukey and Dunn tests were utilized for multiple comparisons. The relationships among numerical variables were examined utilizing Pearson and Spearman correlation analysis. The relationships among categorical variables were defined utilizing the chi-square test. A p-value less than 0.05 was evaluated statistically significant for hypothesis testing.

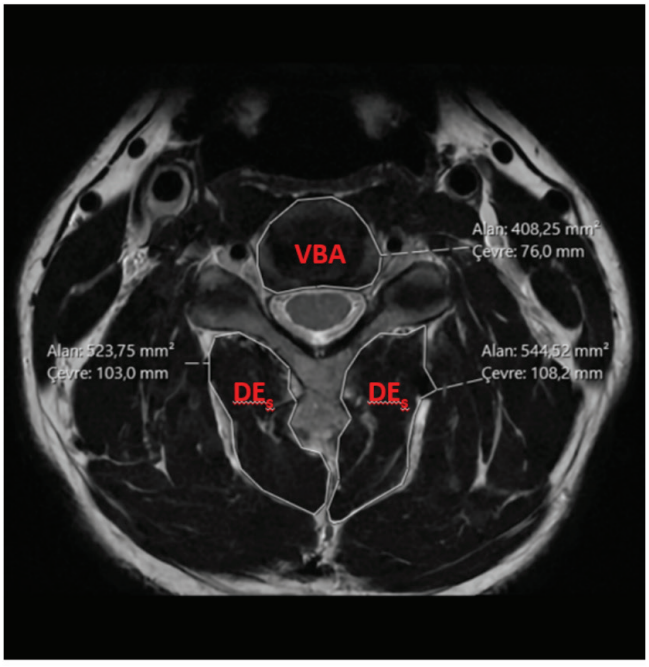

Figure 1. Measurements of the deep extensor muscles areas on axial T2-weighted passing through the C4-C5 disc level

DEs: Deep extensors, VBA: Vertebral body area

Results

One hundred sixty-two patients were included in the research, composed of 98 (60.5%) females and 64 (39.5%) males. The average age of the 162 patients was 42.30±8.8 and the average R-CSA_{DEs} was 1.96±0.45. The degree of disc degeneration at the C4-C5 level was grade 0 in 41 (25.3%) patients, grade 1 in 99 (61.1%) patients and grade 2 in 22 (13.6%) patients. There was no significant difference in age among females and males (p=0.93). But a significant difference was found in the degree of degeneration between genders (p=0.049). The grade 0 degree was statistically significantly more detected in females comparison to males (p=0.022), while the grade 1 degree was statistically significantly more detectedin males than females (p=0.023). No significant difference was detected among genders regarding the grade 2 degeneration. A notable distinction was detected in the mean R-CSA_{DEs} among genders (p=0.038), with males having higher mean R-CSA_{DEs} than females (Table 1).

When examined both in all patients (p<0.001) and separately in female patients (p<0.001) and male patients (p=0.004), it was observed that age increased with the increase in degeneration grade. Significant differences in age were found between grade 0 and grade 1, grade 0 and grade 2, and grade 1 and grade 2 in both total patients and female patients. However, in male patients, significant differences in age were only observed between grade 0 and grade 2, and grade 1 and grade 2. It was observed that there was a decrease in R-CSA_{DEs} value with an increase in degeneration grade in both all patients and female and male patients. In both total patients and female patients, significant differences in R-CSA_{DEs} value were found between grade 0 and grade 2, and grade 1 and grade 2 (p<0.001). But, in male patients, no significant difference in R-CSA_{DEs} value was found among all grades (p=0.130) (Table 2).

There was no correlation found between age and R-CSA_{DEs} value in the total population, as well as when analyzed separately for females and males (Table 3).

Discussion

Neck pain complaints are most common in middle-aged people and female (22,23), has a prevalence rate of 37.2% in the adult population and is increasing gradually (24). Non-specific neck pain diminishes patients' quality of life by negatively impacting

Table 1. Characteristics, disc degeneration and cervical muscle area of study groups

	Total (n=162)	Female (n=98)	Male (n=64)	p-value
Age (year)	42.30±8.83*	43 (35-49.25)**	43 (36.25-47.75)**	0.93
Grade, n (%)				
0	41 (25.3)	31 (31.6)	10 (15.6)	
1	99 (61.1)	53 (54.1)	46 (71.9)	0.049
2	22 (13.6)	14 (14.3)	8 (12.5)	
R-CSA_{DEs}	1.96±0.45*	1.90±0.45*	2.05±0.43*	0.038

*: mean ± standard deviation, **: Median (interquartile range)

Table 2. Relationships between cervical muscle area and age with disc degeneration

	Grade 0	Grade 1	Grade 2	p-value
Total, mean ± SD				
Age (year)	36.29±7.49	42.48±7.63	52.64±6.09	0.001
R-CSA$_{DEs}$	(2.12±0.38)^{a}	(1.97±0.45)^{ab}	(1.65±0.40)^{c}	0.001
Female, median (interquartile range)				
Age (year)	37 (31-42)	43 (38-49)	52 (49.75-56.75)	0.001
R-CSA$_{DEs}$	2.09 (1.85-2.21)^{a}	1.87 (1.52-2.26)^{ab}	1.35 (1.22-1.75)^{c}	0.001
Male, median (interquartile range)				
Age (year)	42.50 (32.75-43.25)	42 (35-47)	54 (48.25-58.75)	0.004
R-CSA$_{DEs}$	2.22 (1.99-2.60)	1.94 (1.72-2.37)	1.93 (1.70-2.06)	0.130

SD: Standard deviation, R-CSA$_{DEs}$: Relative cross-sectional area of the deep extensor muscles
Different letters indicate statistically significant differences

Table 3. Correlation of cervical muscle area and age

Total	R-CSA$_{DEs}$
Age (year)	r-0.088
	p 0.266

R-CSA$_{DEs}$: Relative cross-sectional area of the deep extensor muscles

their social interactions and participation in daily life activities (25). There are very few studies in the literature that compare the degrees of cervical disc degeneration in male and female with neck pain. In a study comparing 31 females and 31 males with neck pain, it was found that females had statistically lower disc narrowing, and protrusion compared to males (26). In the group without severe neck pain, females showed statistically lower disc height narrowing and C4-C5 degeneration degree compared to males (27). Like our study, despite females having a lower degree of disc degeneration compared to males, one of the reasons for their higher clinical presentation might be attributed to females having lower pain perception thresholds than males (28). A study conducted on full-body MRI of 468 individuals indicated that females had less skeletal muscle mass compared to males (29). In accordance with the literature, we can suggest that the greater R-CSA$_{DEs}$ in males compared to females may arise from gender-related differences in total muscle mass (Table 1) (30,31).

As age advances, biochemical changes such as a reduction in proteoglycan content and water within the disc, particularly in the nucleus pulposus, contribute to the progression of degeneration in intervertebral discs (32). Furthermore, genetic, traumatic, biomechanical, and nutritional factors can also contribute to degeneration in the intervertebral discs (33). Numerous studies investigating the relationship between degenerative changes in the cervical region and age have consistently indicated that as age increases, the degree of disc degeneration also increases (18,19,34). In a study conducted on 143 female patients, a significant decrease in R-CSA$_{DEs}$, specifically the multifidus muscle, was observed with an increase in the degree of degeneration after grade 2 (34).

We confirmed an increase in the degree of disc degeneration with age in both female and male. Based on our results, we can say that aging, along with multifactorial effects, is the main pathogenesis of degeneration (35). As one of the first studies examining the relationship between R-CSA$_{DEs}$ value and the degree of C4-C5 disc degeneration, we detected a statistically significant decrease in the R-CSA$_{DEs}$ value as the degree of disc degeneration increased in female patients. However, in male patients, we found that the decrease in R-CSA$_{DEs}$ value with an increase in disc degeneration was not statistically significant (Table 2).

Indeed, as the degree of degeneration in the disc increases, we can say that there is atrophy of the cervical deep extensor muscles (DEs) area in females. The decrease in the R-CSA$_{DEs}$ has been reported to reduce stabilization in the cervical spine and increase the severity of pain and the neck disability index (36). As a result, we can say that disc degeneration affects the R-CSA$_{DEs}$ in males to a lesser extent than in females, and the decrease in R-CSA$_{DEs}$ in females leads to experiencing more pain and consequently seeking medical attention more frequently than males.

Age-related reduction in muscle volume has been observed, particularly after the age of fifty or sixty (32,37). Additionally, it has been noted that the CSA of cervical extensor muscles also decreases after the age of fifty (30). In our study, the average age was 43, and since we had only 29 patients who were over 50 years old, we believe that we could not find any relationship between age and the R-CSA$_{DEs}$ value (Table 3).

Study Limitations

Our study had a few limitations. Firstly, we were unable to inquire about the history of chronic illnesses for example diabetes that could be related to smoking, alcohol use, occupation, and disc degeneration. Secondly, we believe that comparing our data with healthy individuals without chronic neck pain would provide more comprehensive information on this subject.

Conclusion

According to our study, as age advances, the degree of disc degeneration increases, and as the degree of disc degeneration increases, the R-CSA$_{DEs}$ decreases. As a result, there is a weakening tendency in DEs, especially in females with chronic neck pain and intervertebral disc degeneration. Therefore, we believe that strengthening exercises for DEs should be added to rehabilitation

programs, especially for female patients with chronic neck pain, to reduce pain intensity and prevent further disability problems.

Ethics

Ethics Committee Approval: This research received approval from Kocaeli University Non-Interventional Clinical Research Ethics Committee (decision number: KÜ GOKAEK-2023/12.02, date: 13.07.2023). The study was carried out in accordance with the ethical rules of the Declaration of Helsinki.

Informed Consent: Consent was obtained from the patients participating in our study.

Authorship Contributions

Concept: A.T., G.G., Design: A.T., G.G., T.Ç., Data Collection or Processing: A.T., G.G., Ö.Ç., Analysis or Interpretation: A.T., G.G., T.Ç., Ö.Ç., Literature Search: A.T., G.G., Writing: A.T., G.G., T.Ç.

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

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Original Article

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Triglyceride-Glucose Index is a Reliable Predictor of Metabolic Disorder in Gallstones

Trigliserit-Glikoz İndeksi Safra Taşlarında Metabolik Bozukluğun Güvenilir Bir Göstergesidir

Erkan AKSOY[1], Zeynep ERGENÇ[2], Özlem KARACA OCAK[3], Hasan ERGENÇ[2]

[1]Medical Park Hospital, Clinic of General Surgery, Ordu, Türkiye
[2]Ayancık State Hospital, Clinic of Internal Medicine, Sinop, Türkiye
[3]Medicana International Samsun Hospital, Clinic of General Surgery, Samsun, Türkiye

ABSTRACT

Objective: The risk of gallstones is increased in fatty liver, overweight, and metabolic syndrome. The hyperinsulinemic-euglycemic clamp method is considered the best method for insulin resistance (IR); but it is a time consuming and expensive procedure. As an alternative strategy, in this study, we would like to demonstrate the triglyceride-glucose (TyG) index as the predictor of the development of gallstone disease associated with metabolic disorders.

Methods: The TyG was investigated in our study with 1484 patients between the ages of 18-75 who underwent follow-up and/or cholecystectomy due to gallstones. The serum fasting blood glucose and triglyceride levels of the patients were measured in the biochemistry laboratory of our hospital. The bile ducts and gallstones of all patients were evaluated with the Philips Affiniti 50 (The Philips Affiniti 50 Ultrasound; Philips North America Corporation 3000 Minuteman Road M/S 109 Andover, MA 01810, USA) ultrasonography device used by radiologists in the radiology clinic of our hospital. The data obtained were evaluated in SPSS, and $p<0.05$ was considered significant.

Results: Our study includes 1484 (980 female, 504 male) patients. The plasma glucose (124.6±24.5 mg/dL), triglyceride (198.3±36.8 mg/dL), insulin (16.5±5.6 uIU/mL), and Homeostasis Model Assessment of IR 4 level (8±1.2), were at statistically and significantly higher levels in the patients than in the control group ($p=0.001$). It was found that the basal TyG was associated with the primary endpoint incidence of gallstones at significant levels. The

ÖZ

Amaç: Yağlı karaciğer, obezite ve metabolik hastalıklarda safra kesesi taşı görülme riski artmıştır. Hiperinsülinemik-glisemik klemp yöntemi insülin direnci (IR) için "altın standart" bir yöntem olarak kabul edilmektedir, ancak zor ve maliyetlidir. Alternatif bir yöntem olarak daha hızlı ve daha düşük maliyetli biyokimyasal ölçüm olan trigliseride-glukoz (TyG) indeksi, metabolik bozukluklarla ilişkili olan safra kesesi taşı hastalığı tanısında yararlı olabilir. Bu çalışmanın amacı safra kesesi taşı tanısında TyG indeksinin etkinliğini değerlendirmektir.

Yöntemler: Çalışmada, safra kesesi taşı ile takip ve/veya kolesistektomi yapılan 18-75 yaş aralığında olan 1484 olgularda TyG indeksi araştırıldı. Hastaların serum açlık kan glukoz ve trigliserid düzeyleri hastanemiz biyokimya laboratuvarında ölçüldü. Tüm hastaların safra yolları ve safra taşları hastanemiz radyoloji kliniğinde radyologlar tarafından kullanılan Philips Affiniti 50 (The Philips Affiniti 50 Ultrasound; Philips North America Corporation 3000 Minuteman Road M/S 109 Andover, MA 01810, USA) ultrasonografi cihazı ile değerlendirildi. Elde dilen veriler SPSS'de değerlendirildi, $p<0,05$ anlamlı kabul edildi.

Bulgular: Araştırmaya toplam 1484 (980 kadın ve 504 erkek) hasta alındı. Çalışmamızdaki biyokimyasal parametrelerden açlık safra kesinde taş saptanan olgularda plazma glukoz 124,6±24,5 mg/dL, trigliserid 198,3±36,8 mg/dL, insülin 16,5±5,6 uIU/mL, IR'nin Homeostatik Modeli Değerlendirmesi 4,8±1,2 seviyesi kontrol grubuna göre hepsi istatistiksel olarak anlamlı yüksekti ($p=0,001$). Bazal TyG indeksi, primer sonlanım noktası safra kesesi taşı insidansı ile önemli ölçüde ilişkili olduğu saptandı. Tek değişkenli

Address for Correspondence: Hasan Ergenç, Ayancık State Hospital, Clinic of Internal Medicine, Sinop, Türkiye
E-mail: dr.hasanergenc@hotmail.com **ORCID ID:** orcid.org/0000-0003-0519-0264

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ABSTRACT

TyG was related with hazard ratio 1.65 as a continuous variable in univariate analysis (95% confidence interval 1.15-1.98; p<0.001).

Conclusion: High TyG scores were associated with gallstone disease, and it can be used as an identifying marker for screening people who have gallstone disease and as an indication for therapeutic precautions.

Keywords: Triglyceride-glucose index, metabolic disorder, gallstones

ÖZ

analizde, sürekli bir değişken olarak TyG indeksi, risk oranı 1,65 (%95 güven aralığı 1,15-1,98; p<0,001) ile ilişkilendirildi.

Sonuç: Yüksek TyG skorları safra taşı hastalığıyla ilişkilendirilmiştir ve safra taşı hastalığı olan kişileri taramak için tanımlayıcı bir belirteç ve terapötik önlemler için bir gösterge olarak kullanılabilir.

Anahtar Sözcükler: Trigliserid-glukoz indeksi, metabolik hastalıklar, safra taşı

Introduction

Gallstones are common in the world. The prevalence of gallstones has a frequency of 10-15% in developed countries (1). If we identify the chain of events that result in the formation of gallstones, many studies were conducted in the past to elucidate the factors and mechanisms responsible for the nucleation of crystals (2). There are some theories about gallstone formation. But the most accepted theories are the changes in bile composition (oversaturation of calcium bilirubinate, pigment and cholesterol stones), increased crystal nucleation in the presence of mucin and similar factors, and slowing of bile flow (3).

The risk of especially gallstones is increased in fatty liver, obesity and metabolic diseases (4). It has been reported in recent years that metabolic disorders are associated with diseases such as fatty liver, type 2 diabetes mellitus (DM), metabolic syndrome, and hyperlipidemia (5,6). Insulin resistance (IR) is the predictor of the onset of these diseases, and is a widely used marker (7). It is already known that IR is a key factor in the pathogenesis of metabolic abnormalities (8). The hyperinsulinemic-euglycemic clamp (HEC) method is considered as the best method for IR; however, it is a difficult process and costly. As an alternative strategy, we would like to investigate the triglyceride-glucose (TyG) index which is a faster and low-cost biochemical measurement tool, as the predictor of the development of gallstone disease associated with metabolic disorders for the first time.

Methods

The TyG index was investigated in our study with 1484 patients, who were between the ages of 18-75, who underwent follow-up and/or cholecystectomy due to gallstones in our general surgery clinic between 2010-2021. Patients who were pregnant, who had acute inflammation, alcohol use, history of cancer, renal or infectious hepatitis (anti-HCV positive), and liver cirrhosis were excluded from the study. Those who had a history of DM or newly diagnosed diabetic patients, those who used triglyceride-reducing medication (statins, fibrates, omega-3, thiazolidinedione, or insulin) were also excluded from the study.

The demographic characteristics of the patients, their biochemical data, ultrasonographic examination reports of the bile ducts were collected from the patient files and electronic records. The serum fasting blood glucose and triglyceride levels of the patients were measured in the biochemistry laboratory of our hospital. Plasma glucose (cut-off level 70-100 mg/dL) was determined with the glucose oxidase method. The triglyceride (cut-off level 0-150 mg/dL) level was determined with the enzymatic method. Fasting plasma was calculated by using glucose and insulin (cut-off level 0-25 uIU/mL) and Homeostasis Model Assessment for IR (HOMA-IR) [HOMA-IR: fasting insulin (uIU/mL) x fasting glucose (mg/dL)/405] (9). The TyG index was calculated with the formula in [fasting TGs (mg/dL) x fasting glucose (mg/dL)/2] and using fasting triglyceride and glucose (10). The biliary tract and gallstones of all patients were evaluated with the Philips Affiniti 50 (The Philips Affiniti 50 Ultrasound; Philips North America Corporation 3000 Minuteman Road M/S 109 Andover, MA 01810, USA) ultrasonography (US) device used by radiologists in our hospital's radiology clinic. Philips Affiniti 50 brand US device is a well-equipped device that provides radiologists with new levels of clinical knowledge by providing easy examination with advanced organ modeling, image sectioning and proven quantification.

This study was conducted in accordance with the ethical rules with the approval of Medicana International Samsun Hospital Clinical Research Ethics Committee (decision no: 7136, date: 20.05.2021). All participants were informed before the study and were included in the study after their consent was obtained. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

The data was transferred to IBM SPSS Statistics 22 (IBM, Armonk, NY, USA) and processed. Frequency distribution (number, percentage) for categorical variables and descriptive statistics (mean, standard deviation) for numerical variables were provided while assessing the study data. The Kolmogorov-Smirnov test was used to determine if numerical variables followed a normal distribution. For normal distribution variables, independent sample t-test was employed to see if there was a difference between the two groups. For variables that did not fit a normal distribution, the Mann-Whitney U test was utilized. To assess the association between two category variables, the chi-square test was performed. In addition, the cut-offs for the variables were determined using receiver operating characteristic

(ROC) analysis. The statistically significant two tailed p-value was considered as <0.05.

Results

A total of 1484 (980 female, 504 male) patients were included in the present study. The mean age of the patients was 54±12.5 (18-75). The biochemical parameters used in our study, the plasma glucose (124.6±24.5 mg/dL), triglyceride (198.3±36.8 mg/dL), insulin (16.5±5.6 uIU/mL), and HOMA-IR 4 level (8±1.2) were at statistically and significantly higher levels in the patients than in the control group (p=0.001). The clinical data, demographic characteristics, and biochemical data of the cases are shown in Table 1.

The fasting plasma glucose and triglyceride levels were found to be higher at statistically significant levels in the group with gallstones when compared to the group without gallstones in our study (p=0.001). It was found that the basal TyG index was associated with the primary endpoint incidence of gallstones at significant levels. The TyG index was associated with hazard ratio (HR) 1.65 as a continuous variable in univariate analysis [95% confidence interval (CI) 1.15-1.98; p<0.001]. The cut-off value of the TyG index was 4.82 for gallstone disease. Area under the ROC curve (AUROC)=0.789 (95% CI 0.783-0.795). The ≥4.82 TyG Index value identified gallstone disease with a sensitivity of 74.2% and a specificity of 72.5% (Figure 1). The TyG index value was accepted as 4.49; the intersection point with IR with a sensitivity of 82.6% and specificity of 82.1% in previous studies (AUC=0.889, 95% CI: 0.854-0.924) (11).

Discussion

Among the pathogenic factors for gallstones, genetic predisposition, hepatic oversecretion of cholesterol, oversaturated bile, oversaturation and inflamation in the gallbladder, and altered bowel microbiota are mentioned in previous studies (12). The pathophysiology of this disease are associated with IR, overweight, metabolic diseases, and DM (13,14). Gallstones are among the consequences of metabolic disorders. In this respect, using tests and markers to predict metabolic diseases may be predictive for gallstones from the consequences of metabolic diseases.

IR was used as an important tool for metabolic disorders for many years (15). The HEC is the best test for IR measurement. However, it cannot be used commonly in clinical settings because of the complexity of the test process (16). Metabolic disorders are related with the chronic increases in plasma glucose and triglycerides. It was reported in recent studies that the TyG index is associated with IR that is measured with the HEC test at significant levels. It was shown that it performs better than the HOMA-IR evaluation (17,18). In the present study, it was found that the HOMA-IR level was higher in gallbladder stone disease. We can speculate that some gallstones are the result of metabolic diseases.

The pathogenesis of gallstones has not yet been fully understood. New risk factors continue to be identified in addition to previous risk factors (19). In this respect, one of the most important risk factors is IR. Many previous studies showed that non-insulin-dependent diabetic patients have a risk factor in this regard (20).

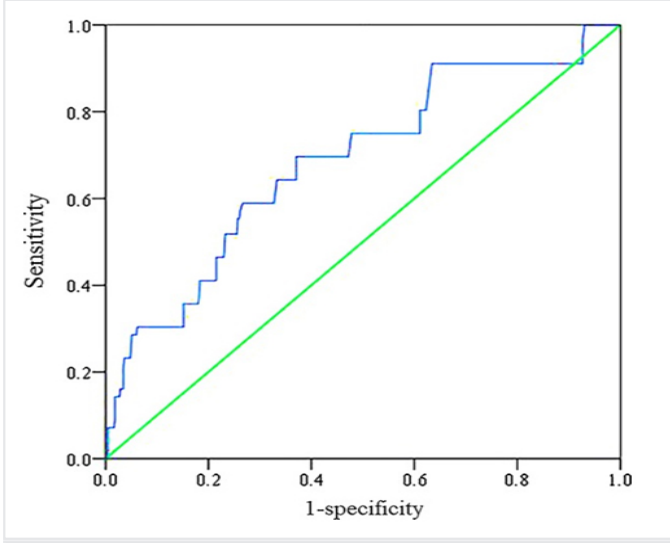

Figure 1. The ROC curves of the triglyceride-glucose index, as a marker for predicting gall stone disease. The AUC of ROC curves of the triglyceride-glucose index to predict gall stone disease (AUC) 0.789 (%95 CI 0.783-0.795; p=0.001).

ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval

Table 1. The relation between clinical, demographic, biochemical characteristics and TyG index values of the participants of both groups

Parameters	Gallstones (n=1484)	Non-gallstones (n=256)	p-value
Age	58.5±12.4	59.1±11.5	0.592
Female, sex (%)	980 (66.03%)	175 (68.35%)	0.494
Fasting plasma glucose (mg/dL)	124.6±24.5	102.5±18.3	0.001
Triglycerides (mg/dL)	198.3±36.8	156.9±24.7	0.001
Insulin (uIU/mL)	16.5±5.6	9.5±1.1	0.001
TyG index	5.06±0.04	4.81±0.03	0.001
HOMA-IR	4.8±1.2	2.3±0.7	0.001

Data are expressed as mean±SD, median or number (%). TyG index: Triglyceride-glucose index, HOMA-IR: Homeostasis model assessment of insulin resistance

In these studies, it was reported that the most important risk was that IR might be the primary factor playing roles in the formation of gallstones (21,22). The relation between gallstones and metabolic syndrome and/or IR syndrome is the subject of recent reports (22,23). However, there are very few data on the relation with IR. It was found in our study that the fasting plasma glucose and insulin values, which are among the metabolic parameters, were statistically higher in the patients compared to the control group. We also detected IR in the group with gallstones.

It was reported in previous studies that the risk factors for cholesterol gallstones were increased waist circumference and central fat deposition. It was shown that these two factors are frequently related with hyperlipidemia (especially hypertriglyceridemia and low-high-density lipoprotein concentrations) (24,25). These increased bile cholesterol concentrations and gallbladder mucin secretion caused by hypertriglyceridemia may constitute the basic steps in the pathogenesis of gallstones (26-28). We showed in our study that the triglyceride level is significantly higher. It might be the preliminary finding of metabolic gallstones.

It was shown in recent studies that the TyG index can predict the incidence of fatty liver disease (29). It was shown that it is the definition and predictor of metabolic syndrome (30). It was also shown to be a risk determinant and predictor in prediabetes, diabetes, coronary syndrome, hypertension, macrovascular complications and obesity, which are the reflections of IR and/or metabolic diseases, and these diseases are strongly associated with the TyG index (31-34). Some gallstones are detected in patients with metabolic syndrome associated with hyperinsulinemia, hypertriglyceridemia, and high glucose levels. IR, metabolic syndrome, and gallstones were investigated very little in previous studies, and the data are very inadequate in this respect. The relation between the TyG index and gallstones was not investigated until now. Our study is the first one in this regard. It was determined in our study that the incidence of gallstone disease, which is the primary endpoint and the TyG index were significantly associated. As a continuous variable, the TyG index was associated with HR 1.65 in univariate analysis (95% CI 1.15-1.98; p<0.001). The cut-off value of the TyG index was 4.82 for gallstone disease. AUROC=0.789 (95% CI 0.783-0.795). The TyG index ≥4.82 identified gallstone disease with a sensitivity of 74.2% and a specificity of 72.5%. We showed that there is a strong relation between gallstone disease and the TyG index.

Conclusion

When the findings of this study were evaluated, it was found that high TyG index scores were associated with gallstone disease. We showed that the TyG index is a definition and predictor of gallstone disease. For this reason, it can be used as an identifying marker for screening people who have gallstone disease and as an indication for therapeutic precautions.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the ethical rules with the approval of Medicana International Samsun Hospital Clinical Research Ethics Committee (decision no: 7136, date: 20.05.2021).

Informed Consent: All participants were informed before the study and were included in the study after their consent was obtained.

Authorship Contributions

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

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Original Article

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The Role of Glyoxal, an Advanced Glycation Product, in Diabetic and Non-diabetic Patients with COVID-19

COVID-19 Geçiren Diyabetli ve Diyabetli Olmayan Hastalarda İleri Glikasyon Ürünü Olan Glioksalin Rolü

Nilgün IŞIKSAÇAN[1], Gülçin ŞAHİNGÖZ ERDAL[2], Duygu TEKSÖZ[3], Pınar KASAPOĞLU[1], Mustafa YAMAN[4], Halime UĞUR[5], Kadriye KART YAŞAR[6]

[1]University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Biochemistry and Immunology, İstanbul, Türkiye
[2]University of Health Sciences Türkiye, Hamidiye Health Sciences Institute, Department of Immunology, İstanbul, Türkiye
[3]University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Biochemistry, İstanbul, Türkiye
[4]İstanbul Sabahattin Zaim University Faculty of Health Sciences, Department of Nutrition and Dietetics, İstanbul, Türkiye
[5]İstanbul Medeniyet University Faculty of Health Sciences, Department of Nutrition and Dietetics, İstanbul, Türkiye
[6]University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, İstanbul, Türkiye

ABSTRACT

Objective: The aim of this study was to investigate the effect on coronavirus disease-2019 (COVID-19) of glyoxal (GO), which is a precursor of advanced glycation end-products, in diabetic and non-diabetic patients, treated in hospital for COVID-19 who were of similar age and disease severity with similar comorbidities.

Methods: The study included 57 patients. Measurement of GO was made with the high-performance liquid chromatography method.

Results: The GO values were found to be higher in the diabetic group than in the non-diabetic group (p=0.001, p<0.01). The length of stay in hospital was longer in the diabetic group (p=0.017, p<0.05). The white blood cell, neutrophil, neutrophil/lymphocyte ratio, procalcitonin, and ferritin values were determined to be higher in the diabetic group than in the non-diabetic group (p=0.006; p=0.005, p=0.017, p=0.011, p=0.030). Although the mortality and intensive care unit admission rates were similar in the diabetic and non-diabetic patients of similar age with similar comorbidities and COVID-19 severity, the total length of stay in hospital was determined to be longer in the diabetic patients.

ÖZ

Amaç: Bu çalışmanın amacı, ileri glikasyon son ürünlerinin öncülü olan glioksalin (GO), koronavirüs hastalığı-2019 (COVID-19) nedeniyle hastanede tedavi gören benzer yaş ve benzer komorbiditeli hastalık şiddetine sahip diyabeti ve diyabeti olmayan hastalardaki etkisini araştırmaktır.

Yöntemler: Çalışmaya 57 hasta dahil edildi. GO ölçümü yüksek performanslı sıvı kromatografi yöntemiyle yapıldı.

Bulgular: Glioksal değerleri diyabetik grupta diyabetik olmayan gruba göre daha yüksek bulundu (p=0,001, p<0,01). Hastanede kalış süresi diyabetik grupta daha uzundu (p=0,017, p<0,05). Beyaz kan hücresi, nötrofil, nötrofil/lenfosit oranı, prokalsitonin ve ferritin değerlerinin diyabetik grupta diyabetik olmayan gruba göre daha yüksek olduğu belirlendi (p=0,006; p=0,005, p=0,017, p=0,011, p=0,030). Benzer yaştaki, komorbiditeleri ve COVID-19 şiddeti benzer olan diyabetik ve diyabetik olmayan hastalarda mortalite ve yoğun bakıma yatış oranları benzer olmasına rağmen, diyabetik hastaların hastanede toplam kalış süresinin daha uzun olduğu belirlendi. Diğer enflamasyon belirteçleriyle birlikte GO'nun diyabetik grupta daha yüksek olduğu belirlendi.

Address for Correspondence: Nilgün Işıksaçan, University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Biochemistry and Immunology, İstanbul, Türkiye
E-mail: nisiksacan@gmail.com **ORCID ID:** orcid.org/0000-0002-0230-6500

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ABSTRACT

Together with other inflammation markers, GO, was determined to be higher in the diabetic group.

Conclusion: The results of this study showed that elevated GO could be associated with a prolonged recovery time in COVID-19.

Keywords: Advanced glycation end-products, COVID-19, diabetes, glyoxal, length of stay in hospital

ÖZ

Sonuç: Bu çalışmanın sonuçları, yüksek GO'nun COVID-19'da uzun süreli iyileşme süresiyle ilişkili olabileceğini gösterdi.

Anahtar Sözcükler: İleri glikasyon son ürünleri, COVID-19, diyabet, glioksal, hastanede kalış süresi

Introduction

The whole world has been affected by the coronavirus disease-2019 (COVID-19) pandemic for more than 2 years and as of December 2021, approximately 696 million people worldwide have contracted this disease and approximately nearly 6.9 million lives have been lost associated with the disease. In Türkiye, nearly 17.2 million people have caught the infection to date, and the number of deaths is approximately 102.174 (1). The morbidity and mortality rates of COVID-19 are known to be increased by advanced age, and comorbidities such as hypertension (HT) and diabetes (2). The frequency of type 2 diabetes is currently increasing in direct proportion to the increasing incidence of obesity and poor nutrition, especially in developed countries (3).

One of the mechanisms leading to damage in several body systems in diabetes is an increase in the level of advanced glycation end-products (AGE) (4). AGEs are a heterogenous substance group in irreversible form as a result of a non-enzymatic reaction between reducing sugars such as fructose and glucose, and proteins, lipids, or nucleic acids (5). In addition to AGE obtained directly from the diet from foods with a high level, the consumption of foods containing high amounts of simple sugars causes endogenous AGE production (6). Glyoxal (GO) is a precursor of AGE. Although the accumulation of AGEs in tissue is known to play a role in the pathogenesis of diseases such as atherosclerosis, neurodegenerative and chronic inflammatory diseases, the accelerated accumulation of AGE in conditions such as diabetes and insulin resistance in particular, leads to the early development of comorbidities (7-9).

The aim of this study was to investigate the effect on COVID-19 of GO, which is a precursor of AGE, in diabetic and non-diabetic patients, treated in hospital for COVID-19 who were of similar age and disease severity with similar comorbidities.

Methods

Approval for this prospective study was granted by the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital's Ethics Committee (desicion no: 2020-12-37, date: 08.06.2020). All the procedures were applied in compliance with the principles of the Helsinki Declaration. Prior to enrollment, each patient provided written informed consent for their participation in the study. The study included patients diagnosed as having COVID-19 from a positive real-time polymerase chain reaction test of a nasal and pharyngeal smear sample, who were admitted for treatment to our hospital between 1 March and 31 May 2021. Patients were excluded from the study if they were aged <18 years, did not wish to participate in the research, had body mass index >30, had received immunosuppressive treatment, had congenital immunosuppression or had received anti-inflammatory treatment within the last 15 days. The treatments of the patients in the study were not changed and no additional drugs were administered for the study.

The patients in the study were separated into 2 groups as those with and without type 2 diabetes according to the American Diabetes Association criteria (10). Care was taken that the groups had similar distribution of age, gender, and comorbidities. In accordance with the hospital rules, patients were only fed from the hospital canteen and received no food from outside the hospital.

On first presentation at the hospital, a 2 mL venous blood sample was taken by an experienced nurse, after at least 8 hours fasting, before the administration of any antiviral or immunosuppressive treatments, including steriods.

Hemoglobin A1c (HbA1c) was measured with the high-performance liquid chromatography (HPLC) method in an Adams Premier Hb 9210 (Trinity Biotech, USA) device.

For the measurement of GO, a system similar to HPLC with some modifications, as explained by Cengiz et al. (11) was used. The HPLC system includes a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan) and a Shimadzu LC 20AT pump. The mobile phase is formed of methanol, water, and acetonitryl (42:56:2, v/v/v) and has a 254 nm wavelength. GO was separated with an Inersil ODS-3, 250x4.6 mm, 5 um column at the rate of 1 mL/min. The temperature of the column oven was 30 °C. The reference ranges in the hospital laboratory were as follows; white blood cells (WBC): 3.7-10.1x10^3/µL, neutrophils (NEU): 1.63-6.96x10^3/µL, lymphocytes (LYM): 1.09-2.99x10^3/µL, and hemoglobin (HGB): 12.9-15.9 g/dL. The upper limit was defined as 0.5 ng/mL for procalcitonin (PCT) and 5 mg/mL for C-reactive protein (CRP). Internal quality control and external quality reliability were applied for the accuracy of the tests applied. A Beckman Coulter AU5800 clinical chemistry analyzer (Beckman Coulter, Brea, CA, USA) was used to measure ferritin levels and an ADVIA 2120 hematology autoanalyzer

(Siemens Healthcare Diagnostics, Erlangen, Germany) was used for complete blood count.

Classification of the lung involvement of the patients was performed using a semi-quantitative scoring system on computed tomography images. Each of the 5 lobes of the lungs were scored from 0-5 as follows; 0: no involvement, 1 <5% involvement, 2: 25%, 3: 26-49%, 4: 50-74%, and 5: >75% involvement. For this study, the patients were separated into 3 groups according to the severity of pneumonia as mild (<25% lung involvement on CT), moderate (26-74% lung involvement), and severe (>75% involvement).

In the classification of patients according to clinical condition, those with mild symptoms and oxygen saturation within normal limits (>98%) were accepted as mild disease, patients with blood oxygen saturation of 98-94% in room air as moderate disease, and the symptoms of severe disease were accepted as evident tachypnea (respiratory rate ≥30/min), hypoxemia (oxygen saturation ≤93%, inhaled oxygen fraction ratio of partial artery oxygen pressure <300), and pulmonary leakage (>50% lung involvement) (12). Patients classified as having mild disease severity were not included in the study and analysis was only made of the two groups of patients with moderate and severe disease severity.

Statistical Analysis

Data obtained in the study were analysed statistically using NCSS software (Number Cruncher Statistical System). Descriptive statistical methods were used and data were stated as mean ± standard deviation, median, minimum, and maximum values, or number (n) and percentage (%). The conformity of quantitative data to normal distribution was assessed with the Shapiro-Wilk test and graphic examinations. In the comparisons of two groups of quantitative variables, the Student's t-test was used for data with normal distribution and the Mann-Whitney U test for data not showing normal distribution. The Pearson's chi-square test and Fisher's exact test were used in the comparisons of categorical data. The level of statistical significance was accepted as $p<0.05$.

The sample size was calculated from power analysis performed using G*Power (v3.1.7) software. At the start of the study, taking 10 subjects in each group, power was calculated as 0.928, and when the pilot study was applied, it was seen to be necessary to have a total of 40 subjects with 20 in each group to obtain $\alpha=0.05$ and 80% power.

Results

The study included 57 patients treated as hospital in-patients for a diagnosis of COVID-19 pneumonia. The patients comprised 36 (63.2%) males and 21 (36.8%) females with a mean age of 63.5 years (range, 26-90 years). The patients were separated into 2 groups of diabetic and non-diabetic patients, with no difference determined between the groups in respect of age and gender distribution (Table 1).

No statistically significant difference was determined between the groups in respect of age, gender, World Health Organization classification of disease severity, admission to ICU, or mortality rates ($p>0.05$). The GO levels were determined to be significantly higher in the diabetic group than in the non-diabetic group ($p=0.001$, $p<0.01$). The length of stay in the hospital was significantly longer in the diabetic group than in the non-diabetic group ($p=0.017$, $p<0.05$) (Table 1).

No statistically significant difference was determined between the groups in respect of the rates of HT, CAD, COPD, malignancy, CRF, and cerebrovascular event (CVE), or in the distribution of pneumonia severity ($p>0.05$). CVE was seen in 1 patient in each group (Table 2).

The WBC, NEU, NLR, PCT, and ferritin values were determined to be statistically significantly higher in the diabetic group than in the non-diabetic group ($p=0.006$; $p=0.005$, $p=0.017$, $p=0.011$,

Table 1. Comparisons between the diabetic and non-diabetic groups of the demographic and clinical data and glyoxal levels

		Diabetic (n=29)	Non-diabetic (n=28)	p-value
Age (years)	Mean ± SD	64.86±11.89	62.21±15.19	[b]**0.466**
	Median (min-max)	64 (43-89)	60 (26-90)	
Glyoxal (ng/mL)	Mean ± SD	25.37±14.99	13.58±7.90	[a]**0.001****
	Median (min-max)	20.7 (3.8-64.8)	11.6 (2.1-34.6)	
Clinical disease status	**Moderate**	15 (51.7)	18 (64.3)	[c]**0.337**
	Severe	14 (48.3)	10 (35.7)	
Length of stay in hospital (days)	Mean ± SD	17.03±9.52	12.50±9.87	[a]**0.017***
	Median (min-max)	14 (5-39)	8 (4-39)	
Admission to ICU	**No**	24 (82.8)	24 (85.7)	[d]**1.000**
	Yes	5 (17.2)	4 (14.3)	
Mortality	**No**	26 (89.7)	26 (92.9)	[d]**1.000**
	Yes	3 (10.3)	2 (7.1)	

[a]Mann-Whitney U test, [b]Student's t-test, [c]Pearson's chi-square test, [d]Fisher's exact test, *p<0.05, **p<0.01, ICU: Intensive care unit, SD: Standard deviation, Min: Minimum, Max: Maximum

p=0.030, respectively). The HbA1c value was mean 8.1% in the diabetic group and 5.5% in the non-diabetic group (Table 3).

Discussion

Although the mortality rate is known to be high in COVID-19 patients with diabetes (13), the current study results showed no statistically significant difference despite the numerically higher number of diabetic patients who developed mortality (3/29 vs. 2/28). This was attributed to the low number of patients. As comorbid diseases were known to affect morbidity and mortality in COVID-19 patients (1), care was taken to have a balanced distribution of additional diseases in the diabetic and non-diabetic groups. Taking into consideration that aging had an effect on AGE, (14) attention was also paid to ensuring similar mean ages in both groups. The disease severity was similar in the patients in both groups as there was a potential effect on the AGE level of systemic changes caused by disease severity. Throughout the hospitalization period, the patients received similar oral or parenteral nutrition, thereby ensuring that the amount of GO, which is the strongest precursor of AGE, was at the forefront of the conditions related to intrinsic reasons (type 2 diabetes) in the patients.

In the clinical table of COVID-19, the role of cytokine storm, increased cytokines and the levels of inflammatory parameters have been shown to have an effect on prognosis and mortality (15). In individuals with diabetes, there is a low-grade chronic inflammation which can facilitate a cytokine storm, and this seems to be the cause of severe COVID-19 pneumonia and ultimately death in many patients (16). Higher levels of inflammatory markers [fibrinogen, CRP, D-dimer, interleukin-6 (IL-6)] have been determined in COVID-19 patients with diabetes compared to those without diabetes. Agents targeting IL-6 have been used in treatment and have provided significant benefits (17). In the current study, WBC, NEU, PCT, and ferritin levels were determined to be significantly higher in diabetic patients, supporting that there was inflammation present. Many studies have shown that high ferritin levels in COVID-19 patients are associated with severe illness and poor prognosis. Ferritin is the primary storage form of iron in the body and can increase in situations such as inflammation or tissue damage. Elevated ferritin levels in COVID-19 patients are generally considered a sign of systemic inflammation and cytokine storm. This condition can lead to overstimulation of the immune response and tissue damage. High ferritin levels

Table 2. Evaluation of additional diseases in the groups

		Diabetic (n=29)	Non-diabetic (n=28)	p-value
		n (%)	n (%)	
Hypertension	Absent	13 (44.8)	15 (53.6)	[c]0.509
	Present	16 (55.2)	13 (46.4)	
CAD	Absent	23 (79.3)	26 (92.9)	[d]0.253
	Present	6 (20.7)	2 (7.1)	
COPD	Absent	27 (93.1)	24 (85.7)	[d]0.423
	Present	2 (6.9)	4 (14.3)	
Malignancy	Absent	28 (96.9)	27 (96.4)	[d]1.000
	Present	1 (3.4)	1 (3.6)	
CRF	Absent	23 (79.3)	27 (96.4)	[d]0.102
	Present	6 (20.7)	1 (3.6)	
Pneumonia severity	Mild	12 (44.4)	8 (19.6)	[c]0.436
	Moderate	11 (40.7)	12 (44.4)	
	Severe	4 (14.8)	7 (25.9)	

[c]Pearson's chi-square test, [d]Fisher's exact test, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure

Table 3. Biochemical analysis results of the groups

		Diabetic (n=29)	Non-diabetic (n=28)	p-value
WBC	Mean ± SD	10863.45±5067.53	7480±3646.44	[b]**0.006****
	Median (min-max)	11100 (3450-23700)	6745 (2440-19430)	
LYM	Mean ± SD	1812.41±1863.42	1545.03±704.19	[a]0.609
	Median (min-max)	1320 (180-10200)	1445 (61-2890)	
NEU	Mean ± SD	8193.45±4668.21	5072.14±3378.21	[a]**0.005****
	Median (min-max)	7910 (1950-18910)	4525 (120-17410)	
NLR	Mean ± SD	8.56±10.14	3.89±3.6	[a]**0.017***
	Median (min-max)	4.8 (0-48)	2.7 (1-19)	
PCT	Mean ± SD	1.06±2.24	0.61±2.70	[a]**0.011***
	Median (min-max)	0.16 (0-10.6)	0.07 (0-14.3)	
Ferritin	Mean ± SD	366.91±307.72	176.64±169.32	[a]**0.030***
	Median (min-max)	281.8 (20.9-1070.4)	143.05 (9-694)	
HbA1c	Mean ± SD	8.90±2.19	5.65±0.36	[a]**0.001****
	Median (min-max)	8.1 (6.5-16.5)	5.5 (5.3-6.2)	

[a]Mann-Whitney U test, [b]Student's t-test, *p<0.05, **p<0.01, ICU: Intensive care unit, SD: Standard deviation, Min: Minimum, Max: Maximum, WBC: White blood cell, LYM: Lymphocyte, NEU: Neutrophil, NLR: Neutrophil/lymphocyte ratio, PCT: Procalcitonin, HbA1c: Hemoglobin A1c

have also been associated with multi-organ dysfunction and increased mortality risk in COVID-19 patients (18). AGE is a group of complex and heterogenous components, which play a role in complications related to diabetes. Just as it may be the cause of complications observed in diabetes, it may also be formed as a result of complications (19). The results of the current study demonstrated a significantly high level of GO, which is a precursor of AGE, in the diabetic group. The elevated GO level and elevated parameters related to accompanying inflammation in the diabetic group of patients diagnosed as having COVID-19 may be a reason for delayed recovery and prolonged length of stay in hospital.

Previous in vitro studies have shown that elevated AGE is a barrier to the formation of a healthy and necessary immune response. Increasing oxidant stress in particular prevents the repair and renewal of tissues (20). These views were supported by the determination of the longer recovery period in the current study of patients with a high level of GO, which is a precursor of AGE, although they had similar disease severity.

The metabolic effects of AGEs in patients, particularly in the context of COVID-19, warrant detailed elucidation. AGEs, formed through non-enzymatic reactions between reducing sugars and macromolecules, exert multifaceted effects on cellular metabolism and signaling pathways. A study by Brownlee et al. (21) highlighted the role of AGEs in exacerbating insulin resistance, impairing glucose metabolism, and promoting the development of diabetic complications. Furthermore, AGEs contribute to chronic low-grade inflammation and oxidative stress, which are key drivers of metabolic dysfunction in diabetes and other metabolic disorders. In COVID-19 patients, elevated AGE levels may exacerbate systemic inflammation and endothelial dysfunction, thereby amplifying the severity of the disease. Understanding the intricate interplay between AGEs and metabolic pathways is crucial for unraveling the pathophysiology of COVID-19 and developing targeted therapeutic interventions (21).

There are endogenous and exogenous sources of AGE in the body, and thus it was aimed in the current study to equalize the exogenous sources of AGE intake throughout the period of hospitalization. The patients were given both oral and parenteral nutrition in hospital and received no other food. The fact that all the patients had the same diet suggested a greater effect of endogenous sources of AGE on the condition.

Study Limitations

There were some limitations to this study, primarily the low number of patients and that AGEs were only examined during the period of hospitalization. The low number of patients prohibited evaluation of the relationship between low GO and mortality. Further studies of larger numbers of patients may be able to determine a statistically significant association between the level of AGE precursors and COVID-19 mortality.

Conclusion

The findings of this study underscore the complexity of COVID-19 outcomes in diabetic patients, revealing a disparity in the length of hospital stay despite comparable mortality and ICU admission rates between diabetic and non-diabetic patients with similar demographic and clinical profiles. Elevated serum AGE levels observed in diabetic COVID-19 patients, along with inflammatory markers, highlight the potential role of AGEs in influencing disease severity and recovery trajectory. These results emphasize the importance of considering metabolic factors, such as AGEs, in the management and prognosis of COVID-19 patients, particularly those with underlying diabetes. Further research is warranted to elucidate the mechanistic links between AGEs and COVID-19 outcomes, paving the way for targeted therapeutic strategies aimed at mitigating the impact of metabolic dysregulation on disease progression and recovery.

Ethics

Ethics Committee Approval: Approval for this prospective study was granted by the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital's Ethics Committee (desicion no: 2020-12-37, date: 08.06.2020).

Informed Consent: Prior to enrollment, each patient provided written informed consent for their participation in the study.

Authorship Contributions

Surgical and Medical Practices: N.I., M.Y., K.K.Y., Concept: D.T., N.I., G.Ş.E., P.K., M.Y., H.U., K.K.Y., Design: D.T., N.I., P.K., Data Collection or Processing: D.T., N.I., P.K., K.K.Y., Analysis or Interpretation: P.K., H.U., Literature Search: D.T., N.I., P.K., Writing: D.T., N.I., G.Ş.E., P.K., M.Y., H.U., K.K.Y.

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

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Original Article

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Investigating the Frequency of Stent Fracture and its Impact on in-Stent Restenosis in Patients Undergoing Carotid Artery Stenting

Karotis Arterine Stent Uygulanan Hastalarda Stent Kırığı Sıklığının ve Stent İçi Restenoz Üzerine Etkisinin Araştırılması

Mehmet ALTUNOVA[1], Ahmet Arif YALÇIN[1], Ayan IŞIK[1], Ferhat Can PİŞKİN[2], Nedim UZUN[3], Anıl ŞAHİN[4], Yusuf DEMİR[1], Sezgin ATMACA[1], Gökhan DEMİRCİ[1], Mehmet ERTÜRK[1]

[1]University of Health Sciences Türkiye, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Department of Cardiology, İstanbul, Türkiye
[2]Çukurova University Faculty of Medicine, Department of Radiology, Adana, Türkiye
[3]University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital, Clinic of Emergency, İstanbul, Türkiye
[4]İstinye University Faculty of Medicine, Liv Bahçesehir Hospital, Department of Cardiology, İstanbul, Türkiye

ABSTRACT

Objective: This single-center study aimed to assess the incidence and predictors of carotid artery Xact stent fractures (SF) and their impact on in-stent restenosis (ISR) during long-term follow-up.

Methods: A cohort of 108 patients (97 males, median age 69.4±8 months) who underwent Xact stent placement for internal carotid artery stenosis between 2013 and 2021 and were diagnosed with SFs through fluoroscopy in 2022 were included. SFs were categorized as types I-V based on fracture characteristics. Follow-up included duplex ultrasound examinations to assess stent patency.

Results: The average follow-up duration was 49.2±24.3 months, with ISR observed in 10 patients. Twenty-three SFs (21.3%) were identified: type I (5 patients), type II (7 patients), type III (3 patients), type IV (6 patients), and type V (2 patients). Calcification and stent length significantly predicted SFs (p<0.001; p<0.028).

Conclusion: Calcification and stent length are associated with Xact SFs, but SFs do not impact ISR during long-term follow-up.

ÖZ

Amaç: Bu tek merkezli çalışmanın amacı, uzun süreli izlem sırasında karotis arter Xact stent kırıklarının (SF) görülme sıklığını ve öngörücülerini değerlendirmek ve bunların stent içi restenoz (ISR) üzerindeki etkisini değerlendirmektir.

Yöntemler: 2013-2021 yılları arasında iç karotis arter darlığı için Xact stent yerleştirilen ve 2022'de floroskopi ile SF tanısı konan 108 hasta (97 erkek, ortanca yaş 69,4±8 ay) kohortuna dahil edildi. SF'ler kırık özelliklerine göre I-V tiplerine ayrıldı. İzlem, stent geçirgenliğini değerlendirmek için dubleks ultrason muayenelerini içeriyordu.

Bulgular: Ortalama izlem süresi 49,2±24,3 aydı ve ISR 10 hastada gözlemlendi. Yirmi üç stent kırığı (%21,3) tanımlandı: Tip I (5 hasta), tip II (7 hasta), tip III (3 hasta), tip IV (6 hasta) ve tip V (2 hasta). Kalsifikasyon ve stent uzunluğu SF'leri anlamlı olarak öngördü (p<0,001; p<0,028).

Sonuç: Kalsifikasyon ve stent uzunluğu, Xact stent kırıkları ile ilişkilidir, ancak SF'ler uzun süreli izlem sırasında ISR'yi etkilememektedir.

Address for Correspondence: Mehmet Altunova, University of Health Sciences Türkiye, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Department of Cardiology, İstanbul, Türkiye
E-mail: dr.mehmetaltunova@gmail.com **ORCID ID:** orcid.org/0000-0001-5351-5022

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ABSTRACT

Keywords: Internal carotid artery, Xact stents, stent fracture, in-stent restenosis

ÖZ

Anahtar Sözcükler: İç karotis arter, karotis stentler, stent kırığı, stent içi restenoz

Introduction

In selected patient groups, carotid artery stenting (CAS) is an increasingly common endovascular treatment method for The carotid artery stenosis (CAS). Developing techniques for access and devices in CAS provide patients with better procedural outcomes through a minimally invasive procedure (1). In cases where surgical access is challenging, CAS may offer advantages over carotid endarterectomy (CEA), resulting in fewer cranial nerve injuries and wound complications (2). Due to repeated motion of the head and neck, the carotid arteries experience daily movements, including flexion, extension, and rotation. Following CAS, these carotid arteries are subjected to constant mechanical stress caused by these movements, which can lead to stent fractures (SF) occurring at the rates ranging from 0% to 39% (3,4). It has been previously shown that SF may be associated with in-stent restenosis (ISR) or other significant adverse clinical events in coronary and peripheral arteries (5). However, the relationship between SF and ISR in patients with carotid stents remains a subject of ongoing debate (6).

The aim of this study is to investigate the frequency of SF in Xact stents, which are the most commonly used type of stent in our clinic for the treatment of internal carotid artery (ICA) stenosis, and to examine their impact on the development of ISR during long-term follow-up. Additionally, it aims to assess potential factors associated with the development of SF in ICA and determine the need for treatment in such cases.

Methods

Study Population

Our research constitutes a retrospective observational cohort study conducted at a single center. Ethical approval was granted by the University of Health Sciences Türkiye, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, and the study adhered to the principles outlined in the Helsinki Declaration (decision no: 2023.08-84, date: 24.10.2023). Over the period spanning January 2013 to December 2021, we enrolled a total of 134 consecutive patients who underwent endovascular intervention using Xact stents for ICA stenosis at our institution. The inclusion criterion was as follows: Availability of all medical records for retrospective analysis and patients who underwent CAS using Xact stents. The exclusion criterions were as follows: Patients with a history of systemic inflammation (n=5), familial hyperlipidemia (n=3), vasculitis (n=2), Burger's disease (n=1), and those with inadequate clinical and preoperative parameters (n=11) were excluded from the study. Additionally, patients who underwent emergency CEA after the procedure (n=4) were not included in the study. After the inclusion and exclusion criteria, a total of 108 patients were included in the study.

Demographic data, laboratory findings, and outcomes were collected from hospital records, file reviews, and telephone interviews. The CAS was routinely evaluated bilaterally using two-sided carotid duplex ultrasonography (DUS), and afterward, patients were verified through computed tomography angiography (CTA) or digital subtraction angiography (DSA). Our vascular team, consisting of an experienced interventional cardiologist, a consulting neurologist, and a cardiovascular surgeon, determined the indication for CAS. CAS was performed on patients with high surgical risk who had asymptomatic CAS of 70-99%, with procedure-related death and stroke rates of less than 3%, and a life expectancy of more than 5 years. Additionally, CAS was performed on patients with symptomatic CAS of 50-69% and procedure-related death and stroke rates of less than 6%. Symptomatic carotid stenosis was defined as the occurrence of symptoms of stroke or transient ischemic attack within the last 6 months. Asymptomatic carotid stenosis was defined as the absence of previously identified symptoms or symptoms occurring more than 6 months ago. Coronary artery disease was defined as having a history of angina pectoris, myocardial infarction, or coronary revascularization. Diagnosis of chronic heart failure required a left ventricular ejection fraction of less than 50% and the presence of one or more of the following: symptoms, clinical signs, radiographic abnormalities, and hospitalization. Chronic kidney disease is defined as a glomerular filtration rate of less than 60 mL/min/1.73 m^2 that persists for more than 3 months. Peripheral artery disease is defined as the presence of arterial lesions in the lower extremities detected by imaging methods along with the presence of claudication, or a history of peripheral revascularization. Stenosis of the contralateral ICA was defined as 50 percent or greater. The distal tortuosity index (TI) was calculated by referencing the course of the ICA from the carotid bulb, where it enters, to the base of the skull, where the carotid canal is located (7). The total length of the ICA's natural anatomical course, which is curved or angled between these two reference points, is considered as the "arch length" when measured. The straight distance measured between the carotid bulb and the base of the skull is referred to as the "cord length". Therefore, the distal TI is expressed with the following formula: TI = [(arch length/cord length - 1) × 100]. TI was calculated by two independent observers from DSA images of patients in the PACS system. Intra-observer and inter-observer differences for SF were less than 5%.

Grading of Calcification

The assessment of ICA calcification was conducted as follows: Circularity (continuous and discontinuous) and thickness (thick ≥1.5 mm and thin <1.5 mm); none (no calcification), mild (thin and discontinuous calcification), moderate (thin and continuous

calcification or thick and discontinuous calcification), and severe (thick and continuous calcification) (8).

Figure 1 shows calculation of TI. In this example, with a total arch length of 86.3 mm and a cord length of 74.9 mm, TI for this patient was calculated as 15.22. TI was calculated with the following formulation:

TI = [(86.3 / 74.9 - 1) × 100] = [(1.1522 - 1) × 100] = [0.1522 × 100] = 15.22

Procedural Data and The Stenting Method

All procedures were performed by two experienced interventional cardiologists. All procedures were performed via femoral access. Prior to obtaining percutaneous femoral artery access, all patients received local anesthesia. Systemic fractionated heparinization (70-100 U/kg) was administered to all patients. To assess the severity of lesions and treatment methods, all patients underwent preoperative conventional angiography and DSA cerebral embolism protection devices (Spider FX - Emboshield NAV6 and Filterwire EZ) were used in all cases, depending on availability. In this study, Xact stents (Abbott Vascular, Santa Clara, CA) were used. In general, pre-dilatation was carried out using balloons ranging from 2 to 5 mm. Post-dilatation was predominantly conducted using a 5 mm balloon, with a range of 4 to 6 mm. To assess stent patency and any residual stenosis, intracranial and extra cranial angiograms were routinely performed at the end of the procedure. The technical success of the procedure was defined as achieving a residual stenosis of 30% or less without any occurrence of dissection or extravasation. All patients were initiated on acetylsalicylic acid (100 mg/day) and clopidogrel (75 mg/day) at least 1 week before the procedure, unless the patient was already using these antiplatelet medications. Patients

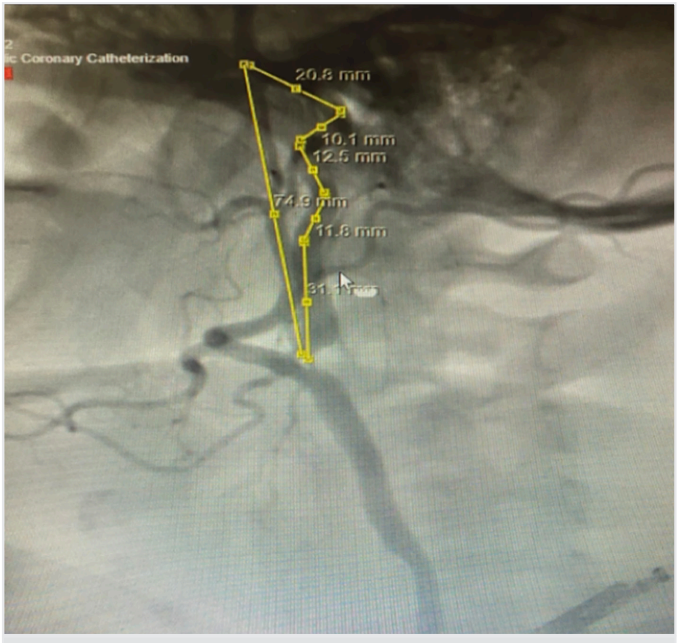

Figure 1. The formula for the distal tortuosity index is indeed as follows: distal tortuosity index = [(arch length/ cord length-1) × 100]

received dual antiplatelet therapy (DAPT) for 3 months, followed by lifelong monotherapy unless there was a cardiac indication for prolonged DAPT. All patients were hospitalized for a minimum of 24 hours of neurological observation. Patients were independently examined by a neurologist before and after the procedure. Patients without any complications were discharged from the hospital one day after the intervention.

Follow-Up

Follow-up examinations were conducted at intervals of 1, 6, and 12 months following the endovascular procedure, followed by annual assessments thereafter.

During the follow-up visits, their clinical status, demographic information, and medical history were re-documented. To evaluate the carotid flows, ISR or carotid stenosis and presence of any SF, bilateral carotid DUS examinations were conducted. In addition to ultrasound, some patients underwent anteroposterior and lateral cervical radiographs to assess SF. Information from these patients' ultrasounds and radiographs were obtained from the hospital records system. ISR was defined as the presence of ≥70% stenosis within the stent. The presence of significant ISR was confirmed using CTA or DSA. Primary patency was defined as an open stent without the need for intervention.

Assessment of SFs

After evaluation of symptoms and DUS, patients were taken to the DSA laboratory to detect SFs, and fluoroscopic images of the ipsilateral neck area were obtained. The images were recorded in standard and neurodevice modes. Close-up high-resolution images were obtained from anterior-posterior, oblique, and lateral angles to examine the integrity of the stent citrate in detail. The fluoroscopic image evaluation was carried out by consensus of two experienced interventional cardiologists. The detected SFs were classified according to the classification proposed by Nakazawa et al. (9) This classification consists of a total of 5 grades: Type I (single strut fracture), type II (multiple strut fractures without stent deformity), type III (multiple strut fractures with stent deformity), type IV (complete stent separation without gap), and type V (stent tearing, complete SFs) (Figure 2). Patients were classified as SF (+) and SF (-) based on the presence of SFs. Intra-observer and inter-observer differences for SF were less than 5%.

Statistical Analysis

The statistical analysis of the study utilized the Statistical Package for the Social Sciences version 26.0 (SPSS Inc., Chicago, Illinois, USA). Various methods were employed to assess the distribution of variables, including visual techniques such as histograms and probability curves, as well as analytical methods like the Kolmogorov-Smirnov test. Numerical variables adhering to a normal distribution were summarized as mean ± standard deviation, whereas non-normally distributed numerical variables were presented as median (interquartile range). Categorical variables were expressed as percentages (%). To compare numerical variables between groups, we employed either Student's t-test or the Mann-Whitney U test, depending on

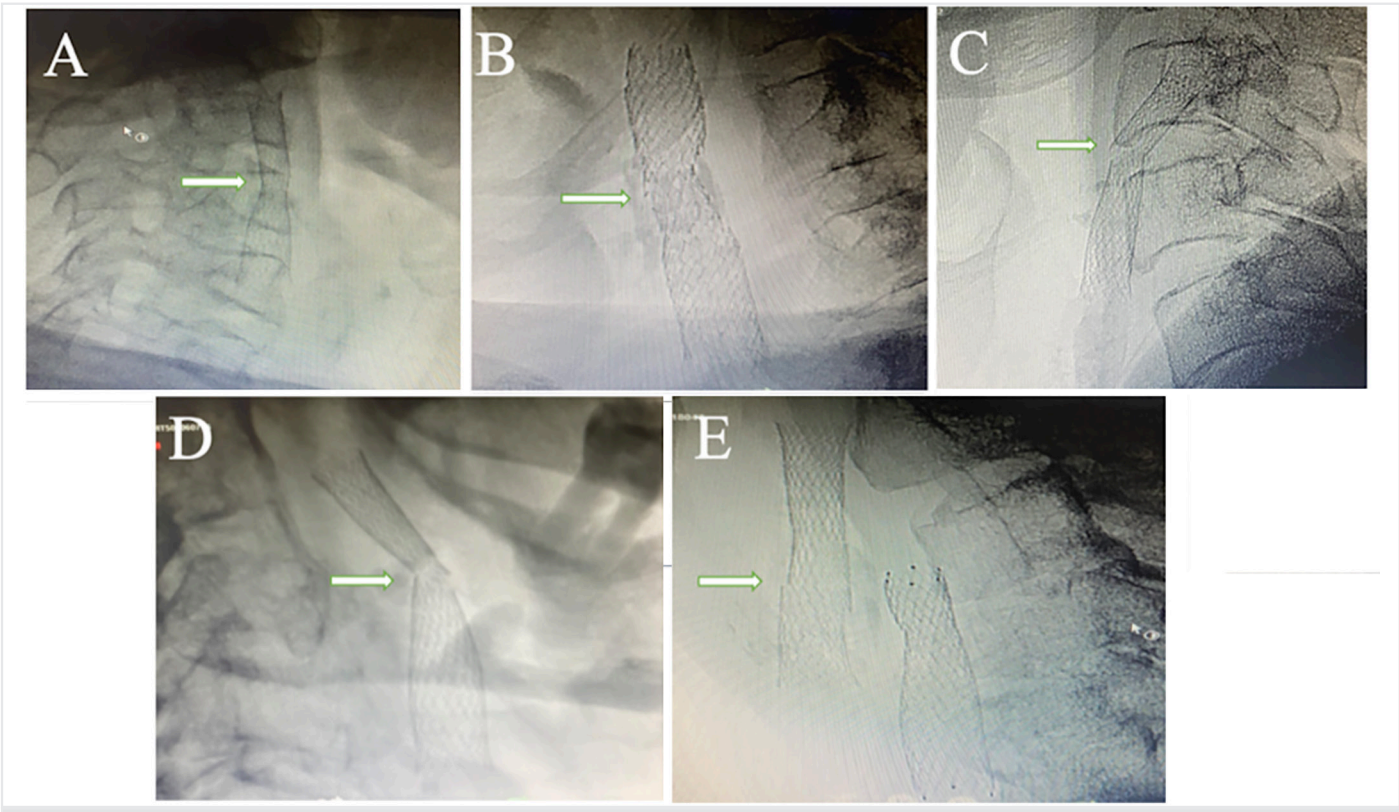

Figure 2. A) Type I (single strut fracture), B) Type II (multiple strut fractures without stent deformity), C) Type III (multiple strut fractures with stent deformity), D) Type IV (complete stent separation without gap), and E) Type V (stent tearing, complete stent fracture)

the distribution of the data. Categorical variables were assessed using either the chi-square test or Fisher's exact test. Event-free survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. Additionally, a univariate and multivariate Cox proportional hazards model was utilized to estimate hazard ratios and 95% confidence intervals (95%) for clinical endpoints. Throughout the study, a significance level of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

The study comprised 108 patients, with a mean age of 69.4±8 years, of whom 97 (74.1%) were male. Patients were categorized into two groups according to the presence or absence of SF. Table 1 displays the fundamental demographic, clinical, and laboratory data of the study cohort. The groups with and without SF exhibited similar demographic, clinical, and laboratory characteristics. However, the presence of stenosis in the contralateral carotid artery [12 (52) vs. 24 (28.2), p=0.031] and the ISR rate [5 (21.7) vs. 5 (5.9), p=0.020] were significantly higher in the group of patients who developed SF. The lesion characteristics and DUS data of the study group are summarized in Table 2. The groups with and without SFs showed similarities in terms of lesion characteristics. However, heavy calcification was significantly higher in the group with SF [17 (73.9) vs. 14 (16.5), p<0.001], whereas less than heavy calcification grades were higher in the SF (-) group [0 (0) vs. 6 (7.1), 0 (0) vs. 25 (29.4), 6 (26.1) vs. 40 (47.1), p<0.001] (Figure 3). Furthermore, based on DUS, ICA/CCA [(1.7±1) vs. (1.1±0.3), p<0.001], peak systolic velocity (PSV) [78 (65-138) vs. 68 (58-85.5), p=0.007], and end-diastolic velocity (EDV) [29 (22-45) vs. 25 (18-30.5), p=0.013] were significantly higher in the SF (+) group.

The outcomes of procedural variables and stent characteristics are presented in Table 3. The groups with and without SF exhibited similarities in relation to these variables. However, the stent length (37.8±4.2 vs. 35.2±5.3, p=0.028) was found to be longer in the group of patients who had SF.

Univariate and multivariate Cox regression analyses were conducted to identify long-term predictors of ISR. Among these parameters, proximal diameter of the stent (p=0.008), post-dilatation (p=0.041), smoking status (p=0.048), and white blood cell count (p=0.039) demonstrated significant associations. A multivariate Cox regression analysis was performed using these variables. As a result of the multivariate Cox regression analysis, there was not any independent predictor for long-term ISR among these variables (Table 4).

The Kaplan-Meier method revealed no significant difference (log-rank p=0.164 between the groups) (Figure 4), indicating that the presence of SFs could not be linked to the development of long-term ISR.

Table 1. Basic demographic, clinical, and laboratory data of study patients

Variables	SF (+) group (n = 23)	SF (-) group (n = 85)	p-value
Male sex, n (%)	18 (78.3)	62 (72.9)	0.606
Age, year, mean (SD)	69.1±8.1	69.4±8	0.871
Diabetes mellitus, n (%)	15 (65.2)	38 (44.7)	0.081
Hypertension, n (%)	14 (60.9)	39 (46.4)	0.220
Dyslipidemia, n (%)	7 (30.4)	23 (27.1)	0.748
Coronary artery disease, n (%)	9 (39.1)	39 (45.9)	0.563
Congestive heart failure, n (%)	2 (8.7)	10 (11.8)	0.678
Chronic kidney disease, n (%)	9 (39.1)	28 (32.9)	0.579
Peripheral artery disease, n (%)	5 (21.7)	12 (14.1)	0.373
History of stroke, n (%)	7 (30.4)	37 (43.5)	0.257
History of TIA, n (%)	3 (13)	8 (9.4)	0.609
History of CEA, n (%)	3 (13)	8 (9.4)	0.609
Current smoking status, n (%)	9 (39.1)	30 (35.3)	0.734
Periprocedural stroke, n (%)	2 (8.7)	11 (12.9)	0.731
Postprocedural stroke, n (%)	2 (8.7)	7 (8.2)	1
SCCA, n (%)	12 (52.2)	24 (28.2)	**0.031**
Contralateral stroke, n (%)	1 (4.5)	5 (6.2)	0.773
Symptomatic related to stroke, n (%)	7 (30.4)	34 (40)	0.402
White blood cells, 10^6/L, mean (SD)	8±1.7	8.1±1.9	0.787
Hemoglobin, g/dL, mean (SD)	12.4±1.5	12.8±1.8	0.398
Serum creatinine, mg/dL, median (IQR)	0.9 (0.7-1.2)	1 (0.8-1.2)	0.615
LDL cholesterol, mg/dL, mean (SD)	92±40.6	92.2±33.8	0.975
CRP, median (IQR)	2.6 (1.3-5.1)	2.6 (1.1-6.3)	0.976
Follow-up time, months	57.3±22.1	47±24.6	0.073
Instent restenosis, n (%)	5 (21.7)	5 (5.9)	**0.020**

Data are presented as percentage, mean ± standard deviation or median (interquartile range). CEA: Carotid endarterectomy, CT: Computed tomography, SCCA: Stenosis of the contralateral carotid artery, TIA: Transient ischemic attack, LDL: Low-density lipoprotein, CRP: C-reactive protein, SD: Standard deviation, SF: Stent fractures, IQR: Interquartile range

Table 2. Lesion characteristics and Doppler ultrasonography data of the study group

Variables	SF (+) group (n=23)	SF (-) group (n=85)	p-value
Lesion length, mm	11.1±5	10±4.9	0.324
DLFCB, mm	7.5±4.2	7±3.7	0.582
Percentage of stenosis, %	83±10.7	80.7±12	0.238
DSFCB, mm	25±8.2	24.4±7.2	0.751
Tortuosity index	13 (10-22)	13 (6.5-20)	0.327
ICA/CCA	1.7±1	1.1±0.3	**<0.001**
Calcification			
No	0 (0)	6 (7.1)	
Mild	0 (0)	25 (29.4)	**<0.001**
Moderate	6 (26.1)	40 (47.1)	
Heavy	17 (73.9)	14 (16.5)	
PSV	78 (65-138)	68 (58-85.5)	**0.007**
EDV	29 (22-45)	25 (18-30.5)	**0.013**
PSV/EDV	3±0.9	3.2±1.3	0.645

Data are presented as percentage, mean ± standard deviation or median (interquartile range). CCA: Common carotid artery, DLFCB: Distance of the lesion from the carotid bulb, DSFCB: Distance of the stent from the carotid bulb, EDV: End diastolic velocity, ICA: Internal carotid artery, PSV: Peak systolic velocity, SF: Stent fractures

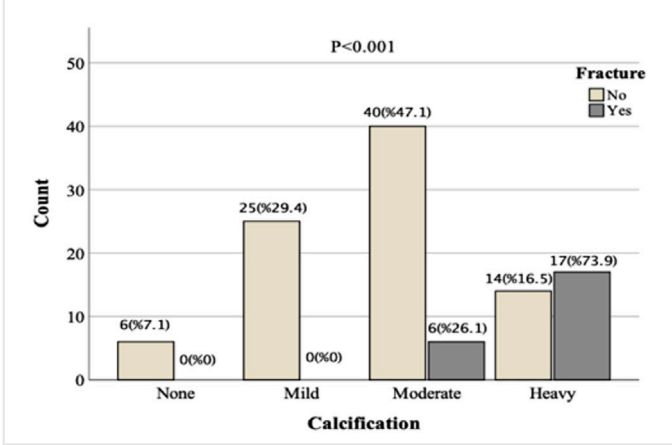

Figure 3. The degree of calcifications according to stent fractures

Discussion

In this study, we investigated and analyzed our clinical experience and results concerning SFs those might be associated with the development of ISR in long-term follow-ups after the implantation of Xact stents for CAS. In this study, among 108

patients who underwent CAS, SFs were detected in 23 (21.9%) patients during long-term follow-up. When patients were divided into two groups based on the presence or absence of SFs, a significantly higher rate of ISR development was found in the group with SFs (p=0.020). In DUS examination, it was observed that in patients with SFs, the lesion causing stenosis in the ICA was more frequently classified as severely calcified (p<0.001). Additionally, in patients with SFs, at the time of SFs detection, PSV and EDV were significantly higher compared to patients without SFs (p=0.007 and p=0.013, respectively). When examining procedural characteristics, it was observed that in patients with SFs, stent length was significantly longer (p=0.028). Regarding the development of ISR, when investigating risk factors, none of the parameters was identified as an independent predictor, moreover SFs did not emerge as a significant factor. Additionally, when Kaplan-Meier analysis was applied, no significant difference was found in terms of the time to ISR development between patients with and without SFs.

Table 3. Procedural variables and informational data of the stent

Variables	SF (+) group (n = 23)	SF (-) group (n = 85)	p-value
Predilation performed, n (%)	8 (34.8)	32 (37.6)	0.801
Postdilation performed, n (%)	16 (69.6)	53 (62.4)	0.523
Stent proximal diameter, mm	8.2±0.9	8.3±0.6	0.585
Stent distal diameter, mm	6.5±0.7	6.5±0.6	0.609
Stent length, mm	37.8±4.2	35.2±5.3	**0.028**
Side of the stent implantation Right ICA Left ICA	 16 (69.6) 7 (30.4)	 41 (48.2) 44 (51.8)	0.069
Predilation balloon size, mm	3.1±0.9	3.6±0.8	0.182
Postdilation balloon size, mm	4.9±0.6	4.8±0.5	0.376
Predilation balloon length, mm	17.6±2.5	19±3.7	0.334
Postdilation balloon length, mm	19.6±12.3	19.6±1.5	0.925

Data are presented as percentage, mean ± standard deviation or median (interquartile range). ICA: Internal carotid artery, SF: Stent fractures

In various studies, the incidence of SF in patients undergoing CAS has been reported to range between 0% and 39% (3,4). While many of these studies employed different stent types, the ACT 1 multicenter randomized trial (8), sponsored by Abbott Vascular, exclusively used the Xact stent, and the SF rate was determined to be 5.4%. In our patient cohort, all patients received the same stent (Xact, Abbott Vascular, Santa Clara, CA). We observed a proportionate rate of SF development (21.9%), consistent with the literature, however, our SF rate was higher compared to the findings of the ACT 1 Multicenter Randomized Trial.

The association between SFs and ISR were studied previously. Sfyroeras et al. (6) analyzed 13 studies examining SFs that developed after CAS (10 case reports and 3 clinical studies). A total of 55 SFs were identified, with the most commonly used stent associated with SFs being the Xact stent [22 SFs (40%)]. In 55% of cases, SFs were linked to ISR. SFs were frequently connected to ISR and generally remained asymptomatic. In our study, similar findings to the study by Sfyroeras et al. (6) were obtained, where the rate of ISR in patients with SFs was higher compared to patients without SFs (21.7% vs. 5.9%, p=0.020). In another study conducted by Weinberg al. (10), they evaluated 1091 CAS procedures, and SFs were identified in 51 patients (5.4%). Xact stents were used in all patients. Weinberg et al. (10) did not find a relationship between CAS - related SFs and adverse events. Additionally, contrary to our study, SFs were not identified as a risk factor for ISR. Moreover, Garcia-Toca et al. (11) evaluated 106 CAS procedures and identified SFs in 8 patients (7.5%). Both open and closed-cell stents were used

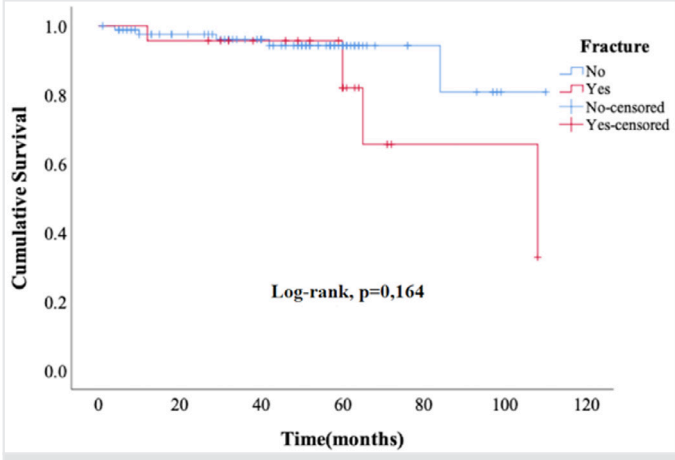

Figure 4. Kaplan-Meier survival curves for stent fractures in long-term stent restenosis

Table 4. Univariate and multivariate Cox regression analyses to identify long-term predictors of restenosis

	Univariate analyses			Multivariate analyses		
	HR	95%CI (lower-upper)	p-value	HR	95%CI (lower-upper)	p-value
Proximal diameter of stent	0.444	0.244-0.807	0.008	0.676	0.339-1.350	0.267
Postdilation	0.242	0.062-0.944	0.041	0.278	0.059-1.302	0.104
Current smoking	3.618	1.012-12.933	0.048	2.700	0.634-11.502	0.179
WBC	1.405	1.017-1.941	0.039	1.399	0.990-1.976	0.057

WBC: White blood cells, CI: Confidence intervals, HR: Hazard ratio; others, see Table 1, Table 2 and, Table 3

in this study, and similar frequencies of SFs were observed in both groups [7 (87.5%) vs. 1 (38.5%), p=0.067)]. Garcia-Toca et al. (11) did not find a relationship between SFs and the development of adverse events. While the rate of ISR was higher in patients with SFs, univariate and multivariate analyses did not identify SFs as a risk factor for ISR. Furthermore, when evaluating the duration of ISR development in long-term follow-ups, no significant difference was found between patients with and without SFs. On the other hand, case reports in the literature indicate that severe cases with SF (Type 3-5) can lead to adverse events and symptoms, necessitating treatment (12,13). Based on these findings, presence of SFs and its association with adverse events lack clinical significance.

In the literature, it has been observed that as the length of the stent placed in the carotid artery and the TI (angle of deviation) between the internal/common carotid arteries increase, the risk of SF also increases (14). Consistent with the literature, in this study, patients who developed SFs after CAS were found to have longer stents (p=0.028). However, there was no significant difference in TI between the group with SF and the group without SF (p=0.327). The head and neck region, similar to the femoropopliteal region, is a highly mobile area. Around the central axis of the cervical vertebra, it undergoes 3-dimensional flexion, extension, and rotational movements. Although these movements are not entirely uniform, they affect the main carotid artery and ICA, exhibiting similar motion mechanisms in these vascular structures. However, studies have shown that the vascular segment undergoing stent placement after CAS loses some of this mobility, while other vascular segments without stent intervention maintain partial mobility (14). Consequently, this lack of alignment results in shear stress and compression on the stent. As the stent length and tortuosity increase, the stent's ability to accommodate this motion mismatch decreases.

The association between the severity of calcification and SF has been studied previously. The study by Chang et al. (3) examined the results of 219 CAS procedures and found that the presence of calcified plaques on direct radiography increased the rate of SF compared to the absence of calcified plaques (62% vs. 15%, p=0.001). Particularly severe calcification of the carotid artery has been identified as a risk factor for complications after CAS. Calcification affects the complete expansion of the stent in the artery. Calcified plaque exerts an external force on the stent, and rigidity increases in calcified vessels. This situation leads to SF and ISR (3,15). In our study, consistent with the literature, the rate of SF in severely calcified plaques was found significantly higher (p<0.001).

Type of SF is another point of view to discuss. In their study, Garcia-Toca et al. (11) classified patients with SFs based on the type of SF and found that the most commonly observed type was Type I-II. As the follow-up duration increases, it is likely that the proportion of more severe Type III-V might increase. As far as we are aware, there is currently no existing literature addressing this particular issue.

The type of SF is another perspective to discuss. Garcia-Toca et al. (11) classified patients with SF based on the SF type and found that the most common type was Type I-II. In our study, 52.1% of cases were Type I-II. It's possible that our longer follow-up period led to an increase in the prevalence of more severe Type III-V SF. However, to the best of our knowledge, there is no study in the literature that addresses this issue.

Study Limitations

This study had several limitations. Firstly, it was retrospective in nature and involved a relatively small sample size. Secondly, only a single type of stent was used for the CAS procedure, and the significance and role of SFs in other open and closed-cell stents were not investigated. Third, the exact date of SF occurrence was not known. Forth, approximately half of the patients had pre-existing computerized tomographic angiography; therefore, fluoroscopic images were used in half of the patient group to assess the presence and degree of calcification. Fifth, some important plaque features, especially those found in vulnerable plaques, were unknown: the presence of lipid core, parietal thrombus, ulceration, intraplaque bleeding, etc. Lastly, lesions with different etiologies resulted in a heterogeneous group of patients.

Conclusion

SFs is a common complication after CAS and the diagnosis of SFs requires careful and regular radiological assessment. SF was found associated with calcification and stent length as these factors increased the occurrence of SF. Additionally, ISR was found associated with SF however none of the parameters analyzed in our study was found as independent predictor. While mild SFs might lack clinical significance, evaluation of larger patient groups is necessary for advanced SF types.

Ethics

Ethics Committee Approval: Ethical approval was granted by the University of Health Sciences Türkiye, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, and the study adhered to the principles outlined in the Helsinki Declaration (decision no: 2023.08-84, date: 24.10.2023).

Informed Consent: Retrospective observational cohort study.

Authorship Contributions

Surgical and Medical Practices: M.A., A.A.Y., M.E., Concept: M.A., A.A.Y., A.I., Y.D., M.E., Design: M.A., F.C.P., A.Ş., Data Collection or Processing: M.A., A.I., S.A., G.D., Analysis or Interpretation: M.A., N.U., Literature Search: M.A., A.A.Y., F.C.P., A.Ş., Writing: M.A.

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

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Case Report

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Unilateral Large Mylohyoid Bridging the Dry Human Mandible

Mandibula'da Görülen Unilateral Geniş Mylohyoid Köprü

Yasin ARİFOĞLU[1], Muhammed PARLAK[1], Fikri ÖZDEMİR[2], Fatih MARAL[1], Elif ZEREN[1], Ural VERİMLİ[3]

[1]Bezmialem Vakıf University Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye
[2]Hitit University Faculty of Medicine, Department of Anatomy, Çorum, Türkiye
[3]Marmara University Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye

ABSTRACT

Forty-five mandibles (90 cases) used in education at the Anatomy Departments of Bezmialem Vakıf University, Hitit University and Marmara University Faculty of Medicine were examined macroscopically and the incidence of mylohyoid bridge was investigated. In some cases, the proximal part of the mylohyoid groove (sulcus mylohyoideus) may appear as a canal through a bony bridge (mylohyoid arc = mylohyoid bridge). Knowledge the frequency of mylohyoid bridge variations will contribute to the literature studies on this subject and will guide the practice of oral surgery and dentistry. Investigating anatomical variations, as well as collecting anthropometric data, is important to draw attention to clinical practices and guide clinicians.

Keywords: Mandible, mylohyoid bridge, sulcus mylohyoideus, canalis mylohyoideus, mandibular foramen

ÖZ

Bezmialem Vakıf Üniversitesi, Hitit Üniversitesi ve Marmara Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalları'nda eğitimde kullanılan 45 alt çene (90 olgu) makroskobik olarak incelenerek mylohyoid köprü görülme sıklığı araştırıldı. Sulcus mylohyoideus'un proksimal kısmı bazı durumlarda arcus mylohyoideus adı verilen kemik bir köprü aracılığıyla kanal şeklinde gözükebilir. Mylohyoid köprü varyasyonlarının bulunma sıklığının bilinmesi, bu konudaki literatür çalışmalarına katkı sağlayacağı gibi klinik uygulamalardaki olası komplikasyonların önlenmesi için oral cerrahi ve diş hekimliği uygulamalarında yol gösterici olacaktır.

Anahtar Kelimeler: Mandibula, arcus mylohyoideus, sulcus mylohyoideus, canalis mylohyoidus, foramen mandibulae

Introduction

The mandible is a strong and mobile bone that forms the skeleton of the lower jaw and consists of a corpus and two rami. On the inner surface of the ramus mandibulae, there is mandibular foramen, and on the front side, there is a protrusion called lingula mandibulae, which is formed by the fusion of the medial and lateral laminae of the compact bone. Under this protrusion is the sulcus mylohyoideus, which starts from the mandibular foramen and moves downwards. Mylohyoid branch of inferior alveolar nerve. courses in sulcus mylohyoideus. This nerve innervates the venter anterior of the mylohyoid and anterior belly of the digastric muscles (1). Studies have reported that the mylohyoid nerve is mainly a motor nerve but also contains sensory fibers (2). In some cases, the proximal part of the sulcus mylohyoideus may appear as a canal through a bone bridge (arcus mylohyoideus = mylohyoid bridge) (3).

The mylohyoid bridge is a hyperostotic derivation that develops from Meckel's cartilage on the sulcus mylohyoideus. In cases where a mylohyoid bridge is observed, the nerves and vessels running in this canal may remain under pressure and may create a barrier during anesthetic injection (4,5).

Address for Correspondence: Yasin Arifoğlu, Bezmialem Vakıf University Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye
E-mail: yarifoglu@bezmialem.edu.tr **ORCID ID:** orcid.org/0000-0002-1895-6436

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Investigating anatomical variations, as well as collecting anthropometric data, is important to draw attention to clinical practices and guide clinicians. This study aimed to determine the frequency of mylohyoid bridge variation on the sulcus mylohyoideus in the mandible and to reveal its clinical importance.

Case Report

Forty-five mandibles (90 cases) used in education at the Anatomy Departments of Bezmialem Vakıf University, Hitit University and Marmara University Faculty of Medicine were examined macroscopically and the frequency of mylohyoid bridge was investigated. In cases where mylohyoid bridge variation was detected, the vertical lengths of the sulcus mylohyoideus and mylohyoid bridge were measured with a digital caliper. During the examinations, a wide mylohyoid bridge was detected on the proximal part of the sulcus mylohyoideus on the left side, extending towards the mandibular foramen, in one of the 90 cases (1.1%). The vertical length of this bridge (between x-y) was measured as 12.41 mm, and the length of the exposed sulcus mylohyoideus (between y-z) was measured as 20.21 mm. In this case, the length of the sulcus mylohyoideus on the right side (between x-z) was measured as 22.7 mm (Figure 1, 2).

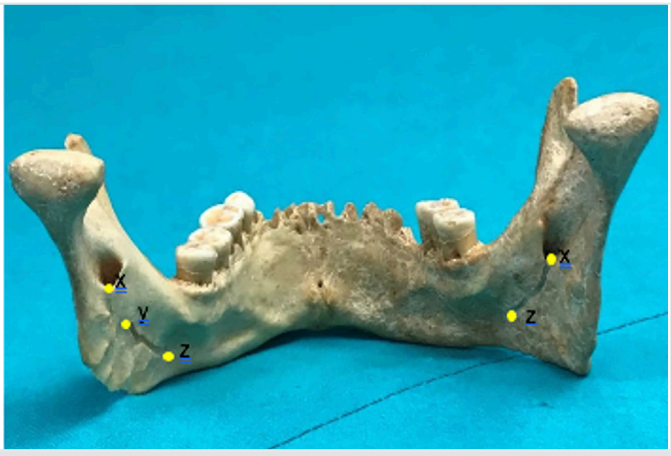

Figure 1. Rear view of the mandible

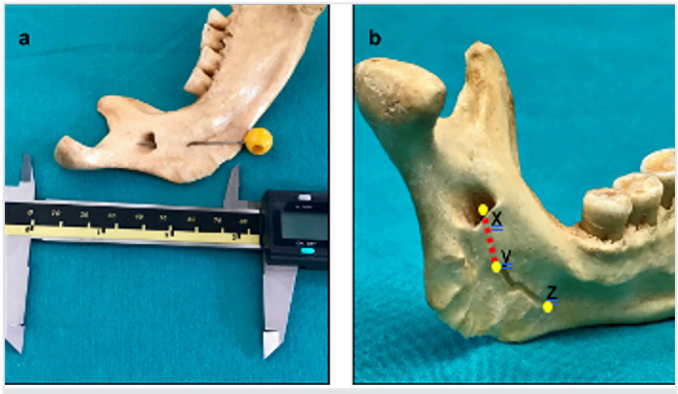

Figure 2. Left ramus mandibulae; a) inner view b) mylohyoid bridge (red line)

Additionally, the transverse diameter of the mandibular foramen was measured in this case on both sides; the average diameter on the left side was 7.04 mm, and the diameter on the right side was 5.57 mm. Informed consent was obtained.

Discussion

It has been reported by many researchers that there is a bone bridge on the sulcus mylohyoideus. In research, the mylohyoid bridge is defined as a morphological variation and is referred to as arcus, ponticulus or canalis mylohyoideus (5).

Bennett and Townsend (3) reported that both lingula and bridge types are present in a mandible.

Rusu et al. (4) found unilateral mylohyoid bridge variation in cone beam computed tomography imaging of a patient. They stated that this variation might prevent the anesthesia of the lower jaw teeth in dental applications.

In our study, in the examination of 45 mandibles (90 cases), a unilateral mylohyoid bridge was found in one case, which was compatible with the results of Rusu et al. (4) and, Bennett and Townsend (3).

Knowing the frequency of mylohyoid bridge variations will contribute to literature studies on this subject and will be a guide in oral surgery and dentistry practices to prevent possible complications in clinical practices.

Ethics

Informed Consent: Informed consent was obtained.

Authorship Contributions

Concept: M.P., F.M., Design: M.P., F.M., Data Collection or Processing: Y.A., M.P., F.Ö., F.M., E.Z., Analysis or Interpretation Y.A., U.V., Literature Search: F.M., E.Z., Writing: Y.A.

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

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Review

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Analysis of the Graduate Theses Related to Palliative Care in Türkiye in Terms of Study Method and Nursing Practices

Türkiye'de Palyatif Bakım ile İlgili Yapılan Lisansüstü Tezlerin Çalışma Yöntemi ve Hemşirelik Uygulamaları Açısından Analizi

Elif Tuğba UYGUN[1], Aylin ÖZAKGÜL[2]

[1]İstanbul University-Cerrahpaşa Institute of Graduate Studies, Fundamentals of Nursing Doctorate Program, İstanbul, Türkiye
[2]İstanbul University-Cerrahpaşa Florence Nightingale Faculty of Nursing, Department of Fundamentals of Nursing, İstanbul, Türkiye

ABSTRACT

This study was conducted to examine the postgraduate theses on palliative care in nursing in Türkiye and to reveal the nursing practices used. The population of the retrospective descriptive study consisted of theses in the field of nursing registered in the National Thesis Center of the Council of Higher Education, while the sample consisted of 55 theses between 2010 and 2022 that met the inclusion criteria among the theses in the field of nursing as a result of the screening made within the scope of PRISMA rules. It was seen that the first thesis on palliative care in nursing was published in 2010. Of the theses obtained, 13 were doctoral (23.6%) and 42 were master's theses (76.4%), 60% were published between 2019 and 2022, 92.8% were conducted with quantitative research methods, 76.5% of these theses used at least one of the descriptive designs and 15.7% were randomized controlled experimental studies. Additionally, it was determined that 36.4% of the theses were sampled by healthcare personnel working in palliative care clinics, and 72.7% did not use a sampling method. Among the interventions implemented by nurses; the most common ones were palliative care training, simulation training/practice, and theory-model based nursing care practice. However, neonatal palliative education, psychoeducation, application of the case management model and face-to-face fan practice were rarely included. In recent years, complementary practices such as ear acupuncture, aromatherapy massage and relaxation exercise, as well as red-white

ÖZ

Bu çalışma, Türkiye'de palyatif bakım üzerine hemşirelikte yapılan lisansüstü tezleri incelemek ve kullanılan hemşirelik uygulamalarını ortaya koymak amacıyla gerçekleştirildi. Retrospektif tanımlayıcı tipte gerçekleştirilen çalışmanın evrenini Yükseköğretim Kurulu Ulusal Tez Merkezi'ne kayıtlı olan, hemşirelik alanındaki tezler oluştururken, örneklemini ise, PRISMA kuralları kapsamında yapılan tarama sonucunda, hemşirelik alanındaki tezler içerisinden dahil edilme ölçütlerine uyan 2010-2022 yılları arasında 55 tez oluşturdu. Palyatif bakım üzerine hemşirelikteki ilk tezin 2010 yılına ait olduğu görüldü. Elde edilen tezlerden 13'ünün doktora (%23,6), 42'sinin yüksek lisans tezi (%76,4) olduğu, %60'ının 2019-2022 yılları arasında yayınlandığı, %92,8'inin nicel araştırma yöntemleriyle gerçekleştirildiği, bu tezlerin içerisinden %76.5'inde tanımlayıcı tasarımlardan en az birinin kullanıldığı ve %15,7'sinin randomize kontrollü deneysel çalışma olduğu görüldü. Ayrıca %36,4'ünün örneklemini palyatif bakım kliniklerinde çalışan sağlık personellerinin oluşturduğu, %72,7'sinde örnekleme yöntemi kullanılmadığı belirlendi. Hemşirelerin uyguladığı girişimler içerisinde; en çok palyatif bakım eğitimi, simülasyon eğitimi/uygulaması, kuram-model temelli hemşirelik bakım uygulaması yer almaktadır. Ancak yenidoğan palyatif eğitimi, psikoeğitim, olgu yönetimi modelinin uygulanması, yüze fan uygulaması, kırmızı-beyaz ışık uygulamasının nadiren yer aldığı saptandı. Son yıllarda auriküler akupresür, aromaterapi masajı ve gevşeme

Address for Correspondence: Elif Tuğba Uygun, İstanbul University-Cerrahpaşa Institute of Graduate Studies, Fundamentals of Nursing Doctorate Program, İstanbul, Türkiye
E-mail: elftugb9@gmail.com **ORCID ID:** orcid.org/0000-0002-8411-769X

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ABSTRACT

light application, were observed. It should be emphasized that thesis studies in palliative care and nursing have increased in recent years, but mostly at the master's level and descriptive type, the number of doctoral level studies is insufficient, and the need for randomized controlled intervention studies to provide evidence for palliative care nursing should be emphasized.

Keywords: Palliative care, nursing, graduate, Türkiye

ÖZ

egzersizi gibi tamamlayıcı uygulamaların olduğu görüldü. Palyatif bakım konusunda hemşirelikteki tez çalışmalarının son yıllarda artığı ancak çoğunlukla yüksek lisans düzeyinde ve tanımlayıcı tipte olduğu, doktora düzeyinde sayıca yetersiz olduğu, palyatif bakım hemşireliğine yönelik kanıt sağlaması amacıyla randomize kontrollü müdahale çalışmalarının yapılmasının gerekliliği üzerinde durulmalıdır.

Anahtar Sözcükler: Palyatif bakım, hemşirelik, lisansüstü, Türkiye

Introduction

World Health Organization (WHO) defined palliative care as follows; "an approach that improves the quality of life of patients and their families facing the problems associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial, and spiritual" (1). Palliative care aims to eliminate or alleviate the symptoms of the patients, starting with the diagnosis of life-threatening problems. Considering the biophysiological, psychological, socio-cultural and economic factors affecting the lives of patients and their families, it supports improving the quality of life in the last stages of life (2,3).

In palliative care, the nurse performs not only the role of caregiver/care provider but also the roles of educator, manager and coordinator. In this respect, palliative care nurses have also important roles and responsibilities such as cooperation with other disciplines in their practices regarding symptom control and their responses to the disease (4). Palliative care nurses have important roles for determining the needs of individuals/families; explaining the concerns of individuals about their lives; giving care for values and preferences and the planning of advanced care (5,6). Palliative care nurses have also necessity for following developments in their fields and performing the role of investigator in research. In recent years, it is stated that the importance of palliative care nursing has increased with the increase in palliative services and the development of practices in the field of palliative care (7-9).

Graduate education in nursing contributes to the delivery of quality nursing care ensuring that up-to-date information is followed and transferred to practices after graduation, the selection of appropriate scientific methods in solving problems related to nursing practice, the development of new hypotheses and the use of evidence-based data obtained as a result of research (10,11). Postgraduate thesis studies have an important role in the formation of scientific and professional knowledge, the dissemination of science and the professional development of nursing (12). Providing the evidence-based care contributes to the strengthening of the scientific foundations of the field and improves nurses' ability to produce solutions to different problems and to think critically and systematically and gives the ability to use qualified maintenance methods (13). The use of scientific research data in care not only allows to increase the quality of care in nursing, but also is important in terms of professional development and allows the creation of health policies (14). Accordingly, graduate education and studies ensure the formation of professional members who produce/develop knowledge in their field (15). It is stated that the professional power of nursing can be increased by synthesizing evidence-based nursing practices and professional knowledge (11). According to the John Hopkins Evidence-Based Nursing Practice model, experimental/randomized controlled studies are considered first-level studies in terms of evidence (16,17). In this context, it is important to examine studies with a high level of evidence.

It is known that scientific and academic studies on the subject are increasing day by day with the increase in chronic diseases and the increasing importance given to palliative care in Türkiye. Graduate thesis studies are studies in which theoretical knowledge and the results of this knowledge are transferred into practice. As a matter of fact, the analysis of scientific theses is important in providing a perspective on the fieldand revealing the general appearance of the examined area (18). From this point of view this study is aimed to guide palliative care nurses and researchers.

It is aimed to examine the graduate thesis studies on palliative care in the field of nursing in our country and to reveal the applied nursing practices with this study. Accordingly, answers to the following research questions were sought in the study:

- What is the distribution of graduate theses in nursing related to palliative care according to their types?

- What is the distribution of graduate theses in nursing related to palliative care according to their years?

- Which research types are preferred in graduate theses in nursing related to palliative care?

- What are the sample groups and sampling methods used in graduate theses in nursing related to palliative care?

- What are the sampling characteristics in graduate theses in nursing related to palliative care?

- What are the nursing practices in graduate theses in nursing related to palliative care?

Methods

This retrospective descriptive study was conducted by using the database of the National Thesis Center of the Council of Higher Education in the field of nursing between 2010 and 2022 (19). During the screening through the Thesis Center, Turkish language, master's and doctoral theses and dissertations covering the field of nursing were screened using the keywords "palliative care", "nursing", "postgraduate", and "Türkiye". Between August 1 and August 13, 2022, all theses that met the screening criteria were included in the study. The process of selecting the theses meeting the inclusion criteria was methodologically arranged according to the PRISMA flowchart (Figure 1).

Inclusion criteria for the study;

- Access to the full text,

- Theses to be accessible,

- To be done by department(s) of the nursing,

- Between the years of 2010 to 2022.

The limitations of the study, were that only the theses registered to the National Thesis Center database system were selected, theses published in full text were included and the low number of theses in the field of nursing.

Ethical aspect of the study, since theses that were accessible to the National Thesis Center database were included in the sampling and it did not involve experimental procedures on humans or animals ethics committee approval was not required.

Data gathering, seventy four theses were found which met the inclusion criteria, were registered in the database for the field of nursing on palliative care. Reviewing the theses 55 theses were included in the study, which met the inclusion criteria. Of the theses to be included in the study as a result of reviewing, 42 were graduate theses and 13 were doctoral theses.

Data analysis, the quality of the study was evaluated using the Critical Appraisal Skill Program. The program includes a checklist that systematically evaluates the results of the study. The questions in the checlist are replied as "Yes" and "No" (https://casp-uk.net/casptools-checklists/). SPSS for Windows Version 20.00 (SPSS Inc., Chicago, IL., USA) packaged software was used in the statistical analysis of the data obtained at the end of the study. The data were evaluated by frequency analysis. The form was developed by the researchers in line with the research questions and the data were evaluated accordingly. Theses included in the study in the content of the form are summarized according to author-advisor, thesis year, the purpose of the study, the type of study, the intervention applied, the sampling method, the group and number of sampling, the evaluation instruments used and the results of the studies in chronological order (Table 6).

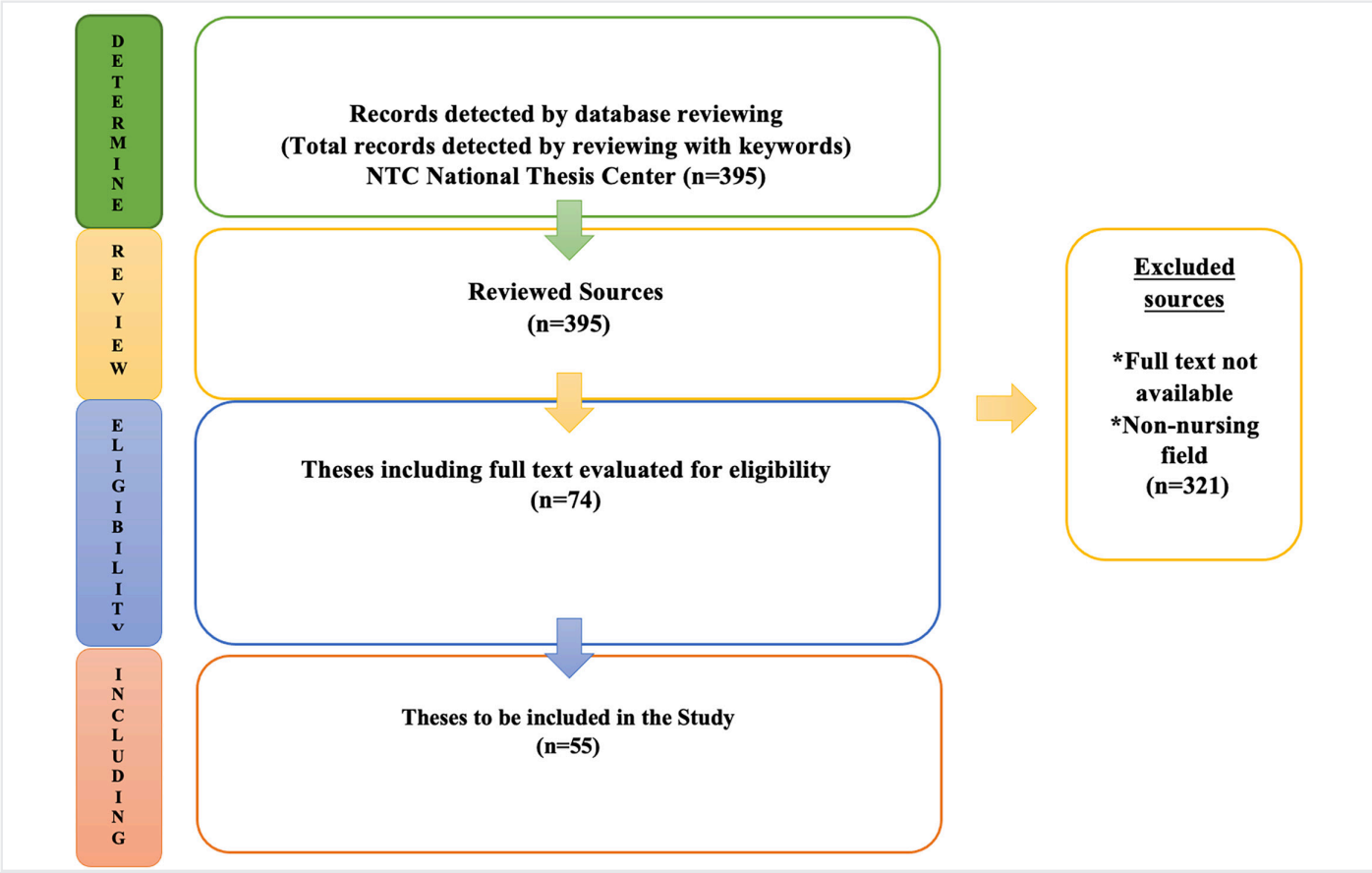

Figure 1. PRISMA flow chart

Findings

In this retrospective descriptive study, 55 theses registered to the National Thesis Center, were included between January 2010 and August 2022, 13 (23.6%) of which were doctoral theses and 42 (76.4%) of which were graduate theses (Table 1). According to the detailed analysis based on years, although the first graduate thesis study on palliative care in the field of nursing in our country belonged to 2010. It was determined that 60% (n=33) of the studies were conducted in 2019 and later (Table 2).

It was determined that 92.8% (n=51) of the thesis studies evaluated were conducted in quantitative design type; the majority (37.3%) (n=19) of the quantitative design types were descriptive and consisted of graduate theses; 8 of the experimental and quasi-experimental studies conducted were doctoral dissertations (Table 3).

It was determined that the thesis studies evaluated consisted of different sampling groups; 36.4% (n=20) of the sampling was conducted with healthcare personnel working in the palliative care unit (Table 4); 72.7% (n=40) of them were carried out without using any method; however, the simplest random sampling method was used among those which were used the method (Table 4). It was determined that the most of the doctoral thesis studies were on patients (n=7) who benefitted from palliative care services; the graduate thesis studies were mostly carried out on the healthcare personnel (n=19) working in the palliative care unit.

Table 1. The types of the theses reviewed (n=55)

	n	%
PhD	13	23.6
Master	42	76.4
Total	**55**	**100**

Table 2. Publication year of the theses reviewed (n=55)

Year	PhD	Master	
	n	n	%
2010	-	2	7.3
2011	1	-	
2012	-	-	
2013	-	1	
2014	-	-	
2015	-	2	32.7
2016	-	3	
2017	-	3	
2018	4	6	
2019	4	9	60
2020	3	6	
2021	-	10	
2022	1	-	
Total	**55**		**100**

It was observed that most of the experimental/quasi-experimental researches (73.3%) which were evaluated were made as doctoral thesis; when nursing practices were examined in theses palliative care education (n=3, 20.0%), simulation (n=2, 13.3%) were mostly discussed, and Watson's Nursing Approach Based on the Human Care Model (n=2, 13.3%) was discussed (Table 5).

Discussion

It was determined that the majority of 55 graduate theses types (76.4%) were conducted as graduate thesis, the number of doctoral dissertations was low and the first thesis was made in 2010 in the context of this study, which was conducted to evaluate the graduate theses related to palliative care in the field of nursing in Türkiye and to determine the nursing practices used in the theses. It was determined that more than half (60%)

Table 3. Research types of the theses reviewed (n=55)

The types of research	PhD	Master			
	n	n	Total	% (quantitative)	%
Quantitative					
Descriptive		19	19	37.3	92.8
Cross-sectional		1	1	2.0	
Experimental	7	1	8	15.7	
Quasi experimental	1	3	4	7.8	
Methodological		2	2	3.9	
Mixed type*	2	15	17	33.3	
Qualitative	1	1	2		3.6
Quantitative + Qualitative (mixed)	2	-	2		3.6
Total	**13**	**42**	**55**	**100**	**100**

It includes at least two types of quantitative research

Table 4. Sampling group and sampling method of the theses reviewed

Sampling group	PhD	Master	%
	n	n	
Patients receiving palliative care	7	6	23.6
Carers/family members of the patient receiving palliative care service	3	16	35
Healthcare personnel working in the palliative care unit	1	19	36.4
Student	2	1	5.4
Sampling method			
Method not used	2	38	72.7
Simple random	4	2	11.0
Stratified	2	-	3.6
Other methods	5	2	12.7
Viewed (n=55)			

Table 5. Nursing practices discussed in the theses reviewed

Nursing practices	PhD n	Master n	%
Palliative care training	2	1	20
Simulation	2		13.3
Watson's approach to nursing care based on HCM	2		13.3
Case management model	1		6.7
Hand fan	1		6.7
Red-white light	1		6.7
Listening to Turkish music accompanied by the tambourine	1		6.7
Auricular acupressure	1		6.7
Aromatherapy massage		1	6.7
Psychoeducation		1	6.7
Relaxation exercise		1	6.7
Total (%)	11 (73.3%)	4 (26.7%)	100

HCM: Human care model

of the theses were carried out in 2019 and afterwards. It was found that the majority of thesis consisted of graduate theses in accordance with our study in many systematic review studies in which the theses on different subjects in the field of nursing are discussed (20,21). It is believed that it is resulted from the low number of students in doctoral programs in our country and the low number of nurses working in this specialty.

Palliative care, which was first defined by WHO in 1986, includes a philosophy of care that has developed from various aspects until today. It is based on holistic and humanistic care. It is known that the emergence of palliative care nursing in the world dates back to the 1990s, when the concept of palliative care emerged. In Türkiye, the concept of palliative care didn't come to the fore until 2000s (1,22). The reason why the first thesis on palliative care in nursing was made in 2010 in our country and the most important reasons for the increase in number over the years was thought to be a result of the increase in the number of clinics providing palliative care services. Especially in the last two decades; development of palliative care nursing has emphasized the concept of palliative care more in national and international studies.

When the research methods used in the theses included in the study were evaluated, it was determined that most of them (92.8%) had a quantitative research design and it was determined that theses were mostly of descriptive type (37.3%), and all of the descriptive theses were graduate thesis. In a study evaluating the trends in nursing research in Türkiye, it was determined that the experimental and quasi-experimental studies among the dissertations were very few (23.5%) and more than half of these studies (66.7%) were doctoral theses, and that graduate theses were mostly conducted in the descriptive type of quantitative research designs (20). In a study conducted in Brazil, it was found that almost half (45.6%) of doctoral dissertations in nursing were conducted descriptively (23). On the other hand, in Sweden, it was determined that doctoral dissertations in the field of nursing mostly had experimental and quasi-experimental designs (24). While in a study evaluating graduate theses in the field of nursing, 39.5% of dissertations were done by non-invasive method, in another study evaluating doctoral dissertation studies, almost half of theses (42%) were conducted in experimental or quasi-experimental type, which was found to be consistent with the results of our study (21,25). While it is determined that graduate theses are more often quantitative in nature and mostly included studies aimed at determining the situation or in relational style, it is seen that the number of studies with experimental design in doctoral dissertations is increasing. In many studies in the literature, it is seen that graduate theses in the field of nursing are mostly designed in descriptive type and this situation stems from the effort to increase the knowledge in the disciplines in which the graduate studies are conducted due to their nature (13,20,26). In addition, it is thought that the reason why descriptive studies are preferred in graduate theses is likely to be related to the short duration of the research. The reasons why randomized controlled studies are preferred in doctoral dissertation are as follows; to contribute to the literature of the research subjects, the necessity of being unique and not studied before, the usability of evidence-based studies in finding answers to social problems, and willing to practice and to develop new methods/models. The fact that experimental designs are at the doctoral level in graduate programs is an expected situation due to the purpose/target and program qualifications of graduate programs. The fact that doctoral dissertations are mostly experimental shows that evidence-based knowledge and nursing practices have become widespread. This finding is stated as an indication that the purposes of graduate doctoral programs conducted in Türkiye have been achieved (20).

In our study, it was determined that the theses conducted in qualitative design and quantitative + qualitative (mixed) design were less frequent and both types were frequently used in the design of doctoral dissertations. It is stated that the mixed method contributes to a better understanding of the research problem than using a single method, which focuses on the use of quantitative methods that support qualitative data or qualitative data that supports quantitative data (27). Qualitative research methods try to reveal people's feelings, thoughts, behaviors and perspectives in a holistic way (28). The use of qualitative studies in the field of nursing is important in supporting quantitative data with experience (29).

While the sampling group of the majority (36.4%) of the graduate theses evaluated within the scope of the study was composed of healthcare personnel working in the palliative care unit, it was determined that doctoral dissertations were mostly conducted on patients benefiting from palliative care services. Researches including palliative patient groupsare fewer in number in our country. In our country, the inability of academic nurses to work in the clinic as clinicians at the same time and concerns about the fact that nurses' inability to study with vulnerable populations leads to more descriptive studies (30). It is difficult to conduct research as palliative care patients are a vulnerable group. In addition to the difficulty of obtaining voluntary

Table 6. Properties of graduate theses containing nursing practices on palliative care in nursing

Author (advisor) year phD	Research design types	Intervention (I) and control (C) group	Sampling method	Sample characteristics (sample and size)	Instruments	Result
Özçelik (43) (Fadıloğlu) 2011	Quasi-Experimental	I=Case management C=Routine care	None used	Cancer patients in the palliative care unit I=22 patients C=22 patients	- Characteristics form - ESAS - KPSS - EORT QLQ CA-30 - Patient and family care satisfaction form - Patient cost registration form - Palliative care variance registration form	Intervention group: - Symptom control (better) - Quality of life (except for physical and cognitive functions) (increased). - Patients and family's satisfaction levels (higher)
Uslu Şahan (36) (Terzioğlu) 2018	Mixed method (Randomized controlled and qualitative research)	I=Simulation (1st group= High validity, 2nd group= Hybrid, 3rd group= High validity and Hybrid) C=No intervention	Quantitative= Stratification method Qualitative=maximum variation sampling method	Students Quantitative I=1st group=21 students, 2nd group=21 students, 3rd group=19 students C=23 students Qualitative Students	- Characteristics form - Questionnaire for determining competencies in palliative care - IEPS4 - T-TAQ5 - Interprofessional gynecological oncology palliative care simulation practice checklist -Semi-structured focus group interview question guide	1st group and 2nd group: - Inter professional education (increased) - Students' knowledge and awareness of palliative care (increased) - The perception of interdisciplinary education (increase), - Understand the importance of teamwork (better)
Çamcı (33) (Oğuz) 2018	Experimental (Randomized Controlled)	I=Routine care and palliative care training C=Routine care	Minimization method of covariate-driven randomization	Chronic heart failure patients in the palliative care I=42 patients C=42 patients	- Information form - ESAS - EQ-5D - LVD-36	Intervention group: - Symptom burden at the first month (better) - The first, third- and sixth-month hospitalization (less)
Seven (38) (Sert) 2018	Experimental (Single-blind Randomized Controlled)	I=Holistic nursing care (Watson's Nursing Approach Based on the Human Care Model) C=Routine care	Simple random sampling method	Palliative care patients I=32 patients C=32 patients	- Information form - VAS - MBS - MRCS dyspnea scale - SF-36 - BAI - HADS - PPHEN	Intervention group: - Dyspnea and anxiety levels (decreased) - Quality of life (increased) - Satisfaction with nursing (increased)
Kocatepe (44) (Can) 2019	Experimental (Randomized Controlled)	I=Hand fan C=Routine care	Simple random sampling method	Lung cancer patients in the palliative care unit I=47 patients C=49 patients	- Identification form - Pulmonary function tests - Blood gas analyses - ECOG performance scale - Vital signs follow-up form - MBS - FACIT-Pal quality of life scale	Intervention group: - Quality of life (increased)

Table 6. Continued

Author (advisor) year phD	Research design types	Intervention (I) and control (C) group	Sampling method	Sample characteristics (sample and size)	Instruments	Result
Boyraz (35) (Yıldız) 2019	Methodological +Quasi Experimental (Single group pretest posttest controlled)	I=Web-based neonatal palliative care education C=No intervention	None used	Neonatal intensive care nurses n=143 nurses	- Information form - NiPCAS - Web-based neonatal palliative care education pretest and posttest evaluation form - Web-based neonatal palliative care education satisfaction evaluation form	NiPCAS (high reliability and adequate validity tool) Intervention group: - Attitudes towards palliative care (positive) - Knowledge level (increased)
Çelik (47) (Usta Yeşilbalkan) 2019	Experimental (Randomized controlled)	I=Bright white light C=Dim red light	Stratified randomization method	Palliative care patients I=26 patients C=26 patients	- Information form - BFI - PSQI Patient follow-up form	Bright White Light: - Fatigue levels (reduced), - Sleep quality and sleep duration (increased)
Kurtgöz (39) (Koç) 2019	Mixed method (Randomized controlled and qualitative research)	I=Watson's Nursing Approach Based on the Human Care Model C=Routine care	Quantitative=simple sampling Qualitative=Homogeneous sampling	Palliative care patients' relatives I=30 patients' relatives C=30 patients' relatives	- Information form - BHS SWBS	Intervention group: - Hopelessness levels (reduced)
Düzgün (50) (Karadakovan) 2020	Experimental (Randomized controlled)	I=Turkish music makams (hicaz and rast) listening by a tambourine C=Routine treatment and care	Stratified randomization method	Cancer patients in the palliative care unit I=30 patients C=30 patients	Information form patient follow-up form - SF-MPQ - GCS - STAI KPSS	Intervention group: - Pain and anxiety (decreased) - Comfort (increased) - Functional capacity (increased)
Alwawi (37) (Vardar İnkaya) 2022	Quasi-Experimental (Randomized controlled)	I= Simulation (1st group=a palliative care nursing theoretical training and Low-reality, 2nd group=a palliative care nursing theoretical training and a standard patient group) C=No control group	Simple random sampling method	Second-year students enrolled in a palliative care nursing course 1st group=35 students 2nd group=35 students	- Demographics form - Palliative care quiz for Nursing test - Student satisfaction and self-confidence in learning scale - Skill evaluation forms	1st group and 2nd group: - Providing a safe environment for students (effective) - Their knowledge, satisfaction, - Confidence and skills (increased)

Table 6. Continued

Author (advisor) year	Research design types	Intervention (I) and control (C) group	Sampling method	Sample characteristics (sample and size)	Instruments	Result
Master						
Gürakan (57) (Yıldırım) 2016	Experimental	I=Aromatherapy or massage 1st group= Routine care and aromatherapy, 2nd group=Routine care and massage C=Routine care	Simple random sampling method	Oncology patients in the palliative care unit I=1st group=11,2nd group=10 patients C=10 patients	- Introduction form - NRS[25] - ECOG performance scale - BFI	Intervention group: - Aromatherapy; pain (reduced), - Back massage; the plasma beta-endorphin level (increased)
Çetin (59) (Türkmen) 2018	Quasi-Experimental (Pretest-Posttest)	I=Psycho education group (seven weeks) C=Routine care	None used	Palliative care patients' relatives I=40 patients' relatives C=20 patients' relatives	- Introduction form - SF-36 - CSI	Intervention group: - Quality of life and the ability to cope with stress (increased)
Altay (34) (Tekkaş Kerman) 2019	Quasi-Experimental (Single group pretest-posttest)	I=Palliative care training (seven weeks) C=No intervention	None used	Oncology nurses I=23 nurses C=23 nurses	- Socio-demographic form - PBBT	Intervention group: - Nurse knowledge and palliative care knowledge level (increased)
Yaman (61) (Büyükyılmaz) 2020	Quasi-Experimental (Single group pretest-posttest)	I=Palliative care training (four weeks) C=No intervention	None used	Palliative care patients' caregivers n=60 caregivers	- Information form - MBI - STAI - VAS - ZCBS	Intervention group: - Care burden, burnout and anxiety levels (decreased)

ESAS: Edmonton Symptom Assessment Scale, KPSS: Karnovsky Performance Status Scale, EORT QLQ CA-30: European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire-30, IEPS: Interdisciplinary Education Perception Scale, T-TAQ: TeamSTEPPS Teamwork Attitudes Questionnaire, EQ-5D: EuroQOL-5D, LVD-36: Left Ventricular Dysfunction-36, VAS: Visual Analog Scale, MBS: Modified Borg Scale, MRCS: Medical research Council dyspnea Scale, SF-36: Short Form-36, BAI: Beck Anxiety Inventory, HADS: Hospital Anxiety and Depression scale, PPHEN: Patient Perception of Hospital Experience with Nursing, ECOG Performance Scale: The Eastern Cooperative Oncology Group Performance Scale,FACIT-Pal quality of life scale: Functional Assessment of Chronic Illness Therapy - Palliative Care quality of life scale, NiPCAS: Neonatal Palliative Care Attitude Scale, BFI: Brief Fatigue Inventory, PSQI: Pittsburgh Sleep Quality Index, BHS: Beck's Despair Scale, SWBS: Spiritual Well-Being Scale, SF-MPQ: Short-form McGill Pain Ouestionnaire, GCS: General Comfort Scale, STAI: State-Trait Anxiety Scale, NRS: Numeric Rating Scale, CSI: New Brief Coping Styles Inventory, PBBT: Palliative Care Knowledge Test, MBI: Maslach Burnout Inventory, ZCBS: Zarit Caregiver Burden Scale

informed consent from this vulnerable group to participate in the study, possible data loss due to reasons such as wanting to withdraw from the study in the continuation of the study or death are stated as the barriers that affect the studies in this field on patients (8,9).

Nursing practices discussed in the theses within the scope of this study are detected that the most commonly palliative care training, simulation training/practice, and Watson's Nursing Care Approach Based on Watson's Nursing Approach Based on the Human Care Model are achieved. Palliative care training has been included in basic, secondary and higher education programs in the last 20 years around the world (31). In addition to the absence of structured training programs in Türkiye, in recent years, the regarding subject has been mentioned in programs such as panels, congresses and symposiums (32). In 2022 for the first time in our country the 1st International Congress on Palliative Care in Nursing was hosted by Malatya İnönü University. Among the theses covered in this study, it was observed that palliative care training reduced the symptom burden and repeated hospitalizations given to patients with chronic heart failure (33). It has been determined that palliative care training given to individuals who care for patients and newborn nurses increases the level of knowledge (34,35). As a result of the findings of the theses dealing with the subject of palliative care training, it has been determined that palliative care training will be appropriate for patients who need palliative care and for nurses who care for them and the content of palliative care education will be developed with current literature.

Palliative care for nursing students with interprofessional simulation training and different simulation applications have been shown to increase the knowledge, awareness, satisfaction, confidence and skills of the teacher by providing an effective and safe learning environment, and it can be useful to better understand the importance of teamwork (36,37).

Considering the models or theories used in theses for palliative care patients in our country, in two doctoral theses Watson's

Human Care Model is used. In these theses, it is determined that nursing care provided in line with the model, it has a positive effect on the care outcomes of the patients and reduces the despair level of the relatives of the patients (38,39). In addition, it was determined that the satisfaction level of patients with nursing care increased (38). In this study, which evaluates the theses related to palliative care, finding that there is limited use of models/theories is supported by many reviews in which nursing theses are evaluated (40,41). However, theories/models offer the opportunity to provide holistic and quality care (42). Therefore, it is important for the development of scientific knowledge that the conceptual framework of doctoral dissertation studies is based on theory/model or that researches based on theory/model are predominantly involved (11). In the process of doctoral education within the scope of the Turkish Higher Education Qualifications Framework, it focuses on the development of students' doctoral theses "to be able to evaluate and use new information in the field with a systematic approach and gain the knowledge and skills to produce scientific solutions to problem" (http://tyyc.yok.gov.tr, accessed date: 21.01.2023). In this context, it is important to support the use of theory/model in palliative care nursing.

Although various models were used in the delivery of nursing care, when the theses covered in this study were evaluated; it was determined that the case management model was used in cancer patients receiving palliative care. It has been determined that this model provides better symptom control, improves quality of life and increases the satisfaction levels of patients and their families, and it is stated that it can be used in palliative care patients (43).

In other theses on palliative care in Türkiye, it was seen that face-fan application, red-white light application, listening to Turkish music accompanied by a tambourine, auricular acupressure application, aromatherapy massage, psychoeducation and relaxation exercise were used. It was determined that face-fan application increased the quality of life of patients for palliative care patients receiving lung cancer treatment in a doctoral thesis conducted to evaluate its effect in the management of dyspnea (44). As a matter of fact, it is emphasized that face-fan application on various patient groups reduces the level of dyspnea, improves exercise performance, increases the duration of exercise, has no side effects and can increase the quality of life of the patient because it is easy to apply (45,46). Another nursing practice in the dissertations discussed is red-white light application. At the end of the application, it was concluded that the application of bright white light reduced the fatigue levels of cancer patients receiving palliative care and had positive results on sleep (46). Many studies on the application of bright white light also showed parallelism with this doctoral thesis on palliative care patients, in which the fatigue level of the patients decreased and their sleep quality improved (48,49).

In another nursing practice, it was determined that Turkish music played with the tambourine reduced pain and anxiety for cancer patients hospitalized in the palliative care service, increased comfort and functional capacity, and were effective in controlling vital signs (50). In the reviewed literature, similar to this study, the benefits of music therapy such as reducing pain levels of individuals, feeling more comfortable, lowering heart rate, reducing anxiety are emphasized (51-53).

Among the theses within the scope of this study, in a doctoral thesis, it was determined that application of auricular acupressure was beneficial in the management of constipation in palliative care patients and increased the quality of life (54). It is possible to encounter similar studies in the current literature (55,56). In this respect, it is thought that auricular acupressure may be also appropriate for palliative care patients.

Another nursing practice is aromatherapy massage. With the help of this application a decrease in pain intensity and an increase in plasma beta-endorphin levels were found in cancer patients (57). In studies evaluating aromatherapy massage, it is emphasized that it has a positive effect on reducing the pain of patients and reduces the level of anxiety, stress and depression (58).

In addition to the applications in experimental studies in the theses evaluated, quasi-experimental psycho education was shown to increase caregivers' quality of life and their ability to cope with stress (59). In the literature, it is seen that psychosocial and spiritual support and symptom control trainings such as psychoeducation are effective on the health status, quality of life and ability to cope with stress of caregivers in different patient groups (60). In another quasi-experimental type thesis; it was concluded that applying relaxation exercises to caregivers reduced the burden of caregiving, burnout and anxiety levels (61). However, no similar study was found specific to the relaxation exercise intervention applied to caregivers of patients receiving palliative care.

Results and Recommendations

As a result of the information obtained from this study; it has been determined that the number of theses conducted in the field of palliative care has increased since 2019. It was determined that the theses discussed were mostly descriptive in nature with a quantitative design type to determine the current situation, and experimental and quasi-experimental studies were mostly doctoral theses. When nursing practices were reviewed, it was seen that palliative care education, training/application for simulation, and Watson's Nursing Approach Based on Watson's Nursing Approach Based on the Human Care Model were mostly discussed. It was determined that nursing practices, which were handled according to the results of all graduate thesis included in this study, positively affected the parameters and variables reviewed in the samples of patients, patient relatives and nursing students. It was observed that theories/models were used limitedly in the theses reviewed, and studies based on theory/models were frequently preferred at the doctoral level.

Accordingly, it is recommended to increase the number of experimental randomized controlled studies with high evidence value, with follow-up appropriate to the graduate education period, in which a cause-effect relationship between variables can be established in studies in the field of palliative care

nursing in Türkiye. At the same time, it is important for the development of nursing science to plan the researches that are planned to be conducted on palliative care based on nursing theories. Increasing research funds for graduate students in order to conduct high-evidence research on palliative care nursing will provide encouragement and support for students to do research.

Ethics

Authorship Contributions

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

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Review

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Phytotherapy in Gastrointestinal Disorders

Gastrointestinal Sistem Hastalıklarında Fitoterapi

Adem AKÇAKAYA[1], Fatma Betül AKÇAKAYA ÖZER[2]

[1]Bezmialem Vakıf University Faculty of Medicine, Department of General Surgery, İstanbul, Türkiye
[2]Bezmialem Vakıf University Faculty of Medicine, Department of Public Health, İstanbul, Türkiye

ABSTRACT

Gastrointestinal disorders include various disorders affecting organs such as the stomach, intestines, and liver, and while they have an important place in human health, their treatment processes are also complex. These disorders constitute an increasing health problem today and pose a significant burden on both health services and individuals. In the treatment of these disorders, phytotherapy (herbal treatment) is considered an important alternative in addition to traditional medicine. The use of phytotherapy has become preferred because it offers more natural options for patients with fewer side effects. Phytotherapy provides positive effects on the digestive system with its many properties such as anti-inflammatory, antioxidant, and antimicrobial features. In this article, various phytotherapeutic agents (e.g., aloe vera, turmeric, bitter melon, pomegranate, St. John's wort, mint, black cumin, berberine, garlic, olive leaf, cinnamon, tea, ginger, carnation and nettle) used in gastrointestinal disorders such as gastroesophageal reflux, peptic ulcer, inflammatory bowel disease, irritable bowel syndrome, colon polyps, colorectal and stomach cancer are discussed and their effects on the digestive system and potential properties in managing symptoms are discussed.

Keywords: Gastrointestinal system, digestive system disorders, phytotherapy, herbal treatment

ÖZ

Gastrointestinal sistem hastalıkları; mide, barsaklar, karaciğer gibi organları etkileyen çeşitli rahatsızlıkları içermektedir ve insan sağlığında önemli bir yer tutmakla birlikte tedavi süreçleri de karmaşıktır. Bu hastalıklar günümüzde giderek artan bir sağlık sorununu teşkil etmekte ve hem sağlık hizmetleri hem de bireyler üzerinde önemli bir yük oluşturmaktadır. Bu hastalıkların tedavisinde geleneksel tıbbın yanı sıra fitoterapi (bitkisel tedavi), önemli bir alternatif olarak değerlendirilmektedir. Fitoterapinin kullanımı, hastalar için daha doğal ve yan etkileri daha az olan seçenekler sunması nedeniyle tercih edilir hale gelmiştir. Fitoterapi; anti-enflamatuvar, antioksidan ve antimikrobiyal gibi pek çok özelliği ile sindirim sistemi üzerinde olumlu etkiler sağlamaktadır. Bu yazıda, gastrointestinal sistem hastalıkları olan gastroözofageal reflü, peptik ülser, enflamatuvar barsak hastalığı, irratable barsak sendromu, kolon polipleri, kolorektal ve mide kanserinde kullanılan çeşitli fitoterapötik ajanlara (örn; aloe vera, zerdeçal, kudret narı, nar, sarı kantaron, nane, çörek otu, berberin, sarımsak, zeytin yaprağı, tarçın, çay, zencefil, karenfil ve ısırgan otu) yer verilmiş sindirim sistemi üzerindeki etkileri ve semptomları yönetmedeki potansiyel özellikleri ele alınmıştır.

Anahtar Sözcükler: Gastrointestinal sistem, sindirim sistemi hastalıkları, fitoterapi, bitkisel tedavi

Introduction

Phytotherapy in Gastrointestinal Disorders

Phytotherapy (the use of medicinal plants for therapeutic purposes) is one of the oldest treatment methods used worldwide. Herodotus documented the first herbal medicine reports dating back to 3000 BC, and physician and philosopher Paracelsus recognized the healing power of plants and referred to the holistic approach for sustainable health by saying, "All meadows, all mountains and hills are pharmacies" (1). The concept of phytotherapy generally refers to the use of medicinal plants or herbal medicines to prevent, treat diseases or improve health conditions. Most phytotherapies have been developed from traditional medicinal plants. This concept summarizes an

Address for Correspondence: Adem Akçakaya, Bezmialem Vakıf University Faculty of Medicine, Department of General Surgery, İstanbul, Türkiye
E-mail: drakcakaya@yahoo.com **ORCID ID:** orcid.org/0000-0003-3116-7033

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evidence-based healing system that is widely practiced worldwide. Compared to nutritional therapy, phytotherapy is more complex due to the use of many products with diverse chemical structures and biological activities. Phytotherapy has an ancient history and is widely used worldwide. Some phytotherapy strategies are well known, having been extensively studied in the fields of chemistry and pharmacology and partially proven by clinical trials.

Epidemiological studies have shown that plant-based diets can have protective effects on diseases such as cardiovascular, gastrointestinal disorders and cancers, and can also be effective on broad health problems such as obesity and diabetes.

In this article, we will examine the plants used in the main gastrointestinal disorders and their methods of action in the light of the literature. The use of phytotherapy in these system diseases has become preferred because it offers more natural options for patients with fewer side effects. Phytotherapy provides positive effects on the digestive system with many properties such as anti-inflammatory, antioxidant, antimicrobial and anticancer features. The diseases discussed and the plants used are explained specifically for the disease.

Phytotherapy in Gastroesophageal Reflux Disease and Peptic Ulcer

Aloe vera is an effective plant used in the treatment of gastroesophageal reflux disease (GERD). GERD is a condition that occurs when stomach acid leaks back into the esophagus (2). Symptoms include heartburn, difficulty swallowing, irritation in the esophagus, a burning sensation in the throat and cough. Aloe vera is a jelly-like substance obtained from the leaves of a cactus-like plant. However, recent studies have shown that aloe vera may also have positive effects on digestive system disorders. The polysaccharides and antioxidants found in aloe vera have the potential to neutralize stomach acid. This feature helps relieve GERD symptoms. The anti-inflammatory properties of aloe vera help reduce irritation and inflammation that may occur in the esophagus. This feature may contribute to the improvement of symptoms such as difficulty swallowing that occur due to GERD. It is thought that aloe vera may have a regulatory effect on the digestive system. It can regulate bowel movements and improve digestive processes. This may be effective in reducing GERD symptoms caused by digestive problems (3). The effectiveness of aloe vera has been demonstrated in various pharmacological and clinical studies. Thus, the potential benefits of aloe vera on GERD and the points to be considered are emphasized. Peptic ulcer disease is a condition characterized by lesions on the inner surface of the stomach or duodenum. These wounds are usually affected by digestive juices such as stomach acid and pepsin, and symptoms can include heartburn, abdominal pain, indigestion, and even bleeding. Peptic ulcers are often associated with factors such as *Helicobacter pylori* infection, long-term use of non-steroidal anti-inflammatory drugs, or excessive alcohol consumption (4).

Turmeric (Curcuma longa) is a plant of which roots grow underground and is a spice widely used in Asian cuisine. The active ingredient of turmeric is curcumin, which has anti-inflammatory, antioxidant, and antiseptic properties (5). These properties suggest that it may be potentially useful in the treatment of peptic ulcer disease. Turmeric can speed up the healing process of ulcers by reducing the inflammatory responses that play a role in the formation of peptic ulcers and the inflammation in the lining of the stomach and intestines. The antioxidants in it can prevent damage to stomach and intestinal cells by combating free radicals. This can slow down the formation and progression of peptic ulcers. Various studies on the potential benefits of turmeric in the treatment of peptic ulcer disease have attempted to prove this effectiveness (6,7).

Bitter melon is one of the herbal treatment methods that may be potentially useful in the treatment of peptic ulcer disease. Bitter melon has anti-inflammatory and antioxidant properties thanks to the various biologically active components it contains. Bitter melon has protective effects on the stomach and intestinal mucosa and can accelerate the healing process of ulcers thanks to these properties. Studies have shown that bitter melon can be effective in the treatment of peptic ulcers by neutralizing stomach acid and protecting the mucosa (8,9).

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a disorder that includes chronic IBD such as Crohn's disease and ulcerative colitis. These diseases are characterized by chronic inflammation and damage to the intestines. Symptoms may include abdominal pain, diarrhea, bloody stools, weight loss and fatigue (10,11). In addition to pharmaceutical drugs, natural and herbal supports are also being investigated in the treatment of patients with IBD.

Pomegranate (Punica granatum L.) is a fruit rich in antioxidants and phytochemicals. Pomegranate peel and seeds, in particular, contain compounds with many health benefits, such as polyphenols (e.g., ellagic acid), anthocyanins, flavonoids, and vitamin C. It is suggested that the anti-inflammatory, antioxidant, and immunomodulatory properties of these compounds may be beneficial in the management of IBD. Ellagic acid, which contains anti-inflammatory and antioxidant effects, can reduce the severity and frequency of IBD symptoms by reducing inflammation in the intestinal wall and can help protect intestinal cells against oxidative stress by fighting free radicals (12,13). Pomegranate, which is among the natural supports in the management of IBD, is considered a potential alternative or supportive treatment option thanks to its anti-inflammatory and antioxidant properties.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a common digestive system disorder and its symptoms include abdominal pain, bloating, gas, diarrhea, and constipation. Although the exact cause of IBS is unknown, factors such as stress, eating habits, and changes in intestinal microbiota are thought to play a role (14). There is an increasing search for non-pharmaceutical, natural supports for managing IBS symptoms.

St. John's Wort (Hypericum perforatum) is a plant that has been used in the treatment of various health problems since ancient

times. It is known especially for treating mental health problems such as depression and anxiety, but in recent years its effects on digestive system disorders have also been investigated (15). The compounds in St. John's Wort help relieve muscle spasms and pain in the intestines, and it is thought that these properties will provide potential benefits in the management of IBS symptoms.

Peppermint oil (Mentha piperita) is another herbal treatment known to be effective in relieving IBS symptoms. The menthol in peppermint oil can reduce muscle spasms in the intestines and relieve symptoms such as abdominal pain, bloating, and gas. Studies have shown that peppermint oil is effective in managing IBS symptoms and improves the quality of life of patients (16).

Black seed oil (Nigella sativa) is another herbal oil that has anti-inflammatory and antispasmodic properties and can relieve IBS symptoms. The thymoquinone content of black seed oil may help manage IBS symptoms by reducing inflammation in the intestines and relieving spasms. Studies investigating the effects of black seed oil on IBS suggest that this oil may be beneficial in reducing symptoms and improving the quality of life of patients (17).

Colon Polyps and Colorectal Cancer

Colon polyps are small, abnormal tissue growths found in the large intestine, and in some cases, they can turn into cancer. Colorectal cancer is cancer of the large intestine or rectum and is a major health problem worldwide (18). The potential benefits of naturally derived compounds are being investigated in the treatment of both colon polyps and colorectal cancer.

Berberine (Berberis Vulgaris) is a natural alkaloid found in many plants. Curcumin is a compound obtained from the turmeric root that has anti-inflammatory, antioxidant, and anticancer properties. These two compounds have properties that may provide potential benefits for conditions such as colon polyps and colorectal cancer.

Berberine is noted for its ability to reduce intestinal inflammation, regulate cell turnover, and stop the growth of cancer cells. Berberine has been shown to have anticancer effects in several studies. Curcumin stands out as an effective tool in preventing colon polyps and cancer development by suppressing inflammation and promoting apoptosis (programmed cell death) processes (19,20).

Pomegranate peel extract may play an important role in preventing colon polyps and colorectal cancer through the high amount of antioxidant compounds it contains. Pomegranate peel contains various antioxidants such as polyphenols, flavonoids, and tannins. These compounds may reduce the risk of cancer by preventing cell damage caused by free radicals. The anti-inflammatory properties of pomegranate peel may also help reduce intestinal inflammation, which may contribute to preventing the development of polyps and cancer (21).

The antioxidant properties of pomegranate peel extract can prevent DNA damage by reducing oxidative stress in cells, thus providing a protective effect against cancer. Studies have shown that pomegranate peel can prevent the growth of cancer cells and promote the apoptosis process. These properties make pomegranate peel extract a potential phytotherapeutic agent in preventing colon polyps and colorectal cancer (22).

Stomach Cancer and Phytotherapy

Stomach cancer is a major health problem worldwide and its treatment processes are complex. Stomach cancer is a type of cancer that occurs as a result of abnormal growth and proliferation in stomach cells. It usually starts in the lining of the stomach and can spread to other tissues over time. Stomach cancer symptoms are often not obvious in the early stages, so the disease is usually diagnosed in advanced stages. Risk factors include *Helicobacter pylori* infection, excessive consumption of salty or smoked foods, obesity, smoking, and family history. Early diagnosis and treatment are important because stomach cancer can be difficult to treat in advanced stages (23). However, research on the positive effects of natural foods and plants on health shows that some foods can reduce the risk of cancer or support treatment processes.

Garlic (Allium sativum) is a plant that has been used for medicinal purposes for thousands of years and is known to have various positive effects on health with the bioactive compounds it contains. Allicin, the main active ingredient of garlic, has antioxidant and antimicrobial properties. In addition, the sulfur compounds found in garlic can also have positive effects on health. Studies on stomach cancer show that garlic consumption can reduce the risk of cancer. Garlic can prevent cell damage and reduce the effects of carcinogenic substances in the body through the antioxidants it contains. In addition, some laboratory studies have shown that garlic can prevent the growth of cancer cells and trigger the apoptosis (programmed cell death) mechanism (24,25). However, more clinical research is needed on the exact effects of these effects on humans.

Resveratrol is a natural polyphenol found in grape skins, red wine, Japanese knotweed (Polygonum cuspidatum) and some other plants. It is known to have antioxidant, anti-inflammatory and anticancer properties. Studies on stomach cancer show that resveratrol can prevent the growth of cancer cells and trigger the apoptosis process. Resveratrol may reduce the risk of stomach cancer by preventing cell damage caused by free radicals and repairing DNA damage (26,27).

The protective and therapeutic effects of resveratrol on stomach cancer involve mechanisms at the cellular level. These mechanisms include suppression of inflammation, reduction of oxidative stress, and regulation of cellular signaling pathways. It has also been suggested that resveratrol may increase treatment efficacy and reduce drug resistance of cancer cells when used in conjunction with chemotherapy (28).

Fatty Liver and Phytotherapy

Fatty liver is a condition characterized by excessive fat accumulation in liver cells. This disease can negatively affect liver

function and, in advanced cases, can develop into liver cirrhosis or liver cancer. Phytotherapy contains natural ingredients that may be potentially beneficial in the treatment of fatty liver.

Olive leaf (Olea europaea L. Folium) extract has strong antioxidant properties and is effective in reducing fat accumulation in liver cells. Oleuropein contained in olive leaf extract has anti-inflammatory and hepatoprotective properties that support liver health (29).

Garcinia cambogia (Garcinia gummi-gutta) extract can reduce fat synthesis and prevent fatty liver through its hydroxycitric acid content. This plant has properties that support weight loss and regulate fat metabolism (30).

Cinnamon (Cinnamomum verum) is another herb recommended for use in the treatment of fatty liver. Cinnamon can support liver health with its anti-inflammatory and antioxidant properties and prevent fat accumulation by reducing insulin resistance (31).

Bitter melon extract has anti-inflammatory and antioxidant properties and may be effective in reducing fat accumulation in liver cells. The components contained in bitter melon may protect liver health and prevent the progression of steatosis (32).

Ellagic acid is a natural polyphenol found in various fruits and vegetables. Ellagic acid, which has antioxidant and anti-inflammatory properties, may support liver health by reducing fat accumulation in liver cells (33).

Curcumin is the active ingredient in turmeric and has strong antioxidant and anti-inflammatory properties. Curcumin may be potentially beneficial in the treatment of fatty liver and may improve liver function (34).

Diarrhea

Diarrhea is a condition characterized by increased bowel movements and increased water content of stool. Some of the plants used in phytotherapeutic treatments for diarrhea are:

Tea (Camelia Sinensis) is one of the plants commonly used in the treatment of diarrhea. The tannins in tea can relieve diarrhea by tightening the intestinal mucosa and reducing bowel movements. In addition, the antimicrobial properties of tea can help control intestinal infections (35).

Pomegranate peel is an effective herbal solution for diarrhea treatment through the tannins it contains. The antimicrobial and anti-inflammatory properties of pomegranate peel can help reduce intestinal infections and inflammation. Studies have shown that pomegranate peel extract is effective in the treatment of diarrhea (21).

Ginger (Zingiber officinale), as well as being widely used in the treatment of nausea and vomiting, can also be effective in the treatment of diarrhea. The antiemetic and antispasmodic properties of ginger can reduce the severity of diarrhea by regulating bowel movements (36).

Clove (syzygium aromaticum) is another effective herb in the treatment of diarrhea. The antimicrobial and anti-inflammatory properties of clove relieve diarrhea by reducing intestinal infections and inflammation. In addition, the eugenol compound contained in cloves helps control diarrhea by reducing intestinal muscle spasms (37).

Constipation

Constipation is a condition characterized by decreased bowel movements and hardening of stools. Phytotherapy offers natural and effective solutions in the treatment of constipation.

Flaxseed oil is a natural product widely used in the treatment of constipation. Flaxseed oil can increase bowel movements and help soften stools through its high fiber content. In addition, the omega-3 fatty acids contained in flaxseed oil support intestinal health and reduce inflammation (38).

Senna (Senna alexandrina) is another plant used in the treatment of constipation. The leaves and fruits of senna contain anthraquinone glycosides that increase bowel movements. These compounds can relieve constipation by encouraging contraction of the intestinal muscles (39).

Nettle (Urtica dioica) has mild laxative properties and can be used in the treatment of constipation. Nettle can relieve constipation by increasing bowel movements and helping to soften stools (40).

Conclusion

Gastrointestinal tract disorders are considered complex conditions in which herbal medicines can be included alongside medical treatment and lifestyle changes. Phytotherapy involves the use of plant-derived compounds, and the effects of these compounds on gastrointestinal health are increasingly being investigated. The chemical composition and clinical studies discussed in my article have shown that some plants may be potentially useful in the management of digestive tract diseases. When the chemical compositions of plants are examined, it has been found that many plants have anti-inflammatory, antioxidant, antispasmodic or antimicrobial properties. For example, it has been found that the anti-inflammatory effects of curcumin, the active ingredient in turmeric, can relieve symptoms of conditions such as stomach ulcers and IBS. Similarly, ginger has been shown to help treat nausea and digestive problems with its antiemetic (anti-vomiting) effects on the digestive system.

The results of clinical studies also support the effectiveness of phytotherapy. However, more scientific research is needed on the effectiveness of phytotherapy, and the methodological quality of such studies and the use of standardized forms of plants are important. In addition, the interactions and side effects of phytotherapy with other treatment methods should also be taken into account.

In conclusion, phytotherapy can be a potentially effective complementary treatment option in the management of gastrointestinal disorders. However, patients and healthcare professionals need to be careful in this regard and create treatment plans that are appropriate for individual situations. Further research and clinical studies will help us understand more about

the effectiveness and safety of herbs, making phytotherapy a reliable option for patients.

Ethics

Authorship Contributions

Concept: A.A., F.B.A.Ö., Design: A.A., F.B.A.Ö., Data Collection or Processing: A.A., F.B.A.Ö., Analysis or Interpretation: A.A., F.B.A.Ö., Literature Search: A.A., F.B.A.Ö., Writing: A.A., F.B.A.Ö.

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